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CLINICAL STUDIES

Association of interleukin 28B and mutations in the core and NS5A region of hepatitis C virus with response to peg-interferon and ribavirin therapy

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Keywords
core region – genotype 1b – hepatitis C virus – interleukin 28B – NS5A

Abbreviations
aa, amino acid; ALT, alanine aminotransferase; HCV, hepatitis C virus; IFN, interferon; IL28B, interleukin 28B; ISDR, interferon sensitivity-determining region; SVR, sustained virological response.

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Abstract

Background and aims: Mutations in the core and NS5A region of hepatitis C virus (HCV) genotype 1b have been associated with response to interferon (IFN) therapy. Genome-wide association studies have revealed that the single-nucleotide polymorphism (SNP) of interleukin 28B (IL28B) contributes to IFN response. The aim of this study was to investigate whether the SNP of IL28B (rs8099917) and amino acid substitutions in the core and NS5A region affect the response to IFN therapy. *Methods:* A total of 299 patients (157 men, 142 women; mean age, 55.9 ± 10.3 years) infected with HCV genotype 1b were studied. The fibrosis stage was diagnosed as F0 ($n=23$), F1 ($n=121$), F2 ($n=62$), F3 ($n=32$) and F4 ($n=7$) by liver biopsy. *Results:* Of the 299 patients, 138 achieved sustained virological response (SVR). On univariate analysis, predictors of SVR were age < 60 years, male gender, higher platelet count, lack of fibrosis, non-Q at core 70, mutant-type interferon sensitivity-determining region (ISDR) and IL28B genotype TT. The factors related to SVR on multivariate analysis were IL28B ($P=0.0001$), fibrosis ($P=0.0111$) and mutations in the core region70 ($P=0.0267$) and ISDR ($P=0.0408$). The best SVR was achieved in patients with non-Q70, mutant-type ISDR and T allele (74.5%), and the worst was achieved in patients with Q70, wild-type ISDR and G allele (8.1%). *Conclusions:* The SNP of IL28B and mutations in the core region and NS5A are associated with IFN responsiveness. Both host and viral factors might be useful for predicting IFN response.

It has been estimated that 170 million worldwide are infected with hepatitis C virus (HCV), which causes chronic hepatitis that can develop into potentially fatal cirrhosis and hepatocellular carcinoma (1). Therefore, HCV infection is a major global health problem. Pegylated-interferon (IFN)-α and ribavirin combination therapy is standard treatment for patients with chronic hepatitis C, but it eradicates HCV for only 50% of patients with genotype1 (2, 3). The difference in response was investigated, and several factors were identified, including age, liver fibrosis, HCV genotype, HCV RNA levels and race (4–7). Viral factors were frequently the focus for investigation of IFN responsiveness, and amino acid (aa) substitutions in the core and NS5A regions were reported as markers that could be used to predict the response to IFN therapy (8–14). However, these relationships were controversial (15, 16), and investigations were limited to viral factors alone to clarify IFN responsiveness. However, host genetic factors, as well as genetic heterogeneity in the HCV genome, contribute to IFN treatment outcomes. Therefore, several genome-wide association studies were performed to understand the host factors that were associated with IFN responsiveness; these revealed that interleukin 28B (IL28B) polymorphisms are strongly associated with response to IFN therapy (17–20). The single-nucleotide polymorphisms (SNPs) of IL28B, rs12979860 and rs8099917 genotypes are significantly associated with the outcome of IFN therapy. Although Caucasians and Hispanics have weak linkage-disequilibrium between these two SNPs, Japanese patients have strong linkage-disequilibrium, with no discrepancy between rs12979860 and rs8099917. Thus, rs8099917, which is strongly associated in Japanese reports, was selected for the present study (21). SNP of IL28B and mutations in the core and NS5A regions had different effects on IFN responsiveness, and their combined use might improve the ability to predict the response to IFN. However, the relationships between IL28B and viral factors such as mutations in the core and NS5A regions are little known. The aim of this study

was to investigate whether the SNP of IL28B and aa substitutions in the core and NS5A regions in patients with HCV genotype 1b affect the response to pegylated-IFN-α 2b and ribavirin combination therapy.

Methods

A total of 432 patients with chronic hepatitis C genotype1b and high viral load who were treated at Nagoya University Hospital, Fujita Health University Hospital and Ogaki Municipal Hospital were enrolled; 299 patients who completed IFN treatment for 48 weeks and had complete clinical data were selected for this study. Patients whose HCV RNA levels were < 100 KIU/ml were excluded. The patients' clinical characteristics are summarized in Table 1. The core region (aa 30–110) and interferon sensitivity-determining region (ISDR) (aa 2209–2248) were examined by direct sequencing. Identification of the SNP of IL28B (rs8099917) was performed by a real-time polymerase chain reaction (PCR) system. Liver biopsy was performed in 245 patients, and fibrosis stage was diagnosed according to the METAVIR criteria (22). Patients received subcutaneous injections of pegylated-IFN-α 2b (1.5 μg/kg) once each week plus oral ribavirin (600 mg for < 60 kg, 800 mg for 60–80 kg, 1000 mg for > 80 kg) daily for 48 weeks. Serum was stored at − 80 °C for virological examination at pretreatment. Patients who were persistently negative for serum HCV RNA at 24 weeks after withdrawal of IFN treatment were considered to have a sustained virological response (SVR). The other patients were considered to have non-SVR. This study was approved by each hospital's ethics committee. Written informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the Declaration of Helsinki.

Virological analysis

The HCV-RNA quantitative viraemia load was determined by PCR. HCV was genotyped by direct sequencing of the 5′-untranslated region and/or E1 regions as described previously (23, 24). Genotypes were classified according to the nomenclature proposed by Simmonds *et al.* (25). Direct sequencing of the HCV core and NS5A-ISDR region was performed as reported previously (9, 14). In brief, RNA was extracted from 140 μl of serum with a commercial kit (QIAamp Viral RNA Kit, Qiagen, Valencia, CA, USA) and dissolved in 50 μl of diethylpyrocarbonate-treated water. RNA (10 ng) was used for reverse transcription with oligo and random hexamer primers using a commercial kit (iScript cDNA Synthesis Kit, Bio-Rad, Hercules, CA, USA). The HCV core region and NS5A-ISDR were amplified by nested PCR. In brief, each 50 μl PCR reaction contained 100 nM of each primer, 1 ng of template cDNA, 5 μl of GeneAmp 10 × PCR buffer, 2 μl of dNTPs and 1.25 U of AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA). Primers for the core region were sense, 5′-GGGAGGTCTCGTA GACCGTGCACCATG-3′ and antisense, 5′-GAGMGG KATRTACCCCATGAGRTCGGC-3′, and primers for the NS5A-ISDR were sense 5′-TGGATGGAGTGCGGTTGCA CAGGTA-3′ and antisense 5′-TCTTTCTCCGTGGAGG TGGTATTG-3′. Amplification conditions consisted of 10 min at 94 °C, followed by 40 cycles of 94 °C for 10 s, 55 °C for 30 s and 72 °C for 30 s in a thermal cycler (GeneAmp PCR System 9700, Applied Biosystems). The second PCR was performed in the same reaction buffer with the first-round PCR product as template and with the following sets of primers: for the core region, sense primer 5′-AGACCGTGCACCATGAGCAC-3′, and antisense 5′-TACGCCGGGGGTCAKTRGGGCCCCA-3′; and for the NS5A-ISDR, sense 5′-CAGGTACGCTCCGGCGTGCA-3′ and antisense 5′-GGGGCCTTGGTAGGTGGCAA-3′. PCR products were separated by electrophoresis on 2% agarose gels, stained with ethidium bromide and visualized under ultraviolet light. PCR products were then purified and sequenced with the second-round PCR primers with a dye terminator sequencing kit (BigDye Terminator v1.1 Cycle Sequencing Kit, Applied Biosystems) and an ABI 310 DNA Sequencer (Applied Biosystems). A mutation mixture was defined as viral mutants that constituted 50% or more of the total viral population.

Table 1. Baseline characteristics of the patients

Clinical characteristics	*N* = 299
Age (years)	55.9 ± 10.3
Sex: male/female	157/142
AST (IU/L)	58.7 ± 48.9
ALT (IU/L)	69.8 ± 66.9
Platelet count (10^4/μL)	16.6 ± 5.3
HCV RNA level (KIU/ml)	1760
The fibrosis stage	(100–7200)
F0, F1, F2, F3, F4	23,121, 62, 32, 7
Body weight (kg)	57.9 ± 12.7

Data are expressed as mean ± standard deviation.
HCV RNA level was shown by median (range).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus.

Genomic analysis

Detection of the SNP of IL28B (rs8099917) was carried out by a real-time PCR system. In brief, genomic DNA was extracted from 150 μl of whole blood using a commercial kit (QIAamp DNA Blood mini Kit, Qiagen) and was dissolved in 50 μl of diethylpyrocarbonate-treated water. DNA (10 ng) was used for PCR with primers and probes from a commercial kit (Taqman SNP Genotyping Assays, Applied Biosystems). The SNP of IL28B (rs8099917) was amplified, and the results were analysed by real-time PCR in a thermal cycler (7300 Real-time PCR System, Applied Biosystems).

Statistical analysis

Data are expressed as means ± standard deviation. The paired *t*-test, the χ^2-test and Fisher's exact test were used

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to analyse differences in variables. A P value < 0.05 was considered significant. Multiple logistic regression models were used to identify factors predictive of SVR. The statistical software used was SPSS software (SPSS Inc., Chicago, IL, USA).

Results

Virological response

Of 299 patients, 35 (11.7%) showed a rapid virological response (RVR), with HCV negativity at 4 weeks, and 172 (57.5%) showed an early virological response (EVR), with HCV negativity at 12 weeks. Overall, 234 patients became HCV negative at the end of treatment (78.3%). However, 138 patients continued to be HCV negative after withdrawal of IFN treatment, and 138 of 299 (46.2%) patients were defined as achieving SVR. Of 35 patients with RVR, 33 (94.3%) achieved SVR. Of 172 patients with EVR, 126 (73.3%) achieved SVR. Of 127 patients without EVR, 115 became non-SVR (90.6%). Thus, RVR and EVR were associated with SVR ($P < 0.001$).

Genetic heterogeneity in NS5A-interferon sensitivity-determining region and response to interferon therapy

The sequence of the HCVJ strain was defined as the consensus sequence, and the approach of counting the number of mutations to the chosen consensus sequence in ISDR was used to analyse the ISDR system as in previous reports (12–14). Seventy-one patients with more than two mutations in the ISDR were defined as mutant type, and the other 228 patients were wild type. SVR was achieved in 41.2% (95/228) of the patients with wild-type ISDR and in 60.6% (43/71) of the patients with mutant-type ISDR ($P = 0.0063$). ISDR was associated with SVR.

Amino acid substitutions in core regions of the hepatitis C virus genome and response to interferon therapy

Eighty-five patients with glutamine in core region 70 were defined as Q-type, and the other 214 patients were non-Q-type, as in previous reports (14). Overall, 118 of 214 patients with non-Q in the core region achieved SVR (55.1%). The SVR rate of patients with Q in core region 70 was 23.5% (20/85). Q70 in core region 70 was significantly associated with poor response to IFN therapy ($P < 0.0001$). The distribution of mutations in the HCV core region at aa 91 was leucine (L), 210 and methionine (M), 89. There were no significant differences between mutations in the HCV core region at aa 91 and SVR.

The prevalence of the single-nucleotide polymorphism of Interleukin28B (rs8099917) T (major allele) and G (minor allele) and response to interferon therapy

The frequencies of the IL28B genotypes were major homozygotes (TT), 219; heterozygotes (TG), 76; and minor homozygotes (GG), 4. The rates of SVR in the patients with TT, TG and GG were 57.9% (127/219), 14.5% (11/76) and 0% (0/4) respectively. The G allele of the IL28B genotype was significantly associated with poor response to IFN therapy ($P < 0.0001$).

Table 2. Association between interleukin 28B genotypes and amino acid substitutions in hepatitis C virus core region and interferon sensitivity-determining region

	ISDR	
	Mutant	Wild
TG/GG	12	68
TT	59	160
P value = 0.0324		
	HCV core region 70	
	Non-Q	Q
TG/GG	35	45
TT	179	40
P value < 0.0001		
	HCV core region 91	
	L	M
TG/GG	46	34
TT	164	55
P value = 0.0044		

The number is patients' number.
HCV, hepatitis C virus; IL28B, interleukin 28B; ISDR, interferon sensitivity-determining region; L, leucine; Q, glutamine; M, methionine.

The relationships between substitutions of aa in the HCV core region, NS5A-ISDR and the SNP of IL28B are shown in Table 2. NS5A-ISDR and both mutations in the HCV core regions were associated with IL28B genotypes. ISDR wild-type and Q70, which were resistant strains to IFN therapy, were more frequently found in patients with resistant TG/GG allele than in those with sensitive TT allele.

Factors associated with sustained virological response

The results of univariate analysis for factors predictive of SVR are shown in Table 3. Patients with SVR were younger than those without SVR. Males were more frequent among SVR patients than non-SVR patients. SVR patients had higher platelet counts than non-SVR patients. SVR was achieved in 23.1% (9/39) of patients with advanced fibrosis and 50.5% (104/206) of patients without advanced fibrosis ($P = 0.0016$). SVR was achieved in 41.7% (95/228) of patients with wild-type ISDR and 60.6% (43/71) of patients with mutant-type ($P = 0.0063$). SVR occurred more frequently in patients without Q70 (55.1%; 118/214) than in those with Q70 (23.5%; 20/85; $P = 0.0001$). Achievement of SVR occurred more frequently in patients with TT allele (58%; 127/219) than in those with TG and GG alleles (13.8%; 11/80;

Table 3. Univariate analysis: factors predictive of sustained virological response

Factors	SVR ($n=138$)	Non-SVR ($n=161$)	P value
Age (years)	53.8 ± 11.5	57.9 ± 8.7	0.0005
Gender: male/female	82/56	75/86	0.0280
ALT (IU/L)	71.3 ± 76.7	68.4 ± 57.6	0.7110
AST (IU/L)	54.6 ± 46.7	62.1 ± 50.6	0.1983
PLT ($\times 10^4/mm^3$)	17.7 ± 5.5	15.6 ± 4.9	0.0008
Fibrosis: F0, 1, 2/3, 4	104/9	102/30	0.0016
HCV RNA level (KIU/ml)	2001.5 ± 1441.2	2168.3 ± 1432.4	0.3705
Core 70: non-Q/Q	118/20	96/65	0.0001
Core 91: L/M	104/106	34/55	0.0771
ISDR: wild/mutant	95/43	133/28	0.0063
IL28B: TT/TG+GG	127/11	92/69	0.0001

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; IL28B, interleukin 28B; ISDR, interferon sensitivity-determining region; L, leucine; M, methionine; PLT, platelet count; Q, glutamine.

Table 4. Factors associated with sustained virological response by multivariate analysis

Factor	Category	Risk ratio	95% CI	P value
IL28B genotype	TT	0.106	0.043–0.259	0.0001
Fibrosis	F3, F4	3.550	1.335–9.440	0.0111
Core70	Q	2.496	1.111–5.604	0.0267
ISDR	Wild	2.206	1.034–4.710	0.0408

Only factor that achieved statistical significance ($P < 0.05$) on multivariate logistic regression analysis are shown.
IL28B, interleukin 28B; ISDR, interferon sensitivity-determining region.

$P=0.0001$). Age, sex, platelet count, liver fibrosis, core 70, ISDR and IL28B were associated with SVR. The same 11 factors used in univariate analysis were used in multivariate analysis. The factors related to SVR on multivariate analysis were IL28B genotype, liver fibrosis, core 70 and ISDR, as shown in Table 4. The other factors were not significant.

The virological response according to interleukin28B genotypes and amino acid substitutions in the 70 core region and interferon sensitivity-determining region

The SVR rates according to IL28B genotypes and aa substitutions in the 70 core region and ISDR are shown in Table 5. The best SVR rate was achieved in patients with non-Q70, mutant-type ISDR and T allele, and the worst response was achieved in patients with Q70, wild-type ISDR and G allele.

Discussion

Viral factors associated with SVR have been the most frequently studied, and several regions, including 5′UTR, core, E2, NS5A and NS5B, have been suggested to play important roles in IFN responsiveness (8–16, 26–31). The aa substitutions in the HCV core and NS5A region would be two major viral factors that have strong associations with IFN response. The ISDR located in the

Table 5. The sustained virological response rate according to interleukin 28B and amino acid substitutions in 70 core region and interferon sensitivity-determining region

Core70/ISDR	IL28B; TT 58% (127/219)	IL28B; GT/GG 13.8% (11/80)
Q/wild 20% (13/65)	35.7% (10/28)	8.1% (3/37)
Q/mutant 35% (7/20)	50% (6/12)	12.5% (1/8)
non-Q/wild 50.3% (82/163)	57.6% (76/132)	19.4% (6/31)
non-Q/mutant 70.6% (36/51)	74.5% (35/47)	25% (1/4)

P-value = 0.0001 by Cochran–Armitage test.
IL28B, interleukin 28B; ISDR, interferon sensitivity-determining region; Q, glutamine.

NS5A region was originally reported in 1996 by Enomoto *et al.* (8) and confirmed by several Asian studies (9, 12–14), but controversial results were reported by Western studies (15, 16). Meta-analysis showed the relationships between ISDR and SVR and suggested that unidentified factors have an effect on IFN responsiveness (32). The ISDR interacts with protein kinase R (PKR) and inactivates replication of HCV *in vitro* (33). Therefore, ISDR heterogeneity plays an important role that may affect response to IFN. However, some reports have not confirmed the interaction between PKR and NS5A (34, 35), and they suggested the PKR-independent effects of NS5A (36, 37). Thus, the effects of aa substitutions of the ISDR are unclear, and investigators searched for other viral factors. The aa substitutions at 70 and 91 in the HCV core region were reported as factors that could predict IFN responsiveness (10). Thus, several studies have reported that combining aa substitutions in the HCV core region and NS5A region could improve the predictive value of SVR in patients with genotype 1b (12–14). These results were useful to develop individualized treatment strategies for chronic hepatitis C patients.

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For instance, in this study, only 20% of patients with Q70 and wild-type ISDR achieved SVR, compared with 70.6% of those with non-Q70 and mutant-type ISDR. However, the majority of patients were classified into those with non-Q70 and wild-type (50.3%), and another factor for improving SVR prediction was considered necessary. Three genome-wide association studies of SVR to pegylated-IFN-α and ribavirin combination therapy for chronic hepatitis C patients with genotype 1 from Japan, the USA and Australia identified SNPs of IL28B associated with IFN responsiveness (17–19). SVR was achieved in 13.8% of patients with IL28B minor allele (TG and GG) and in 58% with IL28B major allele (TT) in this study, and the SNP of IL28B was associated with the response to IFN in patients with HCV genotype 1b, as in previous reports. The effects of both host and viral factors on IFN responsiveness would affect the IFN treatment outcome. Thus, the SNP of IL28B was considered in the analysis of aa substitutions in the HCV core and the NS5A region for improving the prediction of SVR. The strain with the worst SVR outcome was Q70 and wild-type ISDR, with an SVR of 20%. When IL28B was considered in the analysis of patients with Q70 and wild-type ISDR, 8.1% of patients with TG/GG for IL28B achieved SVR compared with 35.7% of those with TT for IL28B. These results indicate the effects of both host and viral factors on IFN responsiveness. The best responders were 47 patients who simultaneously had non-Q70, mutant-type ISDR and TT allele; 35 (74.5%) achieved SVR. The clear suggestion of a correlation between the combination of the SNP of IL28B and aa substitutions in the core region70 and ISDR with IFN responsiveness would not be supported in the non-Q/mutant/G allele and the Q/mutant/G allele groups because of the small number of patients. Both mutations in core region 70 and ISDR were strongly associated with IL28B genotype. Thus, the prevalence of patients with core 70 non-Q, ISDR mutant, and IL28B genotype G was rare, and it was difficult to find these combinations. Patients infected with IFN-resistant strains Q70 and wild-type ISDR could be clearly identified as non-responders to IFN therapy (8.1%) by the IL28B genotype; the positive predictive value for non-SVR was 91.9%. Meanwhile, patients infected with IFN-sensitive strains non-Q70 and mutant-type ISDR showed that the positive predictive value for SVR was 74.5%. Montes-Cano *et al.* (38) reported that the influence of IL28B would be stronger among patients infected with an IFN-resistant genotype (HCV genotype 1) than in those infected with an IFN-sensitive genotype (HCV genotype non-1). The SNP of IL28B would be strongly associated with the response to IFN, especially for poor responders.

Interleukin28B genotype was associated with spontaneous viral clearance, as well as IFN responsiveness (17, 20). A Spanish study found that the prevalence of HCV genotype depends on IL28B genotype and speculated that IL28B would be a candidate to explain HCV genotype differences in the IFN response (38). The IFN-resistant strain (Q70) was detected more frequently in patients with the IL28B minor allele (TG and GG) (56.3%) than in those with the IL28B major allele (TT) (18.3%). The present study showed similar results: patients with IL28B G allele, which is associated with poor response to IFN, seemed to more frequently have the IFN-resistant strain (Q70). Further study is needed to clarify the effect of the IL28B gene on differences in IFN response between each HCV genotype and subgenotype. The IL28B polymorphism might regulate the expression of hepatic interferon-stimulated genes and cause the difference in IFN responsiveness (39). The association between IL28B genotypes and IL28B gene expression is controversial (18, 19, 39). The effects of the SNP of IL28B on gene expression and mechanisms against HCV infection are still under debate. Although the effect of the SNP of IL28B was unclear, as were the aa substitutions of the core region and ISDR, these factors could be used to predict SVR in patients infected with genotype 1b. The SNP of IL28B plays an important role in choosing optimal therapy and avoiding unnecessary treatment.

In conclusion, the SNP of IL28B and aa substitutions in the core region and ISDR were associated with response to IFN in patients with HCV genotype 1b. Combined use of both host and viral factors could improve prediction of the IFN response.

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Original Article

Liver stiffness in extrahepatic cholestasis correlates positively with bilirubin and negatively with alanine aminotransferase

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Aim: Transient elastography is a non-invasive tool to measure liver stiffness (LS), which has been reported to correlate with stage of liver fibrosis. Extrahepatic cholestasis was reported to cause elevated LS, which is considered to be attributed to the increased hydrostatic pressure in the liver. In the present study, the correlation of LS with laboratory data was investigated in extrahepatic cholestasis. The change of LS after biliary drainage was also assessed.

Methods: LS was measured in 29 patients with extrahepatic cholestasis due to carcinomas in 12 and non-neoplastic diseases of biliary tract or pancreas in 17.

Results: In 15 patients, LS was 11.4 kPa or higher which suggested liver cirrhosis in chronic infection of hepatitis C virus. LS significantly correlated positively with serum bilirubin levels ($r = 0.726$, $P < 0.0001$) and negatively with serum aspartate aminotransferase (AST) levels ($r = -0.481$, $P = 0.0082$) and alanine aminotransferase (ALT) levels ($r = -0.631$, $P = 0.0002$). Biliary drainage led to a reduction of bilirubin by 13.5 to 0.9 mg/dL which was significantly correlated with a reduction of LS by 14.3 to 0.5 kPa ($r = 0.524$, $P = 0.0257$).

Conclusion: In extrahepatic cholestasis, the elevation of LS which is probably attributed to the increased hydrostatic pressure in the liver, correlates positively with the accumulation of bilirubin but negatively with damage of hepatocytes indicated by ALT levels. Further studies on the mechanism underlying the elevation of LS should be helpful to elucidate the pathogenesis of extrahepatic cholestasis.

Key words: biliary drainage, Fibroscan, intrahepatic pressure, transient elastography

INTRODUCTION

TRANSIENT ELASTOGRAPHY (TE) is a rapid, non-invasive and reproducible method for measuring liver stiffness (LS) that has been reported to correlate with stages of liver fibrosis in various liver diseases.[1–7] Cut-off values of 6.9–8.8 and 11.4–14.6 kPa were considered to be optimal for discrimination of fibrosis stage 2 (F2) and liver cirrhosis (F4) in patients with chronic hepatitis C virus (HCV) infection, respectively.

However several reports suggested that LS is also affected by inflammatory activity.[8] LS increases during alanine aminotransferase (ALT) flares in patients with chronic viral hepatitis and acute hepatitis.[9–11] ALT levels and grade of inflammatory activity have been reported to correlate with LS in patients with chronic hepatitis C.[6]

So far, only Millonig *et al.* have reported the increase of LS in extrahepatic cholestasis.[12] LS almost always decreased after successful bile duct drainage. They showed that the experimental bile duct ligation of pigs led to elevation of LS to the values suggesting F3 fibrosis, and considered that increased LS is attributed to the increased hepatic hydrostatic pressure. They reported

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the correlation of decrease of LS after bile duct drainage with decrease of bilirubin, while the correlation of LS with laboratory data was not fully elucidated. Extrahepatic cholestasis is caused by benign diseases or carcinomas of the biliary tract or pancreas. The increase of LS in extrahepatic cholestasis may differ between benign diseases and carcinomas, while the differences of increase of LS among the causes of obstruction have not been studied.

In the present study, LS was measured by a Fibroscan in the patients with extrahepatic cholestasis caused by benign diseases and carcinomas of the biliary tract or pancreas. The correlation of increased LS with laboratory data and the causes of bile duct obstruction were assessed. The changes of LS after bile duct drainage were also assessed. This is the first report describing the correlations of LS with bilirubin and ALT levels.

METHODS

Patients

TWENTY-NINE PATIENTS who were admitted with extrahepatic cholestasis to Fujita Health University Hospital from March 2008 to July 2009 were analyzed (Table 1). The underlying diseases were established according to standard criteria using laboratory tests, ultrasound, endoscopic ultrasound, computed tomography (CT) imaging, endoscopic retrograde cholangiopancreatography (ERCP) and percutaneous transhepatic cholangiography (PTC). Twelve patients had carcinomas which were originated from the pancreas in four patients, gallbladder in one, duodenal papilla in one and bile duct in six. Seventeen patients had benign diseases in the biliary tract or pancreas, stones in the common bile duct (CBD) in 13 patients, Mirizzi's syndrome in one, autoimmune pancreatitis in two and retroperitoneal fibrosis in one. With the exception of a patient with carcinoma of the gallbladder, all patients had no apparent hepatic invasion. LS was measured immediately before biliary drainage.

In 18 patients, LS was measured 5–45 days after biliary drainage by stone extraction or stent implementation by endoscopic methods in 16 patients or percutaneous transhepatic cholangio-drainage (PTCD) in two patients.

The study was performed in accordance with the principles of good clinical practice, the principles of the 1975 Declaration of Helsinki and its appendices, and local and national laws. Informed consent in writing was obtained from each patient.

LS measurement

Liver stiffness measurement was performed with a Fibroscan (EchoSens, Paris, France). Ten validated measurements were made on each patient. The results were expressed in kPa. Only procedures with 10 validated measurements and a success rate of at least 60% (ratio of the number of successful acquisitions over the total number of acquisitions) were considered reliable. The median value was considered representative of the liver elastic modulus.

Table 1 Characteristics of the patients with extrahepatic cholestasis

	All	Causes of cholestasis		*P*-value in comparison between benign diseases and carcinomas
		Benign diseases	Carcinomas	
Sex (female/male)	12/17	5/12	7/5	NS
Age (year)	72 ± 11	69 ± 11	75 ± 10	NS
Total bilirubin (mg/dL)	8.7 ± 4.6	6.4 ± 3.7	11.9 ± 3.9	$P = 0.0008$
Direct bilirubin (mg/dL)	6.2 ± 4.0	4.2 ± 3.3	9.0 ± 3.0	$P = 0.0004$
AST (IU/L)	153 ± 104	168 ± 107	131 ± 101	NS
ALT (IU/L)	218 ± 169	237 ± 174	190 ± 167	NS
ALP (IU/L)	1175 ± 664	1003 ± 541	1419 ± 765	NS
γ-GTP (IU/L)	695 ± 604	851 ± 718	474 ± 301	$P = 0.0705$
WBC (/μL)	6966 ± 3243	7024 ± 3265	6883 ± 3354	NS
CRP (mg/dL)	5.1 ± 5.8	6.6 ± 6.4	3.0 ± 4.2	$P = 0.0851$
Liver stiffness (kPa)	11.8 ± 6.5	9.5 ± 4.8	15 ± 7.5	$P = 0.0401$
Diameter of common bile duct (mm)	12.6 ± 4.9	12.2 ± 5.6	13.1 ± 3.8	NS

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; γ-GTP, γ-glutamyl transpeptidase; NS, not significant; WBC, white blood cells.

Table 2 Factors correlating with liver stiffness of the patients with extrahepatic cholestasis

	Linear regression analysis		Multiple regression analysis	
	r	*P*	β	*P*
Cause (benign diseases/carcinomas)		$P = 0.0401$*		NS
Sex (female/male)		NS		
Age (year)		NS		
Total bilirubin (mg/dL)	$r = 0.726$	$P < 0.0001$	$\beta = 0.774$	$P = 0.0005$
Direct bilirubin (mg/dL)	$r = 0.728$	$P < 0.0001$†		
AST (IU/L)	$r = -0.481$	$P = 0.0082$		NS
ALT (IU/L)	$r = -0.631$	$P = 0.0002$	$\beta = -0.014$	$P = 0.0138$
ALP (IU/L)		NS		
γ-GTP (IU/L)	$r = -0.334$	$P = 0.0764$		NS
WBC (/μL)		NS		
CRP (mg/dL)		NS		
Diameter of common bile duct (mm)		NS		
R				0.792
Adjusted R^2				0.599
F				21.9
P				$P < 0.0001$

*Mean values of liver stiffness were compared between the patients with benign diseases and those with carcinomas by Student's *t*-test.
†Direct bilirubin levels were not included because of their close correlation with total bilirubin levels.
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; γ-GTP, γ-glutamyl transpeptidase; NS, not significant; WBC, white blood cells.

Ultrasound examination

Ultrasound examination was carried out to confirm extrahepatic cholestasis and to measure the diameter of the CBD.

Statistical analysis

Comparison between the patients with carcinomas and those with benign diseases was done by χ^2-test or Student's *t*-test. Factors correlating with LS were estimated by χ^2-test or linear regression analysis. Factors independently correlating with LS were assessed by multiple regression analysis.

RESULTS

LS and laboratory data of extrahepatic cholestasis before biliary drainage

LIVER STIFFNESS WAS higher than the designated normal range of 2.4–5.5 kPa in 25 of 29 patients with extrahepatic cholestasis before biliary drainage, and was 11.4 kPa or higher in 15 patients which is a cut-off value for liver cirrhosis of HCV infection determined in our previous study.[13]

Total and direct bilirubin levels and LS were significantly higher in the patients with carcinomas than those with benign diseases ($P = 0.0008$, =0.0004 and =0.0401, respectively) (Table 1).

Correlation of LS with other laboratory data before biliary drainage

Liver stiffness was positively correlated with total bilirubin levels and direct bilirubin levels ($P < 0.0001$ and <0.0001, respectively), while it was negatively correlated with aspartate aminotransferase (AST) levels and ALT levels ($P = 0.0082$ and =0.0002, respectively) (Table 2 and Fig. 1a,b). Serum bilirubin levels significantly correlated negatively with AST levels ($r = -0.410$, $P = 0.0271$) and with ALT levels ($r = -0.489$, $P = 0.0071$) (Fig. 1c).

Multiple regression analysis for the factors independently affecting LS was done with cause of obstruction, total bilirubin levels, AST levels, ALT levels and γ-glutamyl transpeptidase (γ-GTP) levels. Direct bilirubin levels were not included because of their close correlation with total bilirubin levels. Multiple regression analysis demonstrated that total bilirubin levels and ALT levels independently correlated with LS ($P = 0.0005$ and =0.0138, respectively).

In the patients with benign diseases, LS was positively correlated with total bilirubin levels, direct bilirubin levels and diameters of the CBD ($P = 0.0198$, =0.0068

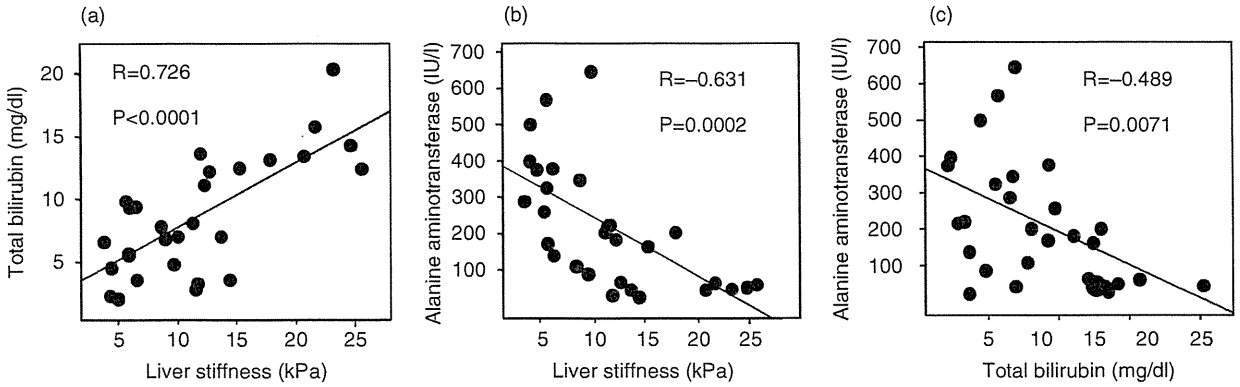

Figure 1 Correlation of liver stiffness with total bilirubin levels and alanine aminotransferase levels before biliary drainage. (a) Liver stiffness was positively correlated with total bilirubin levels ($r = 0.726$, $P < 0.0001$). (b) Liver stiffness was negatively correlated with alanine aminotransferase levels ($r = -0.631$, $P = 0.0002$). (c) Serum bilirubin levels were negatively correlated with alanine aminotransferase levels ($r = -0.489$, $P = 0.0071$).

and =0.0214, respectively), while it was negatively correlated with ALT levels ($P = 0.0153$) (Table 3).

In the patients with carcinomas, LS was positively correlated with total bilirubin levels and direct bilirubin levels ($P = 0.0039$ and =0.0065, respectively), while it was negatively correlated with ALT levels ($P = 0.0065$) (Table 3).

LS after biliary drainage

In 18 patients, LS was measured 5–45 days after biliary drainage by stone extractions in five patients, stent implantations in 11 or PTCD in two. In six of 17 patients whose LS was 5.5 kPa or higher before biliary drainage, LS became lower than 5.5 kPa after biliary drainage. In 10 of 12 patients whose LS was 11.4 kPa or higher before biliary drainage, LS became lower than 11.4 kPa after biliary drainage. LS and laboratory data did not differ significantly between the patients with benign diseases and those with carcinomas after biliary drainage (Table 4).

Decrease of LS after biliary drainage significantly correlated with decrease of total bilirubin levels ($r = 0.524$, $P = 0.0257$) (Table 5).

Table 3 Differences of factors correlating with liver stiffness of the patients with extrahepatic cholestasis between benign diseases and carcinomas

	Benign diseases		Carcinomas	
	r	*P*	*r*	*P*
Sex (female/male)*		NS		NS
Age (year)		NS		NS
Total bilirubin (mg/dL)	$r = 0.559$	$P = 0.0198$	$r = 0.763$	$P = 0.0039$
Direct bilirubin (mg/dL)	$r = 0.629$	$P = 0.0068$	$r = 0.735$	$P = 0.0065$
AST (IU/L)		NS	$r = -0.541$	$P = 0.0695$
ALT (IU/L)	$r = -0.577$	$P = 0.0153$	$r = -0.735$	$P = 0.0065$
ALP (IU/L)	$P = 0.438$	$P = 0.0785$		NS
γ-GTP (IU/L)		NS		NS
WBC (/μL)		NS		NS
CRP (mg/dL)	$r = 0.455$	$P = 0.0666$		NS
Diameter of common bile duct (mm)	$r = 0.569$	$P = 0.0214$	$r = -0.565$	$P = 0.0702$

*Mean values of liver stiffness were compared between female patients and male patients by Student's *t*-test.
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; γ-GTP, γ-glutamyl transpeptidase; NS, not significant; WBC, white blood cells.

Table 4 Characteristics of the patients with extrahepatic cholestasis after drainage

	All	Causes of cholestasis		*P*-value in comparison between benign diseases and carcinomas
		Benign diseases	Carcinomas	
Sex (female/male)	9/9	4/6	5/3	NS
Age (year)	72 ± 11	71 ± 13	74 ± 9	NS
Interval between liver stiffness measurements (days)	23.5 ± 12.4	19.5 ± 12.4	28.5 ± 11.2	NS
Total bilirubin (mg/dL)	2.5 ± 1.7	2.3 ± 1.7	2.7 ± 1.9	NS
Direct bilirubin (mg/dL)	1.0 ± 1.1	0.9 ± 1.0	1.1 ± 1.4	NS
AST (IU/L)	52 ± 47	39 ± 35	67 ± 57	NS
ALT (IU/L)	81 ± 72	61 ± 50	107 ± 90	NS
ALP (IU/L)	667 ± 576	642 ± 537	698 ± 659	NS
γ-GTP (IU/L)	277 ± 265	346 ± 320	190 ± 154	NS
WBC (/μL)	6438 ± 2804	6570 ± 2294	6275 ± 3505	NS
CRP (mg/dL)	1.0 ± 1.4	0.7 ± 0.4	1.4 ± 2.0	NS
Liver stiffness (kPa)	7.9 ± 4.0	7.7 ± 5.0	8.3 ± 2.8	NS

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; γ-GTP, γ-glutamyl transpeptidase; NS, not significant; WBC, white blood cells.

Persistent elevation of LS values despite successful biliary drainage

In two of 12 patients whose LS was 11.4 kPa or higher before biliary drainage, LS remained 11.4 kPa or higher after biliary drainage. In a patient with stones in the CBD whose LS was 21.8 kPa before biliary drainage, LS became 20.4 kPa 14 days after biliary drainage and total bilirubin reduced from 15.9 mg/dL to 4.8 mg/dL. Computed tomography showed no clues of the presence of cirrhosis 11 days after biliary drainage. In the other patient with a carcinoma in the lower CBD whose LS was 24.8 kPa before biliary drainage, LS became 12.0 kPa 36 days after biliary drainage and total bilirubin reduced from 14.4 mg/dL to 2.2 mg/dL. When pancreaticoduodenectomy was done 47 days after biliary drainage, the liver showed no appearance indicating cirrhosis. Neither patients had any etiological factors such as infection of HCV or hepatitis B virus, autoimmune hepatitis, excessive alcohol consumption, diabetes mellitus or metabolic syndrome which cause elevation of LS.

Table 5 Factors correlating with the decrease of liver stiffness of the patients with extrahepatic cholestasis

	r	*P*
Interval between liver stiffness measurements (days)		NS
Decrease of total bilirubin (mg/dL)	$r = 0.524$	$P = 0.0257$
Decrease of direct bilirubin (mg/dL)	$r = 0.461$	$P = 0.0543$
Decrease of AST (IU/L)	$r = -0.432$	$P = 0.0734$
Decrease of ALT (IU/L)	$r = -0.464$	$P = 0.0525$
Decrease of ALP (IU/L)		NS
Decrease of γ-GTP (IU/L)		NS
Decrease of WBC (/μL)		NS
Decrease of CRP (mg/dL)		NS

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; γ-GTP, γ-glutamyl transpeptidase; NS, not significant; WBC, white blood cells.

DISCUSSION

THE PRESENT STUDY demonstrated that LS of patients with extrahepatic cholestasis was higher than the designated normal upper range of 5.5 kPa in 25 of 29 patients and was 11.4 kPa or higher in 15 patients which is a cut-off value for liver cirrhosis of HCV infection determined in our previous study.[13] In six of 17 patients whose LS was 5.5 kPa or higher before biliary drainage, LS became lower than 5.5 kPa after biliary drainage. In 10 of 12 patients whose LS was 11.4 kPa or higher, LS became lower than 11.4 kPa after biliary drainage. Thus, extrahepatic cholestasis caused the elevation of LS, which was reversible when biliary drainage was done in a short period. It is suggested that elevation of LS does not necessarily reflect liver fibrosis, although fibrosis in liver histology was not examined in the present study.

Millonig *et al.* reported that the experimental bile duct ligation of pigs led to a swelling of the liver and LS elevated to the values suggesting F3 fibrosis.[12] After removal of the ligation, LS returned to almost normal values. This experiment indicates that bile duct ligation causes elevation of LS which is probably because of the increased hepatic hydrostatic pressure in the main and is reversible when the bile duct is reopened and the hydrostatic pressure is reduced. Millonig *et al.* also reported that LS is directly influenced by central venous pressure.[14]

The present study demonstrated that the elevation of LS significantly correlated positively with total bilirubin levels and negatively with ALT levels. These correlations were also noted when the patients with carcinomas and those with benign diseases were separately analyzed. Decrease of LS after biliary drainage significantly correlated with decrease of total bilirubin levels. This is the first report describing these correlations. Millonig *et al.* reported only the correlation of decrease of LS after biliary drainage with decrease of bilirubin.[12] The positive correlation between bilirubin levels and LS probably indicates that the higher intraductular pressure causes the higher retention of bilirubin and the higher bilirubin levels. In acute hepatitis, the positive correlation of LS with ALT levels[11,15] and bilirubin levels[10,16,17] has been reported. These positive correlations in acute hepatitis may be attributed to the dominance of inflammatory liver injury instead of increased hepatic hydrostatic pressure.

The negative correlation between ALT levels and LS probably indicates the negative correlation between ALT levels and the hepatic hydrostatic pressure. The leakage of bile fluid from the bile canalicular lumen into the lateral intracellular space and the perisinusoidal space causes the damage of hepatocytes and may also reduce the hydrostatic pressure in the bile duct.[18] The time of admission may also contribute to the negative correlation of ALT levels and LS. The fatigue or abdominal discomfort caused by severe hepatic damage causes the presentation of many patients to hospital before the hepatic hydrostatic pressure raises to the high levels and causes retention of bilirubin. Further studies on the mechanism underlying the elevation of LS, bilirubin levels and ALT levels is needed to elucidate the pathogenesis of jaundice and liver injury in extrahepatic cholestasis.

In the present study, LS did not reduce to the normal levels in more than half of the patients after drainage which should normalize the hydrostatic pressure of the liver. Therefore, not only the increased hydrostatic pressure, but the other features due to impaired bile flow also could be related to the elevation of LS in cholestasis. Extrahepatic cholestasis causes inflammatory features including edema, neutrophil infiltration, proliferation of the biliary epithelial cells and fibrosis.[19–21] This inflammation and fibrosis may also contribute to the elevation of LS, and cause the delay of reduction of LS after drainage.

Fibroscan measures LS without B-mode imaging, while other non-invasive methods such as acoustic radiation force impulse (ARFI)[22] and real-time tissue elastography[23] could measure LS with B-mode imaging. The LS measurement by Fibroscan may be affected by dilated intrahepatic bile ducts in extrahepatic cholestasis, while this effect can be avoided by ARFI or real-time tissue elastography. In the present study, only procedures with 10 validated measurements and a success rate of at least 60% (ratio of the number of successful acquisitions over the total number of acquisitions) were considered reliable. The median value was considered representative of the liver elastic modulus. With this protocol, LS was successfully measured in all the 29 patients examined in the present study. No extreme scattering of the 10 values which might be caused by dilated bile ducts was noted. The measurement depth of Fibroscan was between 25 mm and 65 mm under skin. In this measurement depth of the right lobe, there may be no extremely dilated bile duct. It may be the reason why LS was measured in the present study without any difficulty, although Fibroscan has the weak point of measuring LS without B-mode imaging. The study on LS by ARFI in extrahepatic cholestasis is now under way in Fujita Health University Hospital.

In conclusion, the elevation of LS in extrahepatic cholestasis can be mainly attributed to the increased hydrostatic pressure of the liver. The present study demonstrated that the elevation of LS in extrahepatic cholestasis correlates positively with the accumulation of bilirubin but negatively with damage of hepatocytes indicated by ALT levels. Further studies on the mechanism underlying the elevation of LS are needed to elucidate the pathogenesis of jaundice and liver injury in extrahepatic cholestasis.

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EUS所見より術前診断し，内視鏡的に切除し得た胃Hamartomatous inverted polypの2例

上尾哲也[1)] 石田哲也[1)] 永松秀康[1)] 成田竜一[1)] 高橋 健[1)]
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症 例

EUS所見より術前診断し，内視鏡的に切除し得た胃Hamartomatous inverted polypの2例

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要 旨

症例1は74歳男性．胃穹隆部前壁に粘膜下腫瘤を認めた．症例2は61歳女性．胃体上部前壁に亜有茎性の粘膜下腫瘍様病変を認めた．両症例ともEUSで，第3層に多房性無エコー域を認め，胃Hamartomatous inverted polyp（HIP）と術前診断した．内視鏡的切除にて，粘膜下層に嚢胞状に拡張した腺管群と線維筋成分の増生を認め，いずれも胃のHIPと診断した．胃のHIPは稀な病変で診断が難しいが，EUSはその術前診断に非常に有用であった．また自検例ではESDにて詳細な病理学的検討が可能となり，ESDは今後SMT typeのHIPに有用な治療法となりうると考えた．

Key words Hamartomatous Inverted polyp／EUS／ESD

I 緒 言

胃のHamartomatous Inverted Polyp（以下HIP）は粘膜下層を主体に嚢胞状に拡張した胃腺組織の増生と粘膜筋板に連なった平滑筋の増殖により隆起を形成する病変である．その頻度は極めて稀であり，治療前の鑑別診断としてもあがりにくい．しかしながら，粘膜下に嚢胞状に拡張した腺管群を模倣したEUS所見は，本疾患に特徴的であり診断に有用である．今回われわれは，EUS所見よりHIPと術前診断し，ESD治療を含めた内視鏡的切除により，治療し得た2例のHIPを経験したので報告する．

II 症例1

患者：74歳，男性．

主訴：胃もたれ．

既往歴：高血圧にて降圧剤加療中．

現病歴および経過：平成22年10月，胃もたれを主訴に近医で上部内視鏡検査を受け，胃穹窿部前壁に粘膜下腫瘍様病変を認め，精査加療目的に当科紹介となった．

入院時現症：身長169cm，体重85kg，胸部に異常所見なし．腹部は平坦で軟，圧痛を認めず，腫瘤を触知しなかった．

血液検査所見：特記すべき異常所見を認めなかった．

上部消化管内視鏡検査：胃穹窿部前壁に山田Ⅲ型の腫瘤を認めた（Figure 1-a）．右側臥位による近接にて，表面は健常胃粘膜に覆われた粘膜下腫瘍様の病変であった（Figure 1-b）．EUS検査（7.5Mhz）で，病巣の主座は第3層にあり，内部に多房性無エコー域を認めた（Figure 2）．

上部消化管X線検査：胃穹窿部前壁側に，約15mm大の隆起性病変を認めた．生検所見は異型のない胃底腺粘膜がみられるのみであった．EUS所見よりHIPを疑った．明らかな茎がなくポリペクトミーでは腫瘍が残存する可能性が強いと考え，ESDによる切除を選択し，一括切除した．固

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Two Cases of Hamartomatous Inverted Polyp Preoperatively Diagnosed Based on EUS Findings, and Successfully Treated with Endoscopic Resection.

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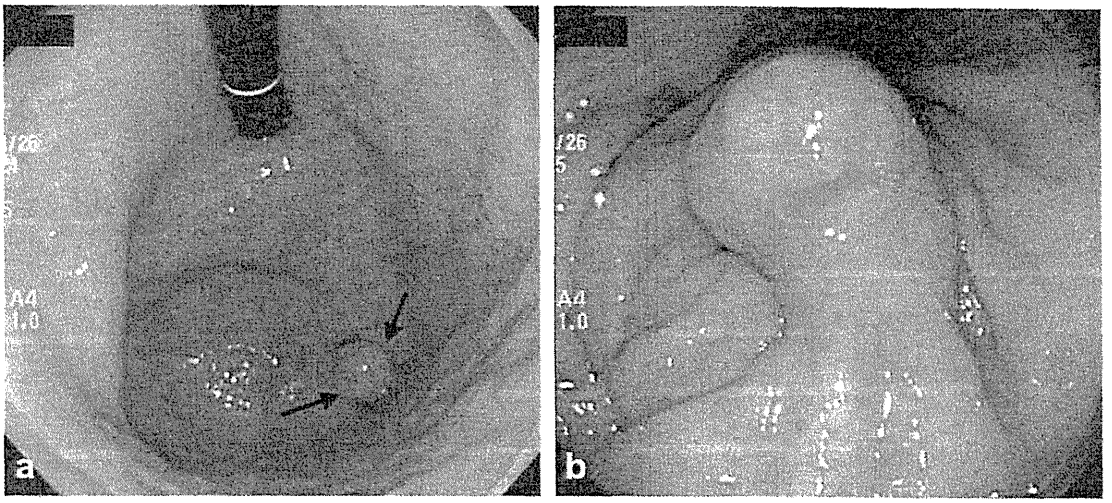

Figure 1　症例1の上部消化管内視鏡所見.
a：胃穹窿部前壁に山田Ⅲ型腫瘤を認めた.
b：近接像では腫瘤表面は健常胃粘膜に覆われた粘膜下腫瘍様の病変であった.

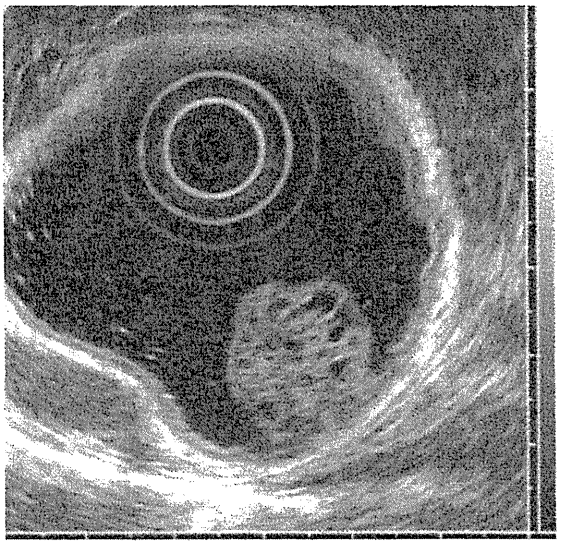

Figure 2　症例1の超音波内視鏡検査所見（7.5Mhz）.
病巣の主座は第3層にあり，多房性無エコー域が内部に認められた.

定切除標本の大きさは18×16mmで，腫瘤割面に大小の嚢胞構造を認めた（Figure 3）．ルーペ像では粘膜下層に多房性嚢胞状に拡張した腺管群とそれをとりかこむように線維筋成分の増生がみられた（Figure 4-a）．腫瘤を被覆する上皮はやや菲薄化した胃粘膜で，筋板を隔てて粘膜下層に種々の程度に嚢胞状に拡張する胃腺管群が認められた（Figure 4-b）．粘膜下層に増生する腺管は腺窩上皮，胃底腺，幽門腺に類似した上皮より構成されており，拡張をともなわずに密に増生した部分も認められた（Figure 4-c）．一部粘膜筋板が欠落した部分で腫瘤を被覆する胃粘膜上皮が落ち込むように粘膜下の拡張した腺管につながる像が見られた（Figure 5-a）．この所見はDesmin染色にてより明瞭に観察された（Figure 5-b）．以上より胃のHIPと診断した.

Ⅲ　症例2

患者：61歳，女性.
主訴：なし.
既往歴：特記事項なし.
現病歴：平成21年1月近医で上部内視鏡検査を受けた際，胃体上部にポリープ様病変を認め，精査加療目的に当科紹介となった.
入院時現症：身長160cm，体重51kg，胸部に異常所見なし．腹部は平坦で軟，圧痛を認めず，腫瘤を触知しなかった.
血液検査所見：特記すべき異常所見を認めなかった.
上部消化管内視鏡検査（Figure 6）：胃体上部前壁に亜有茎性の隆起性病変を認めた．腫瘤表面は平滑でびらん，潰瘍は認めず，ほぼ周囲と同様の正常粘膜に被われた粘膜下腫瘍様であった．EUS検査（20Mhz）にて病巣の主座は第3層にあり，多房性無エコー域が内部に見られた（Figure 7）．生検所見は異型のない胃底腺粘膜がみ

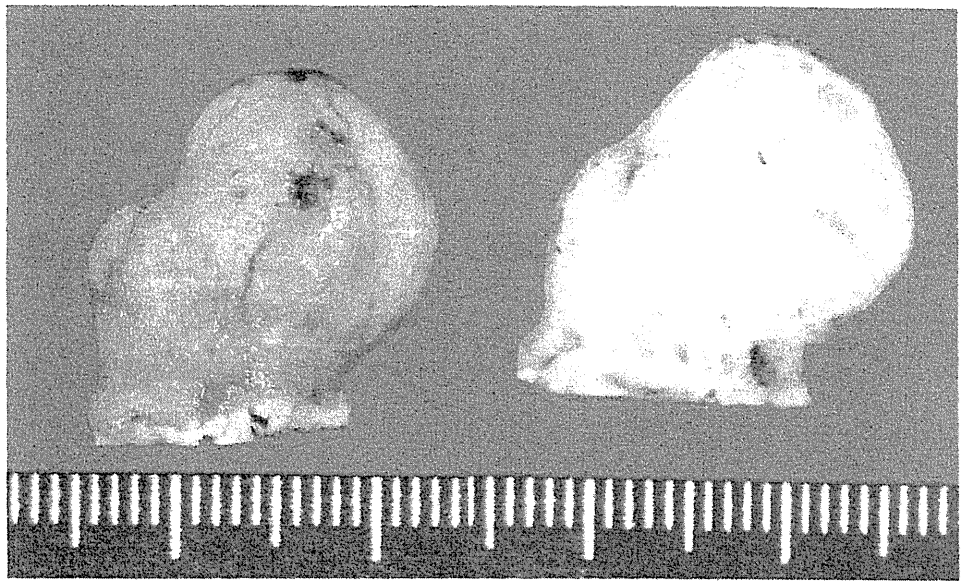

Figure 3 症例1の固定標本の割面.
固定標本での腫瘤の大きさは18×16mmで，腫瘤割面に大小の嚢胞構造を認めた.

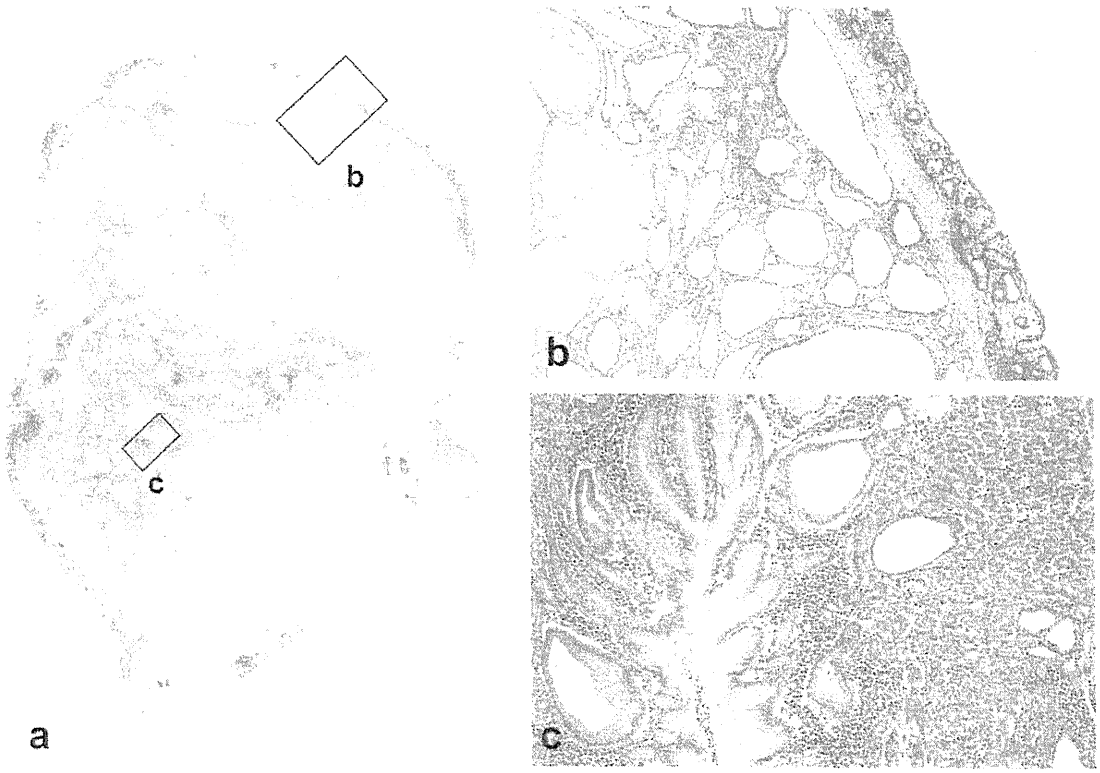

Figure 4 症例1の病理組織学的所見1.
a：粘膜下層に多房性嚢胞状に拡張した腺管群とそれをとりかこむ線維筋成分の増生からなる腫瘤病変を認めた．切除断端に病変の取り残しはない（HE染色ルーペ像）.
b：腫瘤を被覆する上皮はやや菲薄化した胃粘膜で，筋板を隔てて粘膜下層に種々の程度に嚢胞状に拡張する異型のない胃腺管群が認められる（HE染色×40倍）.
c：粘膜下層に増生する腺管は腺窩上皮，胃底腺，幽門腺に類似した上皮より構成されており，拡張をともなわずに密に増生した部分も認められた（HE染色×40倍）.

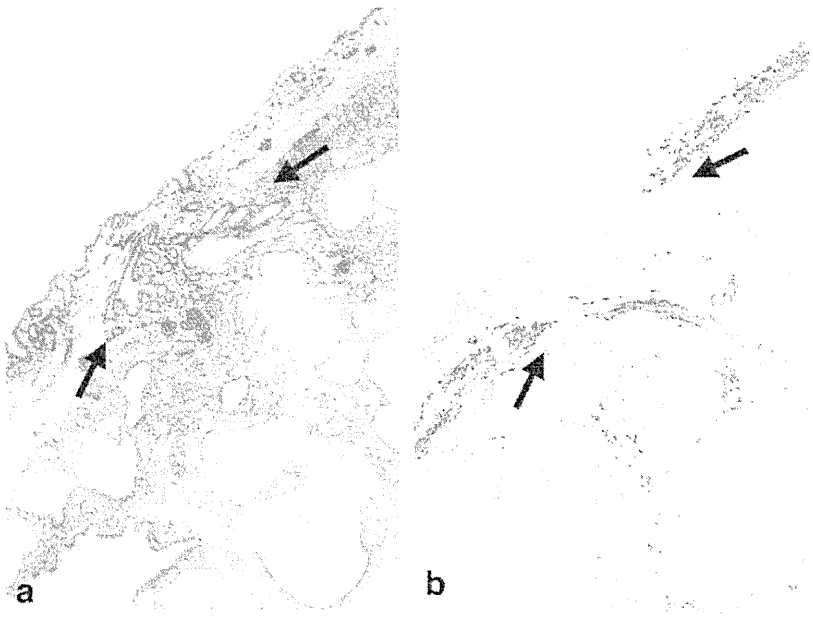

Figure 5　症例1の病理組織学的所見2．
a：一部粘膜筋板が欠落した部分で腫瘤を被覆する胃粘膜上皮が粘膜下に落ち込むような像が見られた（HE染色×40倍）．
b：この所見はDesmin染色にてより明瞭に観察された（Desmin染色×40倍）．

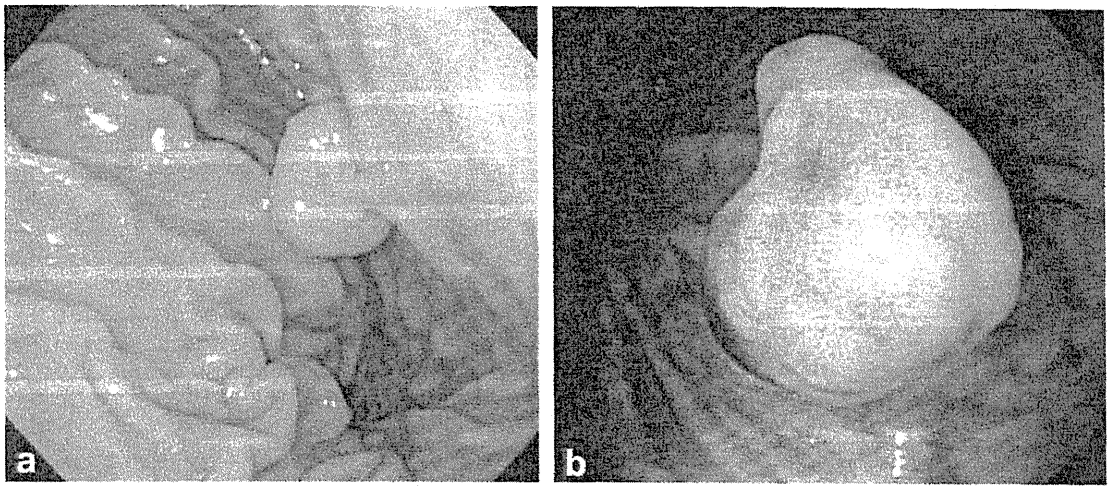

Figure 6　症例2の上部消化管内視鏡所見．
a：胃体上部前壁に亜有茎性の腫瘤病変を認めた．
b：色素散布後の近接像にて，腫瘤は正常粘膜に覆われた粘膜下腫瘍様の形態であった．

られるのみで，上皮に腫瘍性変化はなかった．EUS所見よりHIPを強く疑い，内視鏡的ポリペクトミーを施行した．腫瘤の大きさは15×10mmで，ルーペ像では粘膜下層に多房性嚢胞状に拡張した腺管群とそれをとりかこむように線維筋成分の増生がみられた（Figure 8-a）．粘膜下の嚢胞状に拡張する腺管は腺窩上皮，幽門腺に類似した上皮より構成されており，種々の程度に拡張していた．腫瘤を被覆する上皮はやや菲薄化した萎縮性胃底腺粘膜であった．一部粘膜筋板が欠落した部分で腫瘤を被覆する胃粘膜上皮が落ち込むようにして粘膜下の拡張した腺管につながる像が見られた