Laparoscopic findings of reddish markings predict hepatocellular carcinoma in patients with hepatitis B virus-related liver disease

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HBV, hepatitis B virus; HCC, hepatocellular carcinoma; ALT, alanine aminotransferase; HCV, hepatitis C virus; AST, aspartate aminotransferase.

ABSTRACT

Purpose. For patients with chronic hepatitis due to hepatitis B virus (HBV), factors predicting hepatocellular carcinoma (HCC) other than high levels of HBV DNA and aminotransferase (ALT) are needed to prevent HCC development, as many patients with chronic HBV infection fulfill these conditions. The purpose of this study was to clarify factors predictive of HCC development for those patients.

Methods. The study was a systematic cohort analysis of 303 consecutive patients with hepatitis B e-antigen, receiving laparoscopic examination for assessment of liver disease. Laparoscopic, histological, and clinical characteristics were investigated as related to HCC development.

Results. HCC occurred in 27 patients during a mean follow-up of 8.0 ± 5.0 years, at the age of 37-72 years. Significant associations with HCC development were shown for liver cirrhosis, histological activity grade, reddish markings, and older age. Multivariate analysis revealed that HCC development was strongly associated with older age and male gender (P=0.002 and P=0.043, respectively). HCC occurred more frequently in patients of age ≥30 years even with early stage than in patients of age <30 years (p=0.031). Severe reddish markings, a laparoscopic finding of widespread parenchymal destruction, were highly associated with HCC development in patients of age ≥30 years at diagnosis (odds ratio=1.67, P=0.034), while histological activity grade and ALT level were not (P=0.075, and P=0.69, respectively).

Conclusions. HCC development is associated with older age, male gender, and liver cirrhosis. Reddish markings, rather than histological activity or ALT level, can be useful to predict HCC for HBV patients of age ≥30 years.

Key words: hepatitis B virus; hepatocellular carcinoma; laparoscopy

INTRODUCTION

Hepatitis B virus (HBV) is distributed worldwide, and 400 million people suffer from chronic hepatitis B infection (1). Hepatocellular carcinoma (HCC) and liver failure are frequent among patients with HBV infection. The incidence of HCC development is estimated at 0.8% annually, approximately 100-fold higher than the rate among uninfected people. Half a million patients die of liver-related causes every year (2). Several studies of the prognosis of HBV have shown that persistent elevation of HBV-DNA and alanine aminotransferase (ALT) in serum are highly associated with rapid disease progression and HCC development (3, 4). Host factors such as age, gender, and alcohol intake, and viral factors including hepatitis B e-antigen (HBeAg) and HBV genotype have been implicated as important contributors to disease progression. In Japan, HBV genotype C is predominant over other genotypes, and most HBV patients with chronic hepatitis have been infected perinatally or during early childhood (5). Recent reports have indicated that HBV genotype C is related to poor outcome of slower HBeAg seroconversion (6), earlier disease progression, and more frequent HCC development (7).

Good control of viral replication with nucleoside analogues can decrease liver inflammation and reduce the risk of poor outcomes (8). Such drugs may work, in the short term at least, for most patients in the immune-active phase of chronic HBV infection. However, benefits for long-term survival have not been well-defined. Some patients in young or middle age hesitate to use these drugs due to the possibility of drug resistance and the high cost for medication for life-long use. The presence of HBeAg often indicates active viral replication, and high levels of ALT in the immune-active phase; many patients with HBeAg are thus suitable candidates for use of nucleoside analogues. Predictors for rapid progression to liver cirrhosis and high risk of HCC development should be more clearly defined, to facilitate the selection of HBeAg-positive patients who should be treated immediately with nucleoside analogues.

Laparoscopy provides wide and precise observation of the liver surface. Kalk reported morphological progression from acute hepatitis to cirrhosis (9, 10). Laparoscopic observation with liver biopsy is considered the most accurate method of evaluating liver cirrhosis (11-14). Besides usefulness in evaluating present disease progression, direct observation of the liver surface can provide a large amount of information on disease activity, capsular structural changes, and small lesions on the surface, which can be difficult or impossible to detect on ultrasonography or computed tomography. Studies of patients with hepatitis C virus (HCV) have proposed the importance of

laparoscopic examination and have noted that irregular regenerative nodules, degree of regenerative nodules, and atrophic right lobe can be observed clearly by laparoscopy, and also that those findings represent independent risk factors for HCC development (15, 16). Associations with laparoscopic features have not been well-defined for HBV patients with regard to HCC development.

The purpose of this study was to clarify useful predictive factors of HCC development for HBV patients with HBeAg, by evaluating laparoscopic features, clinical characteristics, and histology with regard to the development of HCC. We revealed that liver cirrhosis, older age, male gender, and a laparoscopic feature of reddish markings were strongly associated with HCC development, and proposed the importance of laparoscopic examination to evaluate the risk of HCC development.

PATIENTS AND METHODS

Patients

This study was a systematic cohort analysis of 303 consecutive patients with HBeAg, and who underwent laparoscopic examination and liver biopsy for the assessment of chronic liver injury at Okayama University Hospital between 1982 and 2002. Presence of HCC was excluded in all patients by imaging examinations with abdominal ultrasonography and computed tomography and by showing normal values of alpha-fetoprotein in serum at the time of diagnosis. Patients suffering from acute hepatitis due to HBV, those with serum positivity for anti-HCV antibodies, and those with daily ethanol intake of >75 g were excluded from the study. The study was performed in accordance with the Helsinki Declaration, and all protocols were approved by the ethics committees of the involved institutes. All patients provided informed consent before enrolment into the study.

Scoring of liver function by using laboratory parameters

In order to estimate the usefulness of laboratory parameters to assess liver function, we selected 5 conventional parameters, and evaluated the score based on these values with histological fibrosis stage. These parameters were scored according to the normal ranges in our institutes as follows: prothrombin time (0, >80%; 1, ≤80%); platelet count (0, >15 $\times 10^4/mm^3$; 1, ≤15 $x10^4/mm^3$); serum level of albumin (0, >3.9 g/dl; 1, ≤3.9 g/dl); serum level of total bilirubin (0, <1.2 mg/dl; 1, ≥1.2 mg/dl); and the ratios of aspartate aminotransferase (AST) and ALT (0, <1.0; 1, ≥1.0).

Histological evaluation

Stage of histological fibrosis and grade of activity were assigned by two pathologists according to the criteria of Desmet et al. (17). All biopsy specimens were obtained under laparoscopic guidance and were more than 1.5 cm long and 2 mm wide. The amount of obtained material was therefore adequate for histological evaluation.

Laparoscopic examination

We selected the following six features for analysis, because these are routinely used for evaluation of disease progression and activity: surface irregularity, whitish markings, vascular proliferation, reddish markings, patchy markings, and fat deposition (18-21). Surface irregularity was evaluated, based on

depression and nodular formation, and classified into three stages: S1, normal or early stage; S2, advanced, pre-cirrhotic stage; and S3: cirrhotic stage. Reddish markings were scored according to location, distribution and color tone of the markings. Whitish markings were defined with their location. These features were assessed as mild or severe based on the total scores as in Table 1. As for vascular proliferation, dilated peripheral portal veins are often observed on liver surface of the patients with chronic hepatitis, and small arteries may become visible when the disease has been progressed. We graded dilated peripheral portal veins as mild and proliferation of small arteries as severe for vascular proliferation. These classifications have been used since Shimada et al. reported their usefulness in 1971 to evaluate disease activity and to predict disease progression for chronic hepatitis (18). Several reports from different institutes have proposed similar classifications by using these features, and revealed these importances for evaluation of disease progression (16, 22, 23). Final laparoscopic findings were evaluated independently by three experienced hepatologists (S.F., B.S., and K.Y.), and discussed for final diagnosis. Figure 1 shows typical laparoscopic features of the liver surface.

Follow-up

All patients received medical check-ups with blood examinations every 2-3 months, and abdominal US or CT every 6 months at least as recommended (24, 25). Patients who had not visited our hospital in the previous 6 months were contacted by letter or telephone and asked to provide details of recent medications by questionnaires. If they visited other hospitals, we also asked them about the results of any imaging studies. For cases in which the patient had died, the date and cause of death were recorded. No patients were treated with nucleoside analogues during follow-up.

Statistical analysis

Data are expressed as mean ±standard deviation (SD) or median (range). Patient laboratory data and laparoscopic findings were compared with histological findings using the Kruskal-Wallis test and canonical correlation analysis. Proportional hazards models were utilized to estimate the effects of patient characteristics on HCC development. Incidence rates of HCC were estimated using the Kaplan-Meier method, and compared with the log-rank test. A value of $P<0.05$ was considered significant. Statistical analysis was performed with JMP software (SAS Institute, Cary, NC).

RESULTS

Patient characteristics

Table 2 lists the clinical characteristics of patients enrolled in this study. Mean age of patients was 34 ±11 years, and 232 patients were male (76.6%). Of the patients, 71.6% had some family history of liver disease. In order to estimate the usefulness of laboratory parameters to assess liver function, we selected 5 conventional parameters, and compared the scores based on these values with histological fibrosis stage (Fig. 2a). Surprisingly, only half of the patients with a total score of 0 (49.1%), representing completely normal in this scoring system, were histologically defined as early stage (fibrosis stage 0 or 1), and 20.5% were advanced, at the pre-cirrhotic or cirrhotic stage (fibrosis stage 3 or 4). These results indicate the necessity for liver biopsy, as conventional laboratory parameters cannot distinguish patients in the early stage from those in the advanced stages, although total scores of laboratory data correlated significantly with stages of histological fibrosis (R=0.46, P<0.0001, canonical correlation analysis). In terms of activity grades, mean ALT levels in patients were very high (156 ± 142 IU/l), and 51.9% of patients with histological grade A1 showed ALT levels ≥80 IU/l (Fig. 2b). ALT levels displayed weak associations with histological activity grade (R=0.14, P=0.013).

Laparoscopic findings at the time of diagnosis

Table 3 provides a summary of laparoscopic features. Frequencies were calculated for each group of surface irregularity. Reddish markings and patchy markings were frequently observed in S2 (74% and 72%, respectively, P<0.001 each). Vascular proliferation was observed less in S2 (73%) than in S1 (86%) or S3 (87%, P=0.018, Kruskal-Wallis tests). Severe vascular proliferation, reflecting proliferation of small arteries was more frequently observed in S3 than S1 or S2, although this increase was not statistically significant (P=0.84). Whitish markings tended to be less frequent, and fat deposition more frequent in S3 than in S1 or S2, but no significant differences were identified (P=0.31 and P=0.061, respectively). Correlations between histological fibrosis stage and laparoscopic surface irregularity were significantly strong (R=0.71, P<0.0001, canonical correlation analysis; Fig. 2c). Reddish markings were significantly associated with histological activity grade as shown in Fig. 2d (R=0.45, P <0.0001).

Risks of HCC development

HCC development was evaluated for 250 patients who were observed for ≥1 year. The accumulated observation was 1991 person-years, accounting for 80% of the total potential follow-up. HCC developed in 27 patients during a mean follow-up period of 8.0 ± 5.0 years, at the age of 37-72 years. The incidence of HCC development was estimated as 5.7% at 5 years of follow-up, 13.5% at 10 years, and 20.6% at 15 years (Fig 3a). Fig. 3b shows cumulative rates of HCC development by age, estimated as 1.3% at 40 years old, 12.3% at 50 years old, and 27.2% at 60 years old. When the patients were divided in three groups according to age at diagnosis (<30 years, 31-39 years, and ≥40 years), there were significant differences in cumulative rates of HCC development among the groups (P=0.003, log-rank test, Fig. 3c), especially between the age groups <30 years, and ≥30 years (P=0.0009, log-rank test). The patient groups of age 31-39 years and age ≥40 years were estimated to have similar risks of HCC occurrence (P=0.57, log-rank test). Furthermore, male patients showed a higher risk of HCC development than females (P=0.008, log-rank test, Fig. 3d), as previously reported (1-7). Table 4 shows evaluations of clinical characteristics, histology, and laparoscopic features, with regard to HCC development using proportional hazards models. Significant associations with HCC development were shown for liver cirrhosis according to histological fibrosis and laparoscopic surface irregularity, high histological activity grade, laparoscopic severe reddish markings, and older age at diagnosis in univariate analysis. Cumulative risks of HCC development were also estimated by the Kaplan-Meier method (Fig. 4). Severity of reddish markings correlated significantly with risk of HCC development (P=0.036, log-rank test), while histological activity grade did not (P=0.054), suggesting some difference between these two parameters. Multivariate analysis, adjusted with a logistic likelihood ratio test, revealed that HCC development was strongly associated with older age and male gender (P=0.002 and p=0.043, respectively). Laparoscopic surface irregularity was not used for multivariate analysis, due to high correlations of laparoscopic surface irregularity with histological fibrosis stage as shown in Fig. 2c.

Sub-group analysis for HCC development

Next, we studied age difference by dividing patients according to age at diagnosis (<30 years, and ≥30 years), and our results in proportional hazards models showed that advanced stages according to histological fibrosis stage and surface irregularity were significantly associated with HCC development for patients of age ≥30 years at diagnosis (P=0.040, and P=0.016, respectively, Table 5). Severe

inflammatory activity with reddish markings also affected HCC development (P=0.034). Therefore we estimated cumulative rates of HCC development, by using the Kaplan-Meier method. Among patients of age ≥30 years at diagnosis, cumulative rates of HCC development were higher in more advanced disease, according to surface irregularity (Fig. 5b, P=0.043, log-rank test). Cumulative rates of HCC development was 37.1% at the 10-year follow-up among the patients in cirrhotic S3 stage, 25.6% among those in pre-cirrhotic S2 stage, and 10.1% among those in S1 stage. Interestingly, the risk of HCC occurrence was significantly higher for those in as early as S1 stage, compared with the patients of age <30 years (Fig. 5c, P=0.031, log-rank test). Actually, none of age <30 years experienced HCC during the 10-year follow-up. Further sub-group analysis in those of age ≥30 years in each laparoscopic stage could not find any significant factors contributing to HCC development. As for the effects of inflammatory activity on HCC development, significant differences in cumulative rates of HCC development were observed among the patients of age ≥ 30 years at diagnosis when stratified by reddish markings (Fig.6, P=0.025, log-rank test), but not by histological activity (P=0.087) or ALT levels (P=0.69).

DISCUSSION

Persistent elevation of HBV-DNA and ALT are associated with rapid disease progression and HCC development (3, 4). Most patients with HBeAg might be candidates for treatment with nucleoside analogues, as the presence of HBeAg often indicates active viral replication and high levels of ALT in an immune-active state of chronic infection. However, due to drug resistance and the high cost of life-long medication, predictors for HCC development should be more clearly defined so that patients can judge the necessity of immediate treatment using nucleoside analogues. We hypothesized that laparoscopic observation of the liver surface might work for this purpose. The present study retrospectively evaluated long-term outcomes for a large systematic cohort of HBeAg-positive patients, focusing on HCC development, using laparoscopic, histological, and clinical characteristics.

In the present study, half patients with early-stage (S1) disease were <30 years old at diagnosis. Cumulative rate of HCC development was 0.0% during the following 10 years, partly because some patients showed seroconversion to negative HBeAg in the following 10 years with cessation of hepatitis. Conversely, the patients who were ≥30 years old in the early stage showed a significantly higher risk of HCC, compared to the patients of age <30 years. Treatment with nucleoside analogues may be worth considering in such patients, although incidence rates were less than those of patients in the pre-cirrhotic stage or cirrhotic stage. Age differences in disease progression have been reported with other chronic liver diseases, including chronic hepatitis C (26), autoimmune hepatitis (27), and primary biliary cirrhosis (28). Our results suggest that age difference plays some role in HCC development among HBV patients, and that patients of age <30 years should be re-evaluated with liver biopsy within 10 years if HBV-DNA and ALT levels remain elevated.

Interestingly, our analysis of the patients of age ≥30 years revealed that a laparoscopic finding of reddish markings correlated significantly with HCC development. Reddish markings were significantly correlated with histological activity, but these parameters showed different influences on HCC development. This was suspected to arise from differences in the origins of these parameters. Ohta et al. performed precise histological analysis of reddish markings with histological reconstruction using serial sections of liver biopsy specimens from cases with reddish markings (22). They revealed that reddish markings correspond to widespread necrosis of hepatocytes, and proposed this finding as a useful index of activity in chronic hepatitis. Shibayama et al. showed that reddish markings did not appear in the early stage of chronic hepatitis with piecemeal necrosis around the portal area, instead

appearing only after hepatic parenchymal destruction subjacent to the liver capsule due to prolonged active hepatitis or repeated acute exacerbations of chronic hepatitis (16, 23). Reddish markings as an index of laparoscopic activity are not equivalent to piecemeal necrosis as an index of histological activity. Progression to liver cirrhosis may occur after the appearance of reddish markings unless the activity of chronic hepatitis can be reduced, because hepatic parenchymal destruction may change the pattern of blood flow in the liver to an increasingly cirrhotic pattern. Reddish markings might be useful not for early detection of HCC, but as warning of transition to liver cirrhosis prior to HCC development. Our results indicate reddish markings as a useful predictor of HCC development.

In terms of liver cirrhosis, our results are consistent with previous reports, showing that liver cirrhosis in histological fibrosis or laparoscopic surface irregularity is strongly associated with HCC development (14). This strong association might explain the results of subgroup analysis among cirrhotic patients, in which no significant predictive factors could be found for HCC development. This reveals that HCC might occur irrespective of other conditions such as liver inflammation, once liver disease has progressed to cirrhosis. Actually, the role of antiviral therapy with nucleoside analogues has not been well-defined for cirrhotic patients with regard to reduced HCC development. We have previously reported that cumulative recurrence rates of HCC after initial and complete treatment for HCC did not differ between lamivudine-treated and control groups (29). Kuzuya et al. supported this finding and suggested that antiviral therapy may improve remnant liver function and increase the chances of receiving available treatment modalities for recurrent HCC (30).

Completely normal values from routine laboratory tests of liver function might suggest a normal liver or only early-stage liver disease, but our analysis showed that only half of patients with such completely normal values were in the early stage. Several investigators have reported noninvasive approaches for quantitative diagnosis of liver fibrosis, using routine laboratory tests, serum fibrosis markers, radiological imaging, and elastography (31), all of which have been in practical use for hepatitis C. Prolonged active hepatitis or repeated acute exacerbations may occur frequently in HBV patients, and might disturb the accuracy of noninvasive quantitation of liver fibrosis (32). Liver biopsy appears warranted for precise evaluation of disease progression, and further examination with laparoscopy would be ideal, even if liver function tests continue to yield normal results.

In conclusion, HCC development is associated with older age, male gender, and liver cirrhosis. Reddish markings, rather than histological activity or ALT level, can be useful to predict HCC for HBV

patients of age ≥30 years at diagnosis. Patients of age ≥30 years even in the early stage may consider treatment with nucleoside analogues because of relatively high risk of HCC development.

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Table 1: Laparoscopic evaluations of reddish markings and whitish markings.

Item	Definition	Score
Reddish markings		
Location	Periportal	1
	Pericentral	1
	Multilobular	2
Distribution	Localized	1
	Sparse	2
	Dense	3
Tone of color	Indistinct	1
	Common	2
	Hemorrhagic	3
Diagnostic classification		
None		0 points
Mild reddish marking		<5 points
Severe reddish marking		≥5 points
Whitish markings		
Location	Spotted	1
	Asteroidal	2
	Network-like	2
Diagnostic classification		
None		0 points
Mild whitish marking		<2 points
Severe whitish marking		≥2 points

370 **Table 2: Patient characteristics at the time of diagnosis.**

	(N = 303)
Age at diagnosis (years)	34 ± 11†
Gender (female/male)	71/232
Family history of liver disease	217 (71.6%)
History of blood transfusion	14 (4.6%)
Liver histology	
Fibrosis stage (1/2/3/4)*	92/90/101/20
Activity grade (1/2/3)*	104/135/64
Laboratory data at diagnosis	
AST (IU/L)	91 ± 73†
ALT (IU/L)	156 ± 142†
Total bilirubin (mg/dl)	0.87 ± 0.53†
Albumin (g/dl)	4.2 ± 0.4†
Platelet count ($\times 10^4/mm^3$)	18 ± 6†

* Histological stage classified by Desmet et al. (20); †: Mean ± SD.

AST, aspartate aminotransferase; ALT, alanine aminotransferase.

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Table 3: Summary of laparoscopic features of HBV patients.

	Surface irregularity*		
	S1 (n=187)	S2 (n=93)	S3 (n=23)
Reddish markings	89 (48%)	69 (74%)	15 (65%)
Severe reddish markings	34 (18%)	34 (37%)	6 (26%)
Whitish markings	51 (27%)	22 (24%)	3 (13%)
Severe whitish markings	34 (18%)	12 (13%)	2 (9%)
Vascular proliferation	160 (86%)	68 (73%)	20 (87%)
Severe vascular proliferation	110 (59%)	57 (61%)	15 (65%)
Patchy markings	28 (15%)	67 (72%)	2 (9%)
Fat deposition	46 (25%)	25 (27%)	11 (48%)

* Surface irregularity, classified in three stages: S1, normal or early stage; S2, advanced, pre-cirrhotic stage; and S3: cirrhotic stage.

Table 4: Analysis of factors predicting HCC development with the proportional hazards model.

	Univariate analysis		Multivariate analysis	
Factors	Odds ratio (range†)	p	Odds ratio (range†)	p
Age at diagnosis (years)	1.06 (1.03-1.10)	<0.001	1.06 (1.02-1.11)	0.002
Gender (male)	3.32 (0.78-14.0)	0.10	4.53 (1.05-19.6)	0.043
Blood transfusion	2.40 (0.56-10.2)	0.24		
Family history of liver disease	1.46 (0.67-3.18)	0.35		
Interferon therapy	0.65 (0.29-1.45)	0.29		
Histological fibrosis stage	1.80 (1.18-2.76)	<0.001	1.21 (0.71-2.07)	0.49
Histological activity grade	1.82 (1.06-3.14)	0.031	1.16 (0.58-2.34)	0.68
AST (≥80 IU/L)	1.32 (0.62-2.83)	0.47		
ALT (≥80 IU/L)	1.06 (0.48-2.37)	0.88		
Surface irregularity	2.45 (1.46-4.09)	<0.001		
Whitish markings	0.77 (0.31-1.90)	0.57		
Vascular proliferation	1.27 (0.48-3.36)	0.64		
Reddish markings	1.66 (1.04-2.65)	0.036	1.45 (0.54-3.90)	0.46
Patchy markings	2.04 (0.96-4.36)	0.065	1.38 (0.57-3.32)	0.48
Fat deposition	1.28 (0.48-3.37)	0.62		

†: 95% confidence interval; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

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Table 5: Analysis of factors predicting HCC development for patients with age ≥30 years with the proportional hazards model.

	Univariate analysis	
Factors	Odds ratio (range†)	p
Histological fibrosis stage	1.57 (1.02-2.40)	0.04
Histological activity grade	1.67 (0.95-2.95)	0.075
AST (≥80 IU/L)	0.72 (0.33-1.57)	0.41
ALT (≥80 IU/L)	1.18 (0.53-2.66)	0.69
Surface irregularity	1.93 (1.13-3.31)	0.016
Reddish markings	1.67 (1.04-2.70)	0.034
Patchy markings	1.54 (0.68-3.48)	0.30

†: 95% confidence interval; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

FIGURE LEGENDS

Figure 1: Laparoscopic features of the patients with chronic viral hepatitis.

Figures show typical pictures of laparoscopic features; laparoscopy of severe reddish markings, showing advanced surface irregularity with densely distributed reddish markings (a), closer view of severe reddish markings in hemorrhagic color which are multilobularly located (b), closer view of mild reddish markings, showing common redness in periportal areas (c), laparoscopy of vascular proliferation (d), and laparoscopy of normal liver (e).

Figure 2: Comparisons of histology, laboratory parameters, and laparoscopic findings.

Histological fibrosis stage was compared with total scores of the five conventional parameters related to liver function with significant correlations (R=0.46, P<0.0001, canonical correlation analysis, Fig 2a): fibrosis stage 1, striped; stage 2, open; stage 3, gray; and stage 4, black. Significantly high correlations were also shown between histological fibrosis stage and laparoscopic surface irregularity (R=0.66, P<0.0001, Fig 2c): fibrosis stage 1, striped; stage 2, open; stage 3, gray; and stage 4, black. As for the activity, alanine aminotransferase (ALT) levels were divided in 4 groups and compared with histological activity grade, showing significant associations (R=0.14, P=0.013, Fig 2b): A1, open; A2, gray; A3, black. Correlations between histological activity grade and reddish markings were significant as shown in Fig 2d (R=0.45, P<0.0001): A1, open; A2, gray; and A3, black.

Figure 3: Cumulative rates of hepatocellular carcinoma (HCC) development.

Fig. 3a shows cumulative rate of HCC development as the follow-up period, estimated by the Kaplan-Meier method, The incidence of HCC development was estimated as 5.7% at 5 years of follow-up, 13.5% at 10 years, and 20.6% at 15 years. Fig. 3b shows cumulative rates of HCC development by age, estimated as 1.3% at 40 years old, 12.3% at 50 years old, and 27.2% at 60 years old. When the patients were divided in three groups according to age at diagnosis (<30 years, 31-39 years, and ≥40 years), there were significant differences in cumulative rates of HCC development among the groups (p=0.003, log-rank test, Fig. 3c). Furthermore, Fig 3d shows significant difference in cumulative rates of HCC development between the female patients and the male patients (p=0.008, log-rank test).

Figure 4: Cumulative rates of hepatocellular carcinoma (HCC) development, stratified by histology and laparoscopic findings.

Figures show cumulative rates of HCC development estimated by the Kaplan-Meier method, stratified by histological fibrosis stage (a), laparoscopic surface irregularity (b), histological activity grade (c), and laparoscopic reddish markings (d). Significant associations with HCC development were shown for liver cirrhosis according to histological fibrosis (p=0.030, log-rank test) and laparoscopic surface irregularity (p=0.002). Severity of laparoscopic reddish markings was significantly associated with HCC development (p=0.036), while that of histological activity grade was not (p=0.054, log-rank test).

Figure 5: Cumulative rates of hepatocellular carcinoma (HCC) development, for the patients of age ≥ 30 years at diagnosis, stratified by disease progression.

Figures show cumulative rates of HCC development, for the patients of age ≥ 30 years at diagnosis, stratified by histological fibrosis stage (Fig. 5a) and surface irregularity (Fig. 5b and 5c). Cumulative rates of HCC development were significantly higher in more advanced diseases, according to surface irregularity (Fig. 5b, P=0.043, log-rank test), but not to histological fibrosis stage (Fig. 5a, P=0.19). The risk of HCC development was significantly higher among the patients of age ≥30 years even in laparoscopic S1 stage at diagnosis, compared with the patients of age <30 years (Fig. 5c, P=0.031, log-rank test).

Figure 6: Cumulative rates of hepatocellular carcinoma (HCC) development, for the patients of age ≥ 30 years at diagnosis, stratified by inflammatory activity.

Cumulative rates of HCC development are shown for patients of age ≥ 30 years at diagnosis, stratified by histological activity (a), laparoscopic Reddish markings (b), and ALT levels (c). Cumulative rates of HCC development showed significant differences when stratified by reddish markings (P=0.025, log-rank test), but not by histological activity (P=0.087) or ALT levels (P=0.69).