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Histological subclassification of cirrhosis based on histological–haemodynamic correlation

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SUMMARY

Background
Determining a relationship between specific histological parameters in cirrhosis and hepatic venous pressure gradient can be used to subclassify cirrhosis.

Aim
To determine the relationship between hepatic venous pressure gradient and specific histological parameters in cirrhosis.

Methods
Forty-seven patients (mean age: 46.2 ± 13.6 years; 36 male) with biopsy-proven cirrhosis and hepatic venous pressure gradient measurements within 1 month of biopsy were studied. The following histological parameters were scored semiquantitatively: nodule size, loss of portal tracts and central veins, portal inflammation, periportal inflammation, bile duct proliferation, lobular inflammation, ballooning, fatty change, cholestasis and septal thickness.

Results
On multiple ordinal regression analysis, small nodule size (odds ratio: 21.0; 95% confidence interval: 2.1–208.2, $P = 0.009$) and thick septa (OR: 42.6; CI: 2.3–783.7, $P = 0.011$) were significantly associated with the presence of clinically significant portal hypertension. A score was assigned to each of the two parameters (nodule size: large = 1, medium = 2, small = 3 and septal thickness: thin = 1, medium = 2, thick = 3). Two subcategories were devised based on the composite score: category A ($n = 12$): score 1–3 and category B ($n = 35$): score 4–6. On ordinal regression, subcategory B (OR: 15.5; CI: 3.3–74.2, $P = 0.001$) was significantly associated with clinically significant portal hypertension.

Conclusion
Small nodularity and thick septa are independent predictors of the presence of clinically significant portal hypertension.

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INTRODUCTION

Cirrhosis is defined histologically by the presence of regenerative nodules surrounded by fibrous tissue. This architectural distortion leads to increased intrahepatic resistance that in turn leads to portal hypertension. Complications of cirrhosis, including oesophageal varices and ascites, develop once portal pressure reaches a threshold level of 10–12 mmHg, as assessed by the hepatic venous pressure gradient (HVPG).[1–3] Cirrhosis denotes the most severe stage of liver fibrosis. However, in cirrhosis, there may be certain histological features indicative of more severe disease. The most direct and accessible evaluation of portal hypertensive syndrome is performed by measuring the HVPG, which has been validated as an indicator of the degree of portal pressure in cirrhosis.[1] HVPG reflects the interaction between hepatic vascular resistance and blood flow and, as such, is thought to indicate disease severity closely. The prognostic value of HVPG has been demonstrated in different settings associated with chronic liver disease and has been shown to correlate with survival, decompensation and development of collaterals.[1, 4–6]

Defining a relationship between specific histological parameters in cirrhosis and HVPG could help subclassify cirrhosis according to its 'severity' as measured by HVPG. Recently, Nagula *et al.* described a subclassification of histological cirrhosis on the basis of severity of portal hypertension that consists of a combination of nodule size and septal thickness, with small nodularity and thick septa being independent predictors of the presence of clinically significant portal hypertension (CSPH).[7] These findings need further evaluation and validation.

This study was undertaken to determine the relationship between portal pressure, as determined by the HVPG and specific histological parameters in cirrhosis and to propose a histological subclassification of cirrhosis.

PATIENT AND METHODS

Patients

Patients who had a liver biopsy specimen (obtained via a transjugular or percutaneous approach) showing a diagnosis of cirrhosis, and HVPG performed within 1 month of each other were included in the study. All specimens were characterized by a length of ≥1.0 cm and width of ≥1.2 mm. In fragmented biopsies, the total length was estimated by adding maximum dimensions of each individual fragment.

Histological assessment

Individual biopsy specimens were scored with the use of the Knodell index, which grades the histological activity of hepatitis on a scale from 0 to 18, with higher scores indicating more severe abnormalities.[8]

Table 1. Morphological assessment of liver biopsies

Histological parameter	Range	Scale
Nodularity		
Small nodules		Nodule size is comparable to width of needle biopsy specimen
Mixed nodules		Presence of both small and large nodules (The presence of even one small nodule would lead to a 'mixed' classification)
Large nodules		Nodule size larger than biopsy width
Portal tracts lost	0–4	0 = absent, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–100%
Central veins lost	0–4	0 = absent, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–100%
Portal inflammation	0–3	0 = absent, 1 = mild, 2 = moderate, 3 = severe
Periportal inflammation	0–3	0 = absent, 1 = mild, 2 = moderate, 3 = severe
Bile duct proliferation	0–2	0 = absent, 1 = present, normal; 2 = present, abnormal, increased
Lobular inflammation	0–3	0 = absent, 1 = mild, 2 = moderate, 3 = severe
Ballooning	0–2	0 = absent, 1 = present, normal; 2 = present, abnormal, increased
Fatty change	0–4	0 = absent, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–100%
Cholestasis	0–2	0 = absent, 1 = present, normal; 2 = present, abnormal, increased
Septal thickness	0–3	0 = absent, 1 = thin, 2 = medium, 3 = thick (Thickness of the predominant type of septae in each specimen)

Table 2. Baseline clinical and laboratory parameters of the study population

Variable	n = 47
Age (year; mean ± s.d.)	46.2 ± 13.6
Male gender, n (%)	36 (77)
Bilirubin (mg/dL; mean ± s.d.)	1.8 ± 1.4
Albumin (g/dL; mean ± s.d.)	3.5 ± 0.7
Platelet count [Lac/cumm; median (range)]	1.4 (0.35–3.2)
AST [U/L; median (range)]	74 (20–400)
ALT [U/L; median (range)]	60 (14–285)
PT prolongation [s; median (range)]	3.7 (0–25)
Child status, n (%)	
A	18 (38)
B	21 (45)
C	8 (17)
Oesophageal varices, n (%)	
Absent	2 (4)
Gr 1	8 (17)
Gr 2	24 (51)
Gr 3	8 (17)
Gr 4	4 (11)
GOV, n (%)	8 (17)
IGV, n (%)	1 (2)
PHG, n (%)	
Mild	15 (32)
Severe	1 (2)
Variceal bleeding history, n (%)	11 (23)
Percutaneous liver biopsy	27
Transjugular liver biopsy	20
Aetiology of cirrhosis, n (%)	
Hepatitis B	21 (45)
Hepatitis C	9 (19)
Alcohol	1 (2)
Hepatitis B plus C	3 (6)
Hepatitis B plus alcohol	2 (4)
Cryptogenic	11 (23)
HVPG (mmHg)	
Median (range)	13 (7–33)
≥10, n (%)	31 (66)

GOV, gastro-oesophageal varices; IGV, isolated gastric varices; PHG, portal hypertensive gastropathy; HVPG, hepatic venous pressure gradient.

The overall Knodell score [Histological Activity Index (HAI)] is the sum of the scores for periportal bridging necrosis (0–10), intralobular degeneration and focal necrosis (0–4), and portal inflammation (0–4). Staging was according to Batts and Ludwig staging,[9] where F1 is portal expansion, F2 is portal septae with or without portal–portal bridging fibrosis, F3 is portal–central bridging fibrosis and F4 is cirrhosis.

Table 3. Median hepatic venous pressure gradient (HVPG) for each histological parameter (n = 47)

Histological parameter	HVPG [mmHg; median (range)]	P-value*
Nodularity		
Small (n = 15)	22 (11–33)	0.001
Mixed (n = 18)	12.5 (8–24)	
Large (n = 14)	9 (7–18)	
Portal tracts lost		
0 (n = 10)	11.5 (8.5–18)	0.326
1 (n = 3)	13 (11–33)	
2 (n = 6)	13.7 (9–30)	
3 (n = 11)	12 (7–24)	
4 (n = 17)	18 (8–28)	
Central veins lost		
0 (n = 3)	9 (8–11)	0.643
1 (n = 5)	12 (8–23)	
2 (n = 4)	19 (9–22)	
3 (n = 12)	12 (8–33)	
4 (n = 23)	14 (7–30)	
Portal inflammation		
0 (n = 0)	–	0.330
1 (n = 26)	12 (7–33)	
2 (n = 11)	20 (9–23)	
3 (n = 10)	13 (8–30)	
Periportal inflammation		
0 (n = 4)	8.5 (7–28)	0.053
1 (n = 15)	9 (8–33)	
2 (n = 11)	20 (9–28)	
3 (n = 17)	13 (8–23)	
Bile duct proliferation		
0 (n = 16)	10.5 (8–26)	0.371
1 (n = 23)	12 (7–30)	
2 (n = 8)	20.5 (9–33)	
Lobular inflammation		
0 (n = 3)	24 (13.5–26)	0.112
1 (n = 19)	12 (8–33)	
2 (n = 19)	13 (7–23)	
3 (n = 6)	13 (9–23)	
Ballooning		
0 (n = 4)	11.2 (8–20)	0.182
1 (n = 24)	12.5 (7–33)	
2 (n = 19)	14 (9–28)	
Fatty change		
0 (n = 32)	11.5 (7–33)	0.460
1 (n = 12)	20 (8–28)	
2 (n = 3)	20 (18–28)	
3 (n = 0)	–	
4 (n = 0)	–	
Cholestasis		
0 (n = 26)	11 (8–23)	0.140
1 (n = 11)	17 (7–33)	
2 (n = 10)	18 (8–30)	

Table 3. (Continued)

Histological parameter	HVPG [mmHg; median (range)]	*P*-value*
Septal thickness		
0 (n = 0)	–	0.001
1 (n = 8)	9 (8–12)	
2 (n = 21)	11 (7–18)	
3 (n = 18)	22 (12–33)	

* One-way ANOVA.

Slides of each biopsy were reviewed by two pathologists (PS and AR) who were blinded to the results of HVPG measurements and they gave a final score to each of the components. Discrepancies in scoring of various lesions were few and minor; they were then reviewed by both pathologists together to arrive at a consensus.

The biopsies were evaluated for the parameters given in Table 1. In case of heterogeneous pattern of sinusoidal fibrosis, the worst pattern was scored. For septal thickness, the thickness of the predominant type of septae in each specimen was scored. Regarding nodule size, the presence of even one small nodule would lead to a 'mixed' classification. Regarding loss of portal tracts and portal veins, we anticipated 4–6 portal tracts and central veins per centimetre of core needle biopsy, therefore, the absence of identifiable portal tracts in the liver biopsy was scored as '4' (maximal abnormality), while their presence, as expected for a normal biopsy (at least 4 portal tracts/cm), was scored as '0'. Similarly, a central vein is expected for each lobule in a normal biopsy and by comparison in each nodule of a cirrhotic liver. The loss of central veins was also subjectively scored on a 0–4 scale. Absence of identifiable central veins in all cirrhotic nodules was scored as '4', while their presence in each cirrhotic nodule was scored as '0'.

Hepatic venous pressure gradient measurements

After an overnight fast, HVPG measurement was carried out using standard procedure. Briefly, under local anaesthesia and in supine position, a venous introducer was placed into the right femoral vein by Seldinger technique. Under fluoroscopic guidance, a 7F balloon-tipped Swan Ganz Catheter (Boston Scientific, MA, USA) was introduced into the main right hepatic vein. Free hepatic venous pressure and wedged (occluded) hepatic venous pressure were measured using Nihon Kohden (Tokyo, Japan) haemodynamic monitor with pressure transducers. Measurements were made in triplicate, and the mean of three readings was taken in every case. If there was a difference of more than 1 mmHg between the readings, all the readings were repeated.[10, 11] Patients were categorized as having CSPH if HVPG was ≥10 mmHg.[1, 3, 4]

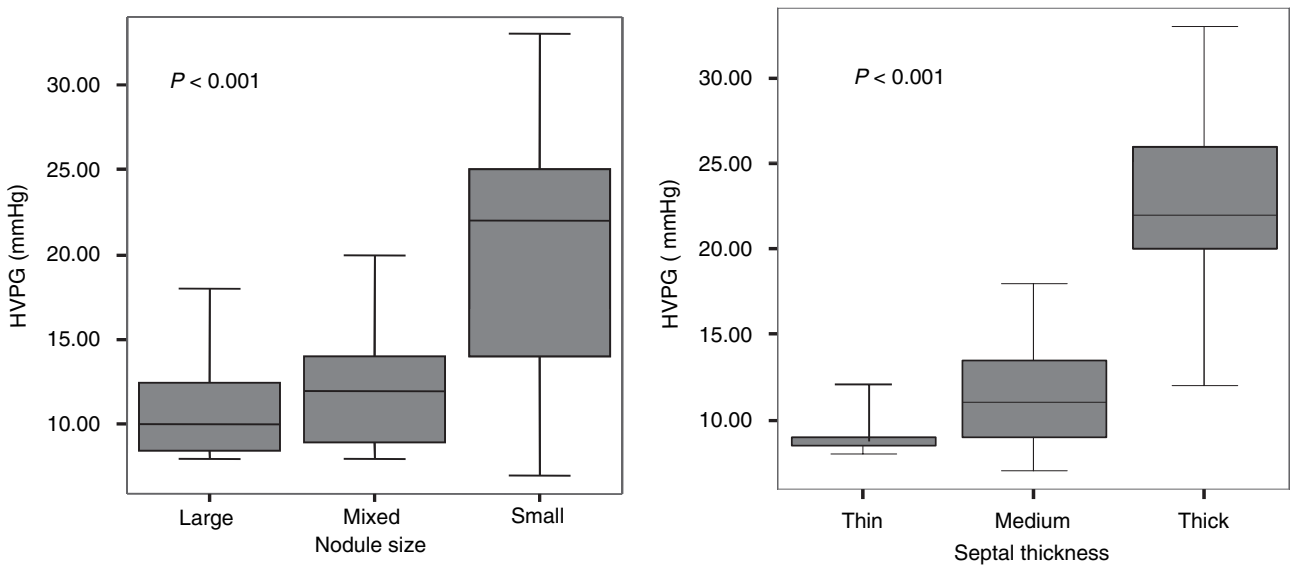

Figure 1. Distribution of hepatic venous pressure gradient (HVPG) according to nodule size and septal thickness, showing median HVPG, 25–75th percentile box and complete range of measurements.

Table 4. Distribution of histological parameters according to the presence or absence of clinically significant portal hypertension (CSPH)

Histological parameter	CSPH (n = 31)	No CSPH (n = 16)	P-value*
Age (years; mean ± s.d.)	46.2 ± 13.9	46.4 ± 14.4	0.960
Male, N (%)	23 (74)	13 (81)	0.725
Nodularity, N (%): small/mixed/large	15(48)/13(42)/3(10)	0.0(0)/5(31)/11(69)	<0.001
Portal tracts lost, N (%): 0/1/2/3/4	6(19)/3(10)/4(13)/9(23)/11(36)	4(25)/0.0(0)/2(13)/4(25)/6(38)	0.782
Central veins lost, N (%): 0/1/2/3/4	1(3)/3(10)/3(10)/7(23)/17(55)	2(13)/2(13)/1(6)/5(31)/6(4)	0.626
Portal inflammation, N (%): 0/1/2/3	0.0(0)/14(45)/10(32)/7(23)	0.0(0)/12(75)/1(6)/3(19)	0.090
Periportal inflammation, N (%): 0/1/2/3	1(3)/(23)/9(29)/14(45)	3(19)/8(50)/2(13)/3(19)	0.053
Bile duct proliferation, N (%): 0/1/2	8(26)/16(52)/7(23)	8(50)/7(44)/1(6)	0.165
Lobular inflammation, N (%): 0/1/2/3	3(10)/11(36)/12(39)/5(16)	0.0(0)/8(50)/7(44)/1(6)	0.396
Ballooning, N (%): 0/1/2	2(7)/14(45)/15(48)	2(13)/10(63)/4(25)	0.286
Fatty change, N (%): 0/1/2/3/4	19(61)/9(29)/39(10)/0.0(0)/0.0(0)	13(81)/3(19)/0.0(0)/0.0(0)/0.0(0)	0.272
Cholestasis, N (%): 0/1/2	15(48)/8(26)/8(26)	11(69)/3(19)/2(13)	0.388
Septal thickness, N (%): 0/1/2/3	0.0(0)/1(3)/12(39)/18(58)	0.0(0)/7(44)/9(56)/0.0(0)	<0.001

* For age: Student's t-test; for others: chi-squared test (Yates correction as required).

Table 5. Histological correlates of HVPG in cirrhotics

Parameter	Spearman's correlation	Significance (two-tailed)
HAI	−0.04	0.77
Nodularity	−0.76	<0.001
Portal tracts lost	0.15	0.31
Central veins lost	0.20	0.18
Portal inflammation	0.15	0.32
Periportal inflammation	0.17	0.24
Bile duct proliferation	0.33	0.02
Lobular inflammation	−0.14	0.34
Ballooning	0.17	0.26
Fatty change	0.25	0.09
Cholestasis	0.29	0.06
Septal thickness	0.81	<0.001

HVPG, hepatic venous pressure gradient.

Table 6. Ordinal regression for prediction of clinically significant portal hypertension from histological parameters

Parameter	OR	95% CI	Significance
Nodularity			
Small	21.0	2.1–208.2	0.009
Mixed	15.8	2.0–122.6	0.008
Large	1		
Septal thickness			
Thick	42.8	2.3–783.7	0.011
Medium	17.3	1.2–252.7	0.037
Thin	1		
Bile duct proliferation			
2	2.4	0.1–53.6	0.578
1	2.2	0.3–15.5	0.415
0	1		

Statistical analysis

The unpaired Student's t-test was applied for comparisons of normally distributed variables. The statistical significance of inter-group differences, for non-normal distributed data, was evaluated by means of Mann–Whitney U-tests. Chi-squared test (Yates correction as required) was used for comparison of categorical variables. One-way ANOVA was used to test for differences among three or more groups. Spearman rank correlation coefficient was used to find correlations between histological parameters and HVPG. Ordinal regression was used to identify the histological factors that

correlated with the presence of CSPH. *P*-values <0.05 were considered statistically significant.

RESULTS

In the period between May 1994 and July 2007, 158 patients with chronic liver disease had a liver biopsy performed within 1 month of HVPG measurement. Four patients were excluded for reasons of fragmented, small specimens. Of the 154 remaining patients, 107 patients had earlier stages of chronic liver disease (precirrhotic). Histological cirrhosis (stage 4) was present in the liver biopsies of 47 patients who were included in the present analysis. These patients were subjected to the haemodynamic study to obtain a basal assessment of portal pressure before enrolling in a primary (n = 6), secondary (n = 11) or early primary prophylaxis (n = 28) protocols for variceal bleeding or for diagnostic purposes (n = 2) to exclude noncirrhotic causes of portal hypertension.

Table 2 shows the baseline clinical and laboratory parameters of the patients. Hepatitis B was the most common aetiological factor present in 21 (45%) cases. The median HVPG was 13.0 mmHg with a range of 7–33; 31 patients (66%) had CSPH.

Correlation between HVPG and histological parameters

Table 3 shows the HVPG values for each histological parameter. HVPG was statistically different among the subcategories of nodule size ($P < 0.001$), and septal thickness ($P < 0.001$) (Figure 1). Table 4 shows the distribution of histological parameters according to the presence or absence of CSPH. Nodule size and septal thickness were found to be significantly different between patients with and without CSPH. Table 5 shows the histological correlates of HVPG in cirrhotics. There was a significant correlation between HVPG and nodule size, septal thickness and bile duct proliferation. These three factors were entered into a multiple ordinal regression analysis with CSPH as the dependent variable (Table 6). Small nodule size and thick septa were significantly associated with the presence of CSPH. Representative micrographs of nodule size and septal thickness are illustrated in Figures 2 and 3.

For subcategorizing the histological cirrhosis a composite score was developed. A score was assigned to each of the two parameters (nodule size: large = 1, medium = 2, small = 3 and septal thickness: thin = 1, medium = 2, thick = 3). Two subcategories of histolog-

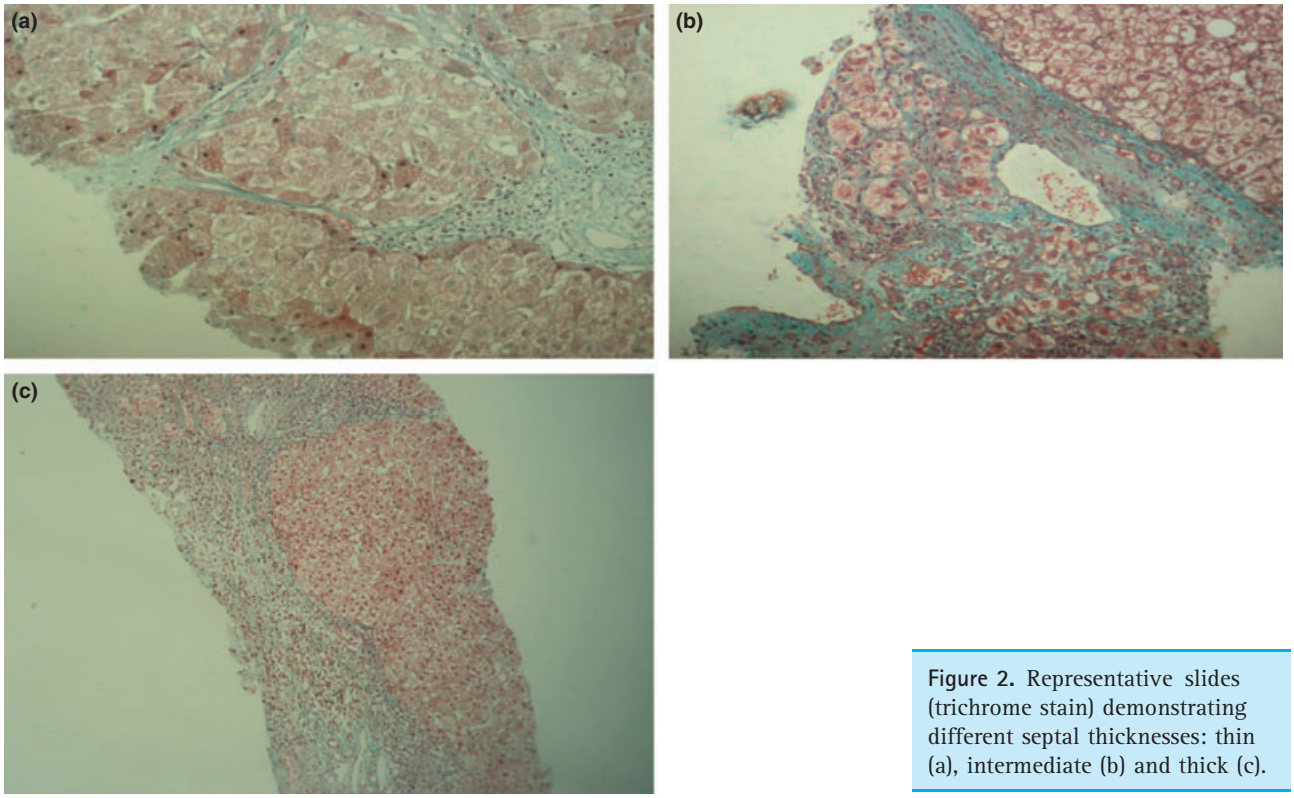

Figure 2. Representative slides (trichrome stain) demonstrating different septal thicknesses: thin (a), intermediate (b) and thick (c).

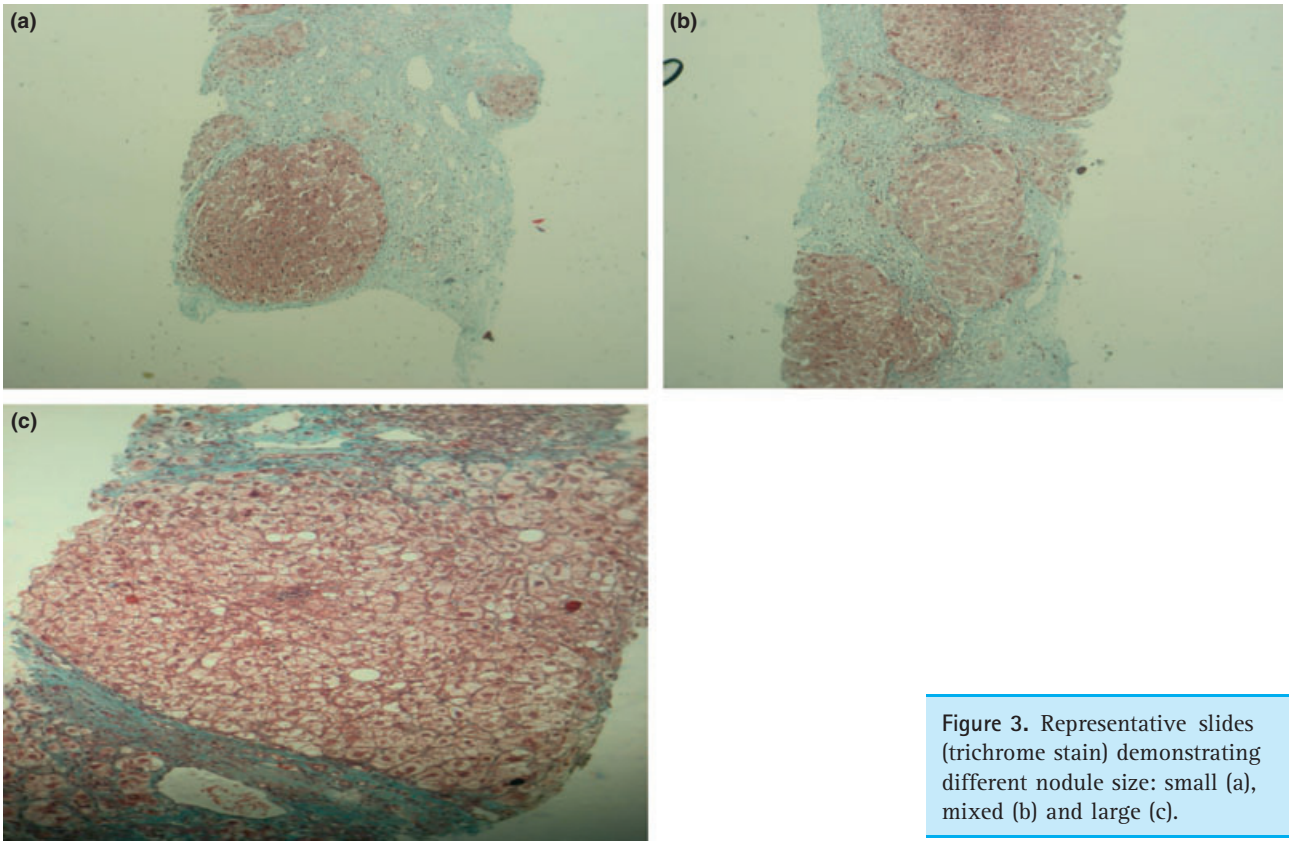

Figure 3. Representative slides (trichrome stain) demonstrating different nodule size: small (a), mixed (b) and large (c).

ical cirrhosis were devised based on the composite score calculated by adding the score assigned to each of the two parameters: Category A: score 1–3 and Category B: score 4–6. Median (range) of HVPG was 9 (7–17) and 17.5 (8–33) mmHg ($P < 0.001$) in subcategory A and B respectively. Subcategory was analysed in ordinal regression as a predictor of CSPH and it was found that subcategory B [odds ratio (OR): 15.5; 95% confidence interval (CI): 3.3–74.2, $P = 0.001$] was significantly associated with the presence of CSPH.

DISCUSSION

The most advanced stage of liver fibrosis is the cirrhotic stage. As most complications of cirrhosis are secondary to portal hypertension, HVPG has been found to be of major prognostic significance. A threshold level of 10 mmHg has been identified as a predictor of the development of complications of cirrhosis (varices, variceal haemorrhage and ascites) and death.[10, 12] We found that septal thickness and nodule size were the two independent predictors of the presence of CSPH. Similar findings were also reported in an earlier study.[7] These findings are consistent with the pathophysiology of portal hypertension. The disease progression from chronic hepatitis to cirrhosis of the liver is associated with an increase in portal pressure.[13, 14] These factors responsible for increase in portal pressure include major angio-architectural modifications involving neo-angiogenesis and the presence of cell types undergoing active contraction in response to an intrahepatic predominance of vasoconstrictor stimuli.[15, 16] As a result, the progressive rise in portal pressure represents a reliable indicator of the tissue changes typical of the cirrhotic liver. Thick septae exert greater obstruction to portal flow and leads to higher HVPG. Small nodule size is also indicative of greater architectural distortion and increased intrahepatic resistance.[7]

Suggestions for subclassification of histological cirrhosis were made earlier;[17] this was based on the characteristics of fibrous septae. In patients with alcoholic liver disease, a positive correlation has been identified between intrahepatic pressure and hepatocyte size and collagen in the space of Disse.[18] Other studies have shown progressive increases in HVPG with increasing severity of liver disease (normal, chronic hepatitis, precirrhosis and cirrhosis).[12, 19–21] However, in a study

no relevant correlation could be found between HVPG and any histological parameter.[22] Another study, found a negative correlation between HVPG and the portal spaces not involved in the process of bridging fibrosis.[23] Recently, Nagula *et al*. described a subclassification of histological cirrhosis based on the severity of portal hypertension that consists of a combination of nodule size and septal thickness, with small nodularity and thick septa being independent predictors of the presence of CSPH.[7] This conclusion is identical to the conclusion of this study but the study population is different. In the study by Nagula *et al*., majority of the patients had alcohol as the aetiology of cirrhosis, whereas in this study, hepatitis B virus was the aetiological factor in the majority of the patients. In addition, we have proposed a scoring system on the basis of nodule size and septal thickness.

In our study, only one patient had pure alcoholic cirrhosis and so our findings may not be applicable to that subpopulation of cirrhosis.

We subcategorized histological cirrhosis into Category A and B on the basis of a composite score derived from nodule size and septal thickness and found that the categorization was a significant predictor for the presence of CSPH. Follow-up studies examining serial biopsies and HVPG measurements need to be undertaken in a larger population to validate the proposed scoring system, which also needs to be verified in larger sample size of patients belonging to different aetiological categories.

In conclusion, small nodularity and thick septa on histology are independent predictors of the presence of CSPH. The histological subclassification of cirrhosis based on a combination of nodule size and septal thickness needs further validation.

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