Nonalcoholic fatty liver disease

Correlation with histology and viral hepatitis

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ABSTRACT

Objective: Nonalcoholic fatty liver disease (NAFLD) involves a heterogeneous group of diseases that places some patients at risk of progression to cirrhosis. In this study, our aim was to investigate the relationships between the histopathological features of NAFLD, hepatic stellate cell activation, and capillarization to find a marker related to fibrosis, for NAFLD.

Methods: We studied liver biopsies from 62 patients with NAFLD, 21 patients with hepatitis B, and 19 patients with hepatitis C from the archives of the Department of Pathology, Gazi University Medical School, between 1997 and 2004. We performed immunoperoxidase stains for α-smooth muscle actin (α-SMA) and CD31 to identify activated hepatic stellate cells and capillarization. We investigated the relationships between histopathological features and both α-SMA and CD31 expressions.

Results: Most NAFLD cases were in low grades and stages. We found a relationship between both necroinflammatory grade and ballooning degeneration, and fibrosis. Pure steatosis did not relate to fibrosis. Immunohistochemical analysis revealed that CD31 expression was significantly higher than α-SMA expression in all groups. We determined a correlation between the fibrotic stage and CD31 expression, but not with α-SMA expression. In NAFLD cases, we detected the highest staining scores of CD31 in zone 3, while the portal/septal area was the dominant zone for control groups. There was no significant zone for α-SMA expression.

Conclusion: Our results suggest that we can use CD31, rather than α-SMA, as a marker of endothelial damage and sinusoidal capillary transformation, both of which might precede fibrogenesis in chronic liver diseases, particularly in NAFLD.

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Nonalcoholic fatty liver disease (NAFLD) is a clinicopathological entity that encompasses a broad spectrum of diseases ranging from simple steatosis to non-alcoholic steatohepatitis (NASH). Ludwig et al[1] first described NASH in 1980 in patients without a history of heavy alcohol use, whose liver biopsies histopathologically mimicked alcoholic hepatitis.[1] In NASH patients, cirrhosis occurs at a rate as high as 26%,[2] and previous studies reported the progression of fibrosis, as detected by sequential liver biopsy, to occur in 37% of the cases.[3] While some patients remained unchanged, a smaller number of patients showed histopathological improvement during follow-up of 1-7 years.[3,4] However, currently there are no distinguishing histopathological features that can assist in predicting progression. We observed the involvement of hepatic stellate cells (HSC) in liver fibrosis by means of their proliferation and myofibroblastic transformation.[5-7] However, we have not entirely investigated the contribution of sinusoidal endothelial cells (EC) to fibrogenesis. Structural and phenotypical changes in sinusoidal ECs accompany the development of capillarization.[8-10]

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One opinion is that damage to the EC or the hepatic stellate cell/endothelial cell matrix in liver diseases may change normal cell-matrix interactions that maintain hepatic stellate cells in a quiescent state.[5] In the present study, we aimed to find a marker related to fibrosis or marker specific in relation to NAFLD. We investigated the relationships between the histopathological features of NAFLD, HSC activation, and capillarization by using α-smooth muscle actin (α-SMA) and CD31 stains. Since NAFLD has a unique fibrosis pattern, we also evaluated zonal predilection of HSC activation and capillarization. We performed statistical analysis and compared our findings with a control group of chronic viral hepatitis B and C cases.

Methods. Sixty-two patients diagnosed with NAFLD were retrospectively studied. Alcohol consumption was a strict exclusionary criterion. Obesity and diabetes were the only underlying causes for NAFLD in the study group. Twenty-one hepatitis-B and 19 hepatitis-C liver biopsy specimens served as the control group. They were all needle biopsies ranging from 1-2 cm in length. Twelve portal tracts were required to include the specimen in the study. All specimens were fixed in neutral buffered formalin and processed routinely. Hematoxylin-eosin and Masson's trichrome stains were reviewed in every case. Each biopsy specimen was evaluated for the following parameters, without the evaluator's knowledge of the clinical and biochemical findings; degree of steatosis, ballooning degeneration of hepatocytes, lobular and portal inflammation, and the extent and location of fibrosis. According to these findings, a global activity index, as described by Brunt et al,[11] was determined for NAFLD cases. Necroinflammatory activity and fibrotic stage of the patients in the control group were evaluated using the Knodell scoring system.[12] The biopsies showing pure steatosis without accompanying ballooning degeneration, or perisinusoidal fibrosis, or both, were diagnosed as NAFLD type 1. The diagnosis of steatohepatitis was made according to the minimal criteria suggested by Brunt et al.[11] Immuno-peroxidase stains for α-SMA (α-SMA-Clone 1A4, NeoMarkers) and CD31 (Clone JC/70A, NeoMarkers) were performed on each biopsy specimen to identify activated hepatic stellate cells and capillarization. Positive staining of portal fibroblasts and muscle cells in the media of hepatic arteries served as a positive internal control for α-SMA. Staining of EC lining portal venules were used as a positive internal control for CD31. We used a double staining technique (Histostain-DS KIT, Zymed) for some NAFLD cases, to reveal 2 distinct antigens in a single tissue. Tissues were immunohistochemically stained with CD31 (Streptavidin-Alkaline Phosphatase Method) and 5-bromo,4-chloro,3-indolylphospate (BCIP) was used as chromogen and then the same slides were stained with α-SMA (Streptavidin-Peroxidase Method) and 3-Amino-9-Ethylcarbazole (AEC) was used as chromogen. The extent and localization of positive staining for α-SMA and CD31 were evaluated independently, without knowledge of the extent of fibrosis on trichrome stains. Steatosis, portal and lobular inflammation, and ballooning degeneration were graded on a scale of 0 (absent) to 3 (severe), individually to reveal the possible impact of each parameter on fibrosis. The number of α-SMA and CD31 positive cells in each biopsy was scored using the system developed by Schmitt-Graff et al.[13] Each zone was scored separately, as follows: 0 = no staining; 0.5± = staining of rare sinusoidal lining cells, occupying less than 1% of the sinusoidal lining in that particular zone; 1+ = staining of sinusoidal lining cells, occupying 2-10% of the sinusoidal lining; 2+ = staining of 11-30% of sinusoidal lining cells; 3+ = staining of more than 31% of sinusoidal lining cells. Fibrous septa and fibrotic portal tracts were also scored as follows: 0 = no staining; 0.5 ± = less than 10% of mesenchymal cells stained; 1+ = 10-20% of mesenchymal cells stained; 2+ = 21-50% of mesenchymal cells stained; 3+ = more than 50% of mesenchymal cells stained. Then α-SMA and CD31 positivity in each area of the biopsy specimens (zone 1, zone 2, zone 3, and portal/septal [PS] areas) were scored separately (with ± scored as 0.5). Mean values of staining scores were determined for statistical studies.

Statistical analysis. Chi-square, Mann-Whitney U tests, and Kruskal-Wallis analysis of variance was applied, as appropriate, to compare continuous variables. Spearman rank of correlation was used to correlate steatosis, and grade and stage of NAFLD with α-SMA and CD31 expressions.

Results. The majority of NAFLD cases were in the early grades and stages of the disease (**Table 1**). Five cases, having had only steatosis, but not ballooning or sinusoidal fibrosis, were graded and staged as 0, since there was no detectable fibrosis on trichrome stains. These cases were categorized as NAFLD type 1. In addition to steatosis and lobular inflammation, perivenular ballooning degeneration, or zone 3 sinusoidal pericellular fibrosis was seen in grade 1 cases, 32 of which were stage 1. Additionally, 4 of the 36 grade 1 specimens showed portal/periportal fibrosis and they were categorized as stage 2. Mallory bodies were scattered in most of the cases, though they did not appear as frequently as in cases of alcoholic steatohepatitis, and spotty lobular necroinflammation was predominantly neutrophilic (**Figure 1**). Specimens having grade 2 activity showed moderate fibrosis and there were 2 stage 1, 8 stage 2, 6 stage 3, and 1 stage 4 in this

group. The biopsies with grade 3 involved severe fibrosis as did stage 3 (1/4) and stage 4 (3/4). Fibrosis was seen mainly in zone 3 perisinusoidal areas, but in advanced cases, portal-periportal fibrosis and septa formations were observed as well (**Figure 2**). **Table 1** shows the histopathological features of the NAFLD patients. Statistical analysis revealed that both lobular inflammation ($p<0.01$) and ballooning degeneration ($p=0.018$) correlated with the fibrotic stage of the disease. However, the degree of steatosis was not significantly related to the degree of fibrosis ($p>0.05$). In both hepatitis B and C cases, the necroinflammation and fibrosis were portal based. The necroinflammatory grade varied from 5-12 in hepatitis B cases; 12 cases showed mild activity, whereas the activity in 7 cases was moderate, and severe in 2 cases (**Table 2**). Hepatitis B surface antigen was stained positively in all cases, whereas hepatitis B core antigen was detected in only 2 of the cases. In the hepatitis C group, 8 cases had mild necroinflammatory activity, 11 cases had moderate activity, and there was no severe hepatitis. There was steatosis in 16 of the cases, involving 10-75% of hepatocytes. Fibrotic stage was low in most of the cases (9 stage 1, and 6 stage 2). There were 2 stage 3 and 2 cirrhotic cases (**Table 2**). The α-SMA expression was seen in portal and periportal fibroblasts, and sinusoidal cells. The α-SMA positivity was detected in 37% (23/62) of the NAFLD cases (**Table 2**). One of the 5 pure steatosis cases displayed α-SMA expression, although there was no detectable fibrotic activity observed histopathologically. While 36% (13/36) of specimens with grade 1 activity showed α-SMA expression, we observed staining in 6 of 17 (35%) grade 2 and in 3 of 4 (75%) grade 3 cases. Statistical analysis revealed that although steatosis itself was not correlated with α-SMA expression ($p=0.17$), steatohepatitic activity was found to be related ($p=0.003$). Although expression of α-SMA was detected in all stages, we could not find any correlation between its expression and the fibrotic stage (**Table 2**, $p=0.37$). The zonal predilection of α-SMA positivity was also analyzed and summarized by rates and scores in **Table 3**. The rate of α-SMA staining was higher in zone 3 than in other zones in the NAFLD group, but difference in rates were not statistically significant between the groups ($p>0.05$). Staining intensities were faint in the majority of the cases; only one case exhibited strong staining in zone 1 and PS area (**Figure 3**). When all NAFLD cases were evaluated according to zonal scores, there were no significant differences between the zones ($p=1.0$, **Table 3**). α-smooth muscle actin expression was detected in 12 of the 21 (57.1%) hepatitis B cases and in 16 of the 19 (84.2%) hepatitis C cases (**Table 2**). The rates and total mean scores of α-SMA expression in hepatitis B and C cases were significantly higher than in the NAFLD group (**Table 3**, $p<0.01$ for hepatitis B and $p<0.0001$ for hepatitis C). We investigated the relationship between the mean scores and zonal distribution of α-SMA expression and the histo-pathological features. Although the most remarkable staining was observed in PS areas in both groups, no statistically significant difference was found between the zones (**Table 3**, $p=0.16$). In hepatitis B cases, the grade of necroinflammatory activity was found to be correlated with both the total mean value ($p=0.004$) and PS expression score ($p=0.007$), but no correlation was found between α-SMA expression and the fibrotic stage in the hepatitis B group ($p=0.54$). In the hepatitis C group, while the highest staining was observed in zone 1 and PS area, the score of zone 3 was slightly higher than zone 2. Since steatosis was present in hepatitis C cases, a possible relationship was investigated, but no correlation was found between steatosis and α-SMA expression. Furthermore, there were no significant differences between the zones (**Table 3**, $p=0.07$). In contrast to hepatitis B, in hepatitis C cases, neither the total mean value nor the zonal expression was related to the histopathological grade ($p=0.71$), but the fibrotic stage of the disease was found to be related to α-SMA expression ($p=0.045$). CD31 expression was observed in sinusoidal lining cells and in portal and septal vascular EC. Forty-nine of 62 (79%) NAFLD specimens were stained positively (**Table 2**). Thirty of the 36 (83.3%) biopsies that had grade 1 activity displayed CD31 staining, whereas 12 of the 17 (70.6%) grade 2 and all 4 (100%) grade 3 cases showed CD31 expression. We found that activity of the disease was related to the expression of CD31 ($p<0.05$). Although CD31 expression was detected in 3 out of 5 pure steatosis cases, there was no significant correlation between them ($p=0.26$). Staining was extensive, and the intensity was strong (**Figure 4**). The rates of positively stained cases were high in all fibrotic stages (**Table 2**). Statistical analysis revealed that CD31 expression was correlated with the stage of the disease ($p<0.05$). Zonal distribution of CD31 expression was also examined, and summarized as rates and scores in **Table 4**. Zone 3 was found to have the highest score (0.67 ± 0.68, **Table 4**) and it was statistically significant for NAFLD ($p=0.01$). CD31 was positively stained in 12 of the 21 (57.1%) hepatitis B cases and 14 of the 19 (73.6%) hepatitis C cases (**Table 2**). The total mean scores of the viral hepatitis groups were lower than the total mean score of the NAFLD group, but there was no significant difference (**Table 4**). Similar to α-SMA expression, histopathological grade was found to be related to CD31 expression in hepatitis B cases ($p=0.05$), while no correlation was detected in hepatitis C cases ($p=0.94$). Conversely, the fibrotic stage of the disease was significantly correlated with CD31 expression in hepatitis C

Table 1 - Degree of steatosis, grades and stages in nonalcoholic fatty liver disease (NAFLD) cases (n=62).

Features	Number of patients (%)
Steatosis (%)	
<33	20 (32.2)
33-66	22 (35.6)
>66	20 (32.2)
Grade	
0	5 (8.1)
1	36 (58.1)
2	17 (27.4)
3	4 (6.4)
Stage	
0	5 (8.1)
1	34 (54.8)
2	12 (19.4)
3	7 (11.3)
4	4 (6.4)

Table 2 - The α-smooth muscle actin (α-SMA) and CD 31 positivity according to fibrotic stages in nonalcoholic fatty liver disease (NAFLD), hepatitis B virus (HBV) and hepatitis C virus (HCV).

Fibrotics stages	Number of cases (%)	
	a-SMA	CD31
NAFLD (n=62)		
Stage 0 (n=5)	1 (20)	3 (60)
Stage 1 (n=34)	12 (35.3)	29 (85.2)
Stage 2 (n=12)	5 (41.6)	9 (75)
Stage 3 (n=7)	3 (42.8)	5 (71.2)
Stage 4 (n=4)	2 (50)	3 (75)
HBV (n=21)		
Stage 1 (n=8)	8 (100)	8 (100)
Stage 2 (n=5)	3 (37.5)	3 (69)
Stage 3 (n=5)	1 (20)	1 (20)
Stage 4 (n=3)	-	-
HCV (n=19)		
Stage 1 (n=9)	6 (66.7)	5 (55.5)
Stage 2 (n=6)	6 (100)	5 (83.3)
Stage 3 (n=2)	2 (100)	2 (100)
Stage 4 (n=2)	2 (100)	2 (100)

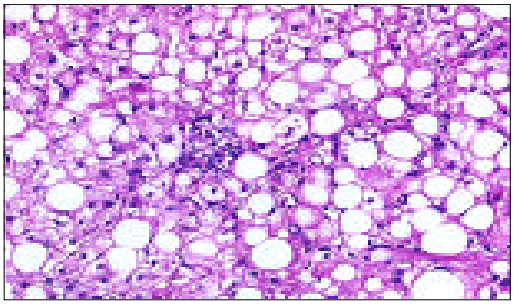

Figure 1 - Nonalcoholic fatty liver disease with lobular neutrophilic inflammation, hematoxylin and eosin x 100.

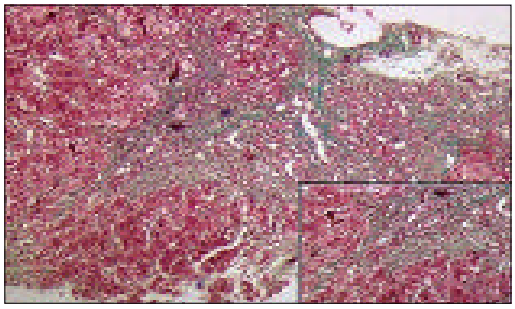

Figure 2 - Pericellular and sinusoidal fibrosis in nonalcoholic fatty liver disease, Masson's trichrome stain, 200 x (inset: portal-periportal fibrosis and septa formations, trichrome stain 400 x).

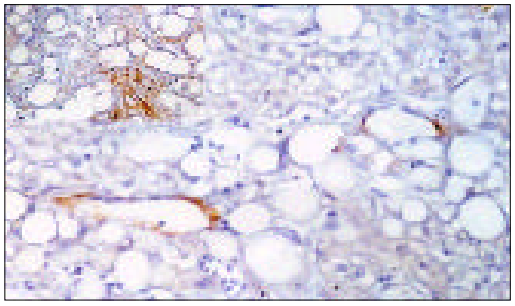

Figure 3 - Scant α-smooth muscle actin (α-SMA) positive staining in nonalcoholic fatty liver disease (NAFLD), (ABP, 3-Amino-9-Ethylcarbazole [AEC], 400 x) (inset: increased α-SMA expression in NAFLD, ABP, AEC, 400 x).

Table 3 - The distribution of zonal scores (mean±SD) and rates of α-smooth muscle actin (α-SMA) expression in a-SMA (+) nonalcoholic fatty liver disease (NAFLD), hepatitis B virus (HBV) and hepatitis C virus (HCV).

a-SMA (+)	Zonal scores and rates (%)				Total mean score
	Zone 1	Zone 2	Zone 3	Portal/septal area	
NAFLD (n=23)	0.15 ± 0.35 (52)	0.008 ± 0.22 (30.4)	0.1 ± 0.2 (69.5)	0.1±0.4 (47.8)	**0.54±0.12**
HBV (n=12)	0.19 ± 0.29 (58.3)	0.19 ± 0.29 (58.3)	0.16 ± 0.24 (58.3)	0.38±0.38 (100)	**0.92±0.19**
HCV (n=16)	0.34 ± 0.37 (62.5)	0.18 ± 0.34 (31.2)	0.26 ± 0.34 (50)	0.47±0.38 (81.2)	**1.26±0.20**

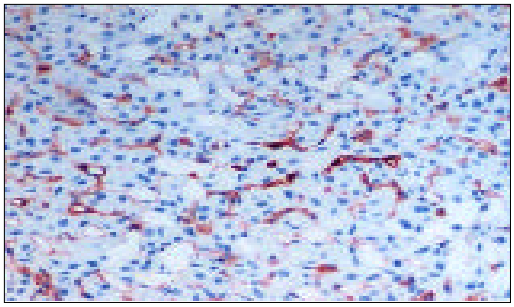

Figure 4 - Prominent capillarization in nonalcoholic fatty liver disease with CD 31, ABP, 3-Amino-9-Ethylcarbazole 200 x.

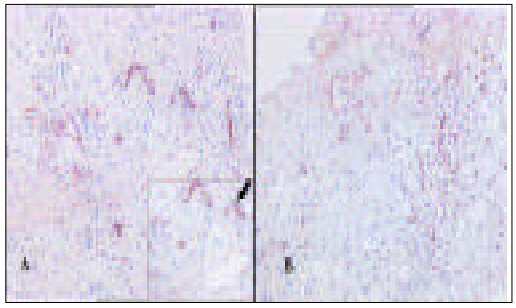

Figure 5 - Double staining of prominent CD31 expression (3-Amino-9-Ethylcarbazole 200 x) and scant a-smooth muscle actin expression (inset, BCIP 400 x, arrow) in nonalcoholic fatty liver disease cases.

Table 4 - The distribution of zonal scores (mean±SD) and rates of CD 31 expression in CD 31(+) nonalcoholic fatty liver disease (NAFLD), hepatitis B virus (HBV) and hepatitis C virus (HCV).

CD31 positive groups	Zonal scores and rates (%)								Total mean score
	Zone 1		Zone 2		Zone 3		Portal/septal area		
NAFLD (n=49)	0.40 ± 0.50	(63.2)	0.30 ± 0.50	(53)	0.67 ± 0.68	(85.7)	0.30 ± 0.60	(36.7)	**1.81 ± 0.24**
HBV (n=12)	0.33 ± 0.45	(91.6	0.38 ± 0.72	(50)	0.59 ± 0.68	(58.3)	0.40 ± 0.37	(100)	**1.70 ± 0.39**
HCV (n=14)	0.34 ± 0.64	(42.8	0.21 ± 0.50	(28.5)	0.23 ± 0.51	(26.3)	0.71 ± 0.60	(92.8)	**1.50 ± 040**

(p=0.011) but not in hepatitis B (p=0.82). Zonal analysis of CD31 expression highlighted that zone 3 and PS area were significant regions for hepatitis B (p=0.001 for zone 3, p=0.05 for PS area), whereas the PS area was significant for hepatitis C (p=0.001). Overall, in the NAFLD group, the rate of CD31 positivity was significantly higher (79%) than the rate of α-SMA positivity (37%) (p<0.005). Double staining of some NAFLD cases revealed the co-expression of α-SMA and CD31 only in the minority of sinusoids, while prominent CD31 expression was detected in the majority (**Figure 5**). Furthermore, the mean scores of CD31 staining were higher than α-SMA mean scores in all study groups (**Tables 3 & 4**) and after statistical analysis, the difference between the total mean scores of α-SMA and CD31 expression was found to be significant for each disease (p=0.000 for NAFLD, p=0.01 for HBV, p=0.0012 for HCV). Upon comparison of each zone's α-SMA and CD31 expression, CD31 seemed to have higher scores in all zones (**Tables 2 & 3**) and except for the PS area, the difference between each zone was significant (p=0.002 for Z1, p=0.000 for Z2, p=0.000 for Z3, and p=0.09 for PS). Statistical analysis revealed that zone 3 was a significant zone for CD31 expression in NAFLD (p=0.01). In contrast, none of the zones were significant for α-SMA expression, in both hepatitis B and C cases, PS area was found to be statistically significant (p=0.05 for hepatitis B, p=0.001 for hepatitis C).

Discussion. We once thought that NAFLD was an innocent disease. Currently we recognize NASH, a type of NAFLD as a chronic progressive form of liver disease, with reports of cirrhotic changes in such cases.[2,3,14] Although there are some well-known clinical parameters for progression, there is no description of the exact histopathological feature of the development of fibrosis. In some patients, fibrosis can exist in the presence of little or no necroinflammation, and yet these patients can progress to cirrhosis. Previous studies suggest steatosis as a cause of oxidative stress in NASH, and with stimulation of fibrogenesis by the mediation of lipid peroxidation, independent of either necrosis or inflammation.[2,4,14,15] In the present study, we did not observe fibrotic activity in pure steatosis cases and we were unable to show a correlation between steatosis and fibrosis. However, in addition to necroinflammation, we found a strong correlation between the degree and presence of ballooning (proof of hepatocyte injury) to the fibrotic stage. Previous reports suggest an independent association between ballooning and both sinusoidal and perivenular fibrosis.[9] Similar to Gramlich's results,[9] our data supports the necessity of the inclusion of ballooning degeneration in the

diagnosis of the progressive form of NAFLD or NASH. Previous reports suggest that hepatocytes, which are undergoing oxidative stress, release radical oxygen synthetase, which stimulates HSC proliferation and transformation into α-SMA expressing myofibroblast-like cells.[6,7] However, the precise mechanisms of fibrogenesis remain unclear,[16] with no exclusion of the possibility of an early contribution to matrix production by sinusoidal EC. Endothelial cells are likely to participate in activation of the fibrotic process, both by production of cellular fibronectin and via conversion of transforming growth factor (TGF) from the latent to the active profibrogenic form.[8,17] To reveal the cellular characteristics of the fibrotic process, in our study we used α-SMA expression for HSCs and CD31 expression for sinusoidal ECs. We observed CD31 expression rather than α-SMA expression in all study groups. Furthermore, although both α-SMA and CD31 expression seemed to increase with the augmenting fibrotic stage of the diseases, we found a significant relation only between CD31 expression and the fibrotic stage, in both NAFLD and hepatitis C. When compared with previous reports, our α-SMA staining rates were lower. This might be due to the immunohistochemical technique, or the clone of antibody we used, or both. On the other hand, CD31 expression was surprisingly high. Unfortunately, there are no studies comparing α-SMA and CD31 expressions, particularly in non-tumoral liver diseases. According to our results, we found CD31 to be a more predictive marker than α-SMA in showing the ongoing cellular changes that might result in fibrosis. The formation of basal lamina and the development of capillarization is nonspecific reactions with different activating mechanisms. Substantial changes in the sinusoidal endothelium always accompany the occurrence of capillarization. Mak et al[10] showed a significant decrease in the number of fenestrate and porosity of ECs in non-cirrhotic alcoholic livers, both in the presence and absence of collagenization of the space of Disse. The existence of a significant blood-hepatocyte barrier may itself cause hepatocellular isolation and deranged nutrition of hepatocytes due to capillarization of sinusoids, which in turn, may cause TGF ß mRNA elevation, the major profibrogenic cytokine in liver.[8,17] Previous observations report capillarization as an early lesion in most alcoholics, independent of parenchymal necrosis, observed even in livers without any signs of damage, except mild steatosis.[7] In the present study, we used CD31, as a marker of both capillarization and the phenotypical conversion of sinusoidal endothelium. It is one of 3 proteins (CD62, CD34, and CD31) that are characteristic of most capillary ECs in the body and always undetectable in non-injured hepatic sinusoids.[18] We detected CD31 expression in most of the cases in all our study groups, with a strong relation to the fibrotic stage, especially in NAFLD. However, CD31 expression did not always correlate with the histopathological evidence of fibrosis. Three of 5 pure steatosis and 19 of the 34 stage 1 case, in which fibrosis was mild, displayed remarkable CD31 positivity. This finding was compatible with Sztark's[19] suggestion that capillarization can occur without established fibrosis.[19] Previous reports suggest mismatch for α-SMA.[7] The authors stressed the dynamic nature of HSC activation that precedes fibrosis and that the number of activated cells can decrease or revert to a quiescent state, or undergo apoptosis. Likewise, the phenotypical conversion of ECs may either follow HSC reversal to a quiescent state or precede their activation. In our study, mismatched cases expressed only CD31 and did not express α-SMA, suggesting the possibility of an early contribution of sinusoidal ECs to fibrosis. Unfortunately, this immunohistochemical study had limitations and was not capable of revealing the chronology of events. The pattern and initiation of fibrosis differs in chronic liver diseases.[5,15,20] Our results relating to the zonal distribution of α-SMA and CD31 expressions in NAFLD suggested that zone 3 may be the crucial zone, not only for fibrogenesis, but for HSC activation and the conversion of sinusoidal ECs as well. Likewise, pure steatosis cases expressed CD31 only in zone 3. We detected the highest score of α-SMA in zone 1 (0.15±0.35), although most of the cases displayed α-SMA positivity in zone 3 (69.5%).

In contrast to NAFLD, the majority of the viral hepatitis cases expressed both markers, predominantly in PS areas with high scores and rates, followed by zone 1 and 3 expressions. Statistical analysis revealed that while CD31 expression in zone 3 was significant for NAFLD and related to the stage of the disease, CD31 expression in PS area was significant for viral hepatitis. Currently there is no standard analysis that can distinguish at-risk progressive fibrosis. We have long considered liver biopsy analyzed with connective tissue stains the gold standard for assessing liver histopathology, disease activity, and fibrosis. However, liver biopsy provides only static data and does not reveal the underlying pathogenetic mechanism. Although the phenotypical conversion of sinusoidal lining cells can be an earlier event, the results of this cross-sectional study were not sufficient to endorse CD31 expression as a preceding marker for fibrosis. Immunohistochemistry has limitations for detection of the expressions, and we did not have follow up biopsies to reveal the progression. However, since we found CD31 expression significantly related to the fibrotic stage of the diseases with different zonal predilections, we can use this as an additional stain

in chronic liver diseases to identify the patients at risk of progression to fibrosis, and it may provide a new insight into the pathogenesis of fibrosis

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