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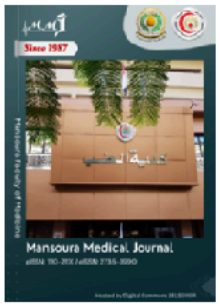

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HISTOPATOLOGICAL FEATURES OF CHRONIC HEPATITIS C VIRUS PROVED BY PCR: COMPARATIVE STUDY WITH CHRONIC HEPATITIS B.

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ABSTRACT

Chronic hepatitis C infectionunlike hepatitis B virus infection has not been shown to have any particular histological markers. This study aims to determine the set of features ,if any, that distinguishes HCV from HBV infections histologically. Fourty liver biopsies of chronic HCV infection proved by PCR and 47 liver biopsies of chronic HBV infection proved serologically were screened for eight histological features. The features observed included bile duct abnormality (bile duct damage and/or ductular proliferation), lymphoid structures (lymphoid follicles and /or aggregates) in the portal tracts, small droplet fatty change, large droplet fatty change, activation of sinusoidal inflammation, liver cell multinucleation, liver cell dysplasia, and periportal Mallory body-like material. The best set of histological features most likely to be associated with HCV rather than HBV by the logistic regression analysis was bile duct abnormality [odds ratio (OR) 2.99 & 95% Confidence Interval (CI) 1.01-8.9], Lymphoid follicles and/or aggregates (OR 3.4 & 95% CI 1.5-10.9), small droplet fatty change (OR 4.9 & 95% CI 1.7-14.3) and Mallory body-like material (OR 6.38 & 95% CI 1.7-24.4) . It seems that these set of histological features are of atmost importance in tha diagnosis of hepatitis C infection.

INTRODUCTION

The recent advances in molecular pathology have led to the detection of hepatitis C virus (HCV) by polymerase chain reaction (PCR)1 . HCV is currently diagnosed by first and sec-

ond generation tests for antibodies (anti -HCV) to structural and non structural proteins of the virus2

Unlike HBV, no histological marker of chronic HCV infection has been shown3 . However, a number of histological features have been cited for their potential diagnostic value4 .

A number of reports are issued describing the histological features in chronic HCV infection which are controversial (5,6,7,8,9,10,4,11) Furthermore, their diagnosis was based on serologic study only. Since it was observed that some serolgically positive cases were negative by PCR and it is now generally accepted that PCR is more definitive for diagnosis of HCV than serological tests alone, we were stimulated to search for the diagnostic significance of these histological features in a patient proved to be PCR positive for HCV.

MATERIAL AND METHODS

This study was performed on biopsies obtained from :1- Forty patients with positive serum for HCV antibodies and confirmed later by PCR . All the patients had elevated ALT and AST for at least 6 months. 2- Forty seven patients negative for HCV and positive for HBV markers. Patients with negative viral markers were excluded from this study. Also we excluded all cases proved serologically positive for HCV antibodies and /or negative by PCR. Also, these positive serologically for both HCV & HBV were excluded.

The liver biopsies were obtained by percutaneous Tru-cut needles after receiving informed consent The specimens were fixed in buffered formalin for at least 24 hours. They were routinely processed and embedded in paraffin wax. Sections were cut at 4 u and stained with haematoxylin and eosin, periodic acid Schiff with and without diastase pre-treatment, Masson trichrome, silver stain for reticulin fibres and orcein stain. Three to five unstained slides of each specimen were also obtained for possible additional stains including immunostains for HBSAg and HBCAg..

The serum samples were collected and screened serologically for HBSAg, HBCAg, HBSAb, HBCAb and HCVAb. The HCVAb positive cases were confirmed by PCR in Department of Immunology, Microbiology, Pathology and Infectious diseases, Division of Clinical Virology, Huddinge

University Hospital, Sweden. The confirmation was done both quantitativly and qualitativly .

The eight histological features used for comparison were defined and graded according to Lefkowitch et al., (1993)[4] as follows:

1. 1-Bile duct abnormalities :- This includes bile duct damage and/or bile ductular proliferation . In bile duct damage (Figure 1) the interlobular bile ducts were surrounded by a lymphocyte-plasma cell infiltrate . These inflammatory cells migrate through the basement membrane into or between the epithelial cells. The damage consists of loss of epithelial cells or epithelial vacuolisation or combination of both 12,,13 . Bile ductular proliferation (Figure 2) was distinguished by increased number of bile ductules in the periphery of the portal tract near the limiting plate. The bile duct abnormality was graded 0, absent; 1, few; 2, moderate; 3, marked.
2. Lymphoid follicles and/or aggregates in portal tracts:- The presence or absence of a lymphoid follicle (Figure 3) consisting of a germinal centre with surrounding small lymphocytes or of a densely packed aggregate of small lymphocytes within a portal tract typically near an interlobular bile duct (Figure 4) was assessed 4
3. Large droplet fatty change:- The presence of macrovesiculaur vacuoles of lipid droplets occupying the majority of the hepatocyte cytoplasm compressing the nucleus to the cell periphery was graded 0, absent; 1, mild; 2, moderate; 3, marked.
4. Small droplet fatty change: The presence of small lipid vacuoles within hepatocytes having nearly central nuclei(Figure 5) was graded 0, absent; 1; mild; 2, moderate; 3, marked.
5. Mallory body-like material: The presence or absence of clumped esinophilic material related to hepatocytes in the periportal area was assessed (Figure 6).
6. Liver cell dysplasia: It was indicated by enlarged hepatocytes with atypical nuclei showing hyperchromatism, multiple nuclei with prominent nucleoli. It was graded 0, absent; 1, mild; 2, moderate; 3, marked.

7-Liver cell multinucleation: The presence of giant hepatocytes containing three or more nuclei indicated

liver cell multinucleation.

8-Activation of sinusoidal inflammatory cells: The prominence of lymphocytes and Kupffer cells within sinusoids (Figure 7) in a 'beads on a string' pattern as in infectious mononucleosis was graded o, absent; 1, mild; 2, moderate; 3, severe 14.

Statistical analysis :

Association between each histologic feature in chronic HBV and HCV status was determined. Data were analysed statistically by backward stepwise logistic regression15 , using a current SPSS statistical package and the level of significance was determined to be less than 0.05 throughout the study. The odds ratio at 95 % confidence level was calculated to estimate the relative likelihood of the HCV infection . The predictive value of paired and single lesions for chronic HCV infection were estimated.

RESULTS

The frequency of each histological feature in biopsy specimens from the HCV and HBV groups were shown in Table 1. It was found that the difference between HCV and HBV groups in large droplet fatty change, liver cell dysplasia and liver cell multinucleation , were statistically insignificant ($P > 0.05$)(Table 1). Mallory body-like material, bile duct abnormality, activation of sinusoidal inflammatory cells, lymphoid aggregates and/or follicles, and small droplet fatty change were found to be statistically significant ($P < 0.05$) (Figure 8).

Backward stepwise logistic regression to the base line data was used to determine the best set of histological lesions associated with HCV. It was observed that the best set of histological lesions that was statistically more likely to be associated with HCV than HBV, consists of the following histological features; lymphoid aggregates and/or lymphoid follicles, small droplet fatty change, Mallory body-like material and bile duct abnormality (Table 2). The small droplet fatty change grade 1 was not significantly different between HCV (22.5%) and HBV (29.8%), while grade 2 and 3 were significantly different (HCV 50% and HBV 8.5%). The activation of sinusoidal inflammatory cells although it was statistically significant ($P < 0.005$), but it was excluded from the equation by the regression analysis.

No single histological feature was

found to be diagnostic of HCV in all specimens examined. However, single variant analysis showed that the Mallory body-like material was seven times more associated with HCV than HBV (Table 3). On doing paired lesion's analysis , we found that, the association of small droplet fatty change with Mallory body-like material was twenty times more associated with HCV than HBV (Table 4).

Table 1. Liver Biopsy Features in HBV and HCV

FEATURE	HCV/40	% VCVH	HBV/47	HBV %
BDA	21	52.5	10	21.3
LF/A	20	50	9	19.1
LDF	24	60	28	59.6
SDF	29	72.5	18	58.3
MBL	16	40	4	8.5
LCD	15	37.5	13	27.7
LCM	9	22.5	6	12.8
ASI	36	90	26	55.3

BDA, bile duct abnormality, LF/A lymphoid follicles and / or lymphoid aggregates; LDF, large droplet fatty change; SDF, small droplet fatty change; MBL, Mallory body-like material; LCD, liver cell dysplasia; LCM, liver cell multinucleation; ASI, activation of sinusoidal inflammation..

Table 2. Backward stepwise logistic regression

Feature	P. Value	OR	95% CI
SDF	0.004	4.9	1.7-14.3
LA/F	0.04	3.4	1.5-10.9
MBL	0.007	6.38	1.7-24.4
BDA	0.05	2.99	1.01-8.9

SDF, small droplet fatty change; LA/F, lymphoid aggregate and/or follicles; MBL, Mallory body like material; BDA bile duct abnormality; OR, odds ratio; P value<0.05; CI, confidence interval.

Table 3. Predictive Value of Single Histological Features for Chronic HCV.

Feature	P. Value	OR	95% CI
MBL	o.001	7.17	2.15-23.9
SDF	0.0018	4.2	3.44-10.5
LA/F	0.0031	4.2	1.6-1o.9
BDA	0.003	4.01	1.6-10.4

SDF, small droplet fatty change; LA/F, lymphoid aggregate and/or follicles; MBL, Mallory body like-material; BDA, bile duct abnormality; OR, odds ratio; P value<0.05; CI, confidence interval.

Table 4.Predictive Value of Paired Histological Features for Chronic HCV.

Feature	P. Value	OR	95% CI
SDF+MBL	0.005	19.7	2.4-18.17
BDA+SDF	0.006	15	3.16-69.9
LA/F+SDF	0.001	13.74	2.8-63.6
BDA+LA/F	0.001	13.4	2.8-63.6
MBL+BDA	0.016	13.3	1.7-85.4
LA/F+MBL	0.024	11.4	1.4-59.9

SDF, small droplet fatty change; LA/F, lymphoid aggregate and/or follicles; MBL, Mallory body-like material; BDA, bile duct abnormality; OR, odds ratio; P value<0.05; CI, confidence interval.

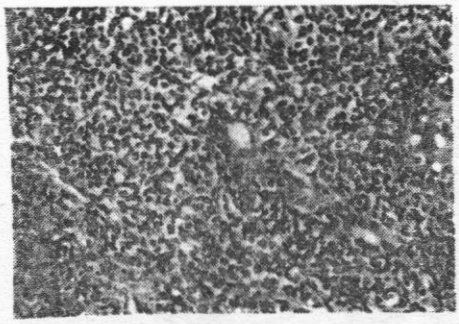

Figure 1. Portal tract with damged bile duct in chronic hepatitis C in the form of vacuoles within the duct epithelium and intraepithelial ductal infiltration by lymphocytes. The background shows dense lymphocytic infiltrate. (H&E: original magnification X 400)

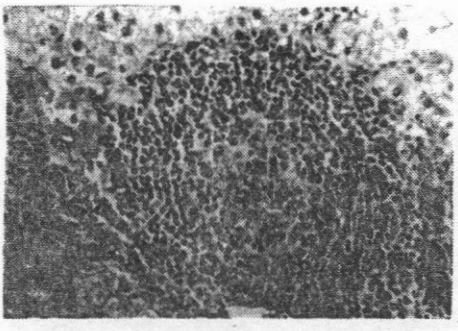

Figure 3. A portal tract in chronic HCV infection showing lymphoid follicle having active germinal center. (H&E original magnification X 400).

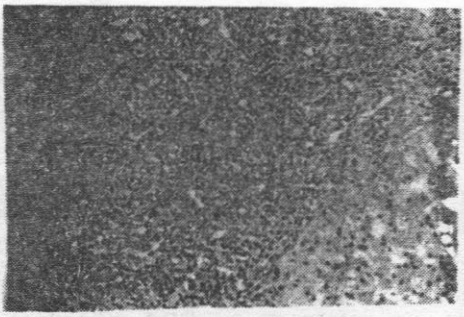

Figure 2. Expanded portal tract in chronic hepatitis C with moderate to marked bile ductular proliferation and mild portal inflammation. (H&E original magnification X 200)

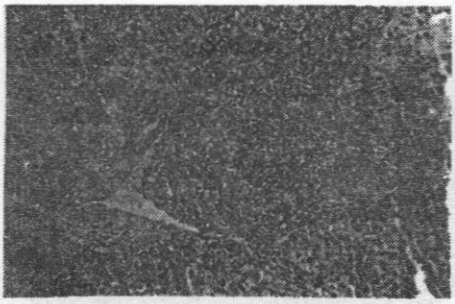

Figure 4. A portal tract in chronic hepatitis C infection showing lymphoid aggregate. (H & E Original magnification X 200).

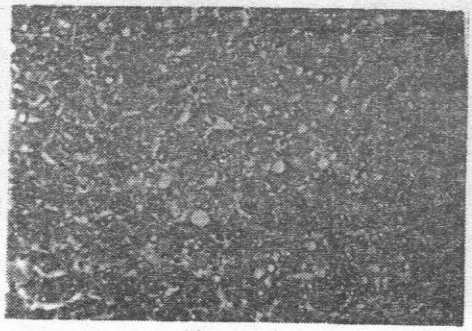

Figure 5. PAS stained section of HCV group showing mild to moderate small droplet fatty change and occasional large droplet fatty change with mild sinusoidal lymphocytosis. (H & E Original magnification X 200)

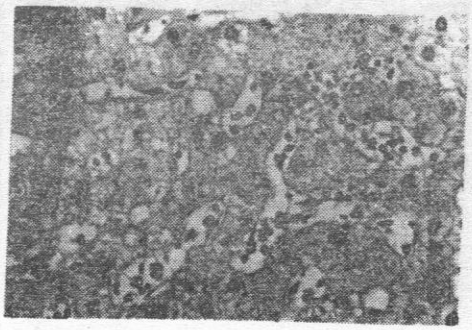

Figure 7. Activation of sinusoidal inflammation in chronic hepatitis C showing moderate sinusoidal lymphocytosis and mild Kupffer cell hyperplasia. (H & E Original magnification X 200).

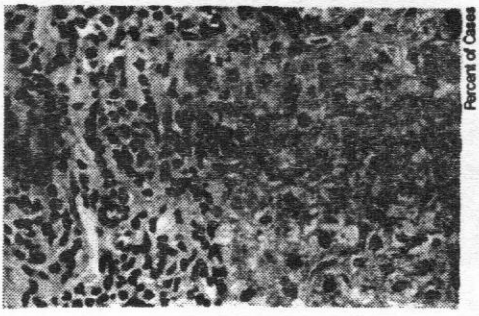

Figure 6. Esinophilic periportal Mallory body-like material with mild to moderate lymphocytic infiltrate in chronic hepatitis C. (H & E Original magnification X 400)

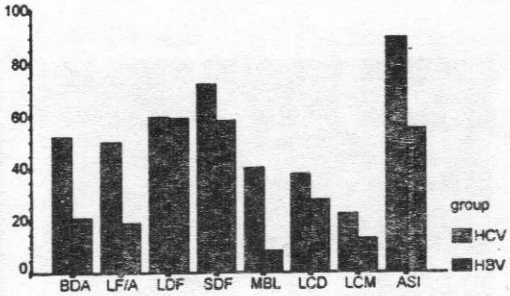

Figure 8. Frequency of 8 histological features in HCv and HBVgroups BDA, bile duct abnormality, LF/A lymphoid follicles and / or lymphoid aggregates; LDF, large droplet fatty change; SDF, small droplet fatty change; MBL, Mallory body-like material; LCD, liver cell dysplasia; LCM, liver cell multinucleation; ASI, activation of sinusoidal inflammation.

DISCUSSION

Up to the moment of writing this paper and to the best of our knowledge , HCV has not been shown in liver tissue by electron microscopy and there is no pathognomonic hepatocellular change for HCV analogous to the ground glass HBSAg inclusions. In this work we tried to evaluate the histological markers of HCV infections proved positive for HCV by PCR.

In this study , we used two groups of patients with chronic HCV and HBV. We observed that the presence of bile duct abnormality (Bile duct damage and/or bile ductular proliferation), lymphoid follicles and/or aggregates in portal tracts, small droplet fatty change and Mallory body-like material within hepatocytes increase the likelihood that HCV rather than HBV is present[16,18,4] in their studies on the morphology of HCV infection in serologically positive cases stressed upon the importance of lymphoid aggregates and/or follicles, bile duct abnormality and fatty change as histological markers for HCV infection. Most of them did not specify the type of fatty change and their work was based on serologically positive cases.

None of these features alone is pathognomonic of HCV as bile duct abnormality occurs in a variety of hepatic diseases including primary biliary cirrhosis (PBC), drug toxicity and liver transplant rejection 12. In PBC and several other conditions, the bile duct lesions are destructive leading to bile duct loss and finally biliary cirrhosis . On the other hand bile duct damage in chronic HCV infection ,although it was very severe, it is usually not associated with bile duct loss. Also in HCV infection, bile ducts were associated with bile ductular proliferation4). We found BDA in 52.5% of HCV patients while it was 31.2 % and 10.9 % in the studies of 4,11 respectively.

Similarly, lymphoid follicles and/or aggregates in portal tracts may be seen in liver tissue from chronic liver diseases ,other than HCV, including PCB (usually at the site of bile duct loss) and autoimmune(lupoid) chronic active hepatitis (CAH)17. The proposed markers of autoimmune CAH from HCV may be difficult, however the presence of abundant plasma cells in the portal -periportal inflammatory infiltrate, clusters of periportal liver cell organised into liver cell rosettes and an architecturally ad-

vanced lesions with cirrhosis may be helpful in the diagnosis. Other proposed markers include severe necrosis and inflammation, piece meal necrosis, hepatocyte multinucleation and broad areas of parenchymal collapse18 .

The well-formed lymphoid follicles and/or aggregates are seen in a variety of chronic inflammatory diseases, often of autoimmune nature as rheumatoid arthritis and Hashimoto's thyroiditis. Their presence in chronic HCV may reflect an ongoing immunologic reaction19 . The differentiation of acute and chronic HCV on pure histological background may be difficult because these lymphoid structures could be found in early stages of acute HCV infection 7 . We found these well formed lymphoid follicles and or aggregates in 50% of HCV cases which was in accordance with the findings of others 11,4 .

Fatty change , particularly small droplet fatty change was described in the work of others 9,20,21, in their studies on delta virus infection. Other study4 reported that large droplet fatty change was more likely to be associated with HCV than small droplet fatty change. In this study, we found that the mild degree of small droplet and large droplet fatty change were found in HBV more than in HCV (HCV 22.5% and HBV 29.8%) (HCV 20% and HBV 25.5%). So the mild forms of small droplet fatty change is not a helpful marker for HCV. As regards the moderate and sever forms of small droplet fatty changes, they were found in association with HCV more than HBV (50% and HBV 8.5%). Fatty change in HCV was both of large and small droplet fatty change. The difference in the type of fatty change may be due to some differences occurring between the Saudi and the European populations 11.

The presence of periportal Mallory body-like material in biopsies for HCV patients was exlained by the alteration of fixation of liver biopsy specimens in Bouin's solution8 , but all the specimens in our study were fixed in buffered formalin.. Periportal Mallory body-like material was found in 17.6% of HCV patients and none of the HBV patient specimens4. This is in marked contrast with the findings of the present study where the similar figures were 40% of HCV patients and 8.5 % in HBV patient specimens.

Regarding the association of

paired lesions, we found that small droplet fatty change when associated with Mallory body -like material was most likely to be associated with HCV about twenty times (odds ratio 19.7) more than to be associated with HBV. The second in order was duct abnormality when associated with small droplet fatty change. Lef Large droplet fatty change when associated with bile duct damage was the first paired lesions that were most likely to be associated with HCV about 8 times than to associated with HBV.(odds ratio 7.79) 4

On doing the predictive value of single lesions associated with HCV, the Mallory body - like material was the first (odds ratio 7.17) then small droplet fatty change and lymphoid aggregates and/or follicles having the same odds ratio(4.2). Other author found that Mallory body like material was observed only in HCV and none in HBV4.

Finally we concluded that although no single histological marker for HCV as in HBV infection, however, a combination of bile duct abnormality, lymphoid aggregates and or follicles in the portal tracts, periportal Mallory body-like material and small droplet fatty change(moderate and marked degrees) are helpful markers for HCV infection. Also the activation of the sinusoidal inflammatory cells if present in association with the set of the histological markers is another helpful histological feature for HCV .

Additional studies on histological marker or markers on liver biopsy specimens with HCV infection based on detection of the virus genome in formalin fixed paraffin embedded tissue blocks will be necessary to determine the reliability of this set of histological markers as a helpful set of markers for both retrospective and prospective studies of the pathology of chronic HCV infection.

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