ORIGINAL ARTICLE

Effect of the number of portal area on modified histological activity index of viral hepatitis and histological findings

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Abstract

Aim: Viral hepatitis is one of the most important causes of chronic hepatitis. Liver biopsy is used to verify clinical diagnosis and to evaluate necroinflammation and fibrosis. Biopsy is the guide for therapy and can be performed also after treatment to assess the effect of therapy on liver. This paper aimed to explore histopathological characteristics of biopsy samples, which had been referred to our department with the clinical diagnosis of chronic viral hepatitis, in reference to Ishak Modified Hepatic Activity Index (IMHAI), as well as to compare inflammatory scores and stages in the groups created according to the number of portal area (PA).

Materials and Methods: The study included 107 patients that underwent liver biopsy in 2011 being diagnosed with chronic viral hepatitis. Age, gender and type of viral hepatitis were retrospectively reviewed and histological findings such as IMHAI inflammatory score and stage, hepatosteatosis and ground glass hepatocytes were re-assessed by two pathologists.

Results: Of the present cases, 97 had chronic hepatitis B, 5 had chronic hepatitis C, and 5 had chronic hepatitis BDThe group with PA number of 2-4 consisted of 8 cases and the group with PA number of 11 and over consisted of 37 cases.

Conclusion: Statistical analysis performed by comparing IMHAI inflammatory score and stage with PA revealed that score and stage were significantly higher in PA ≥ 11 groups as compared to PA 2-4 group.

Key words: Chronic hepatitis, liver biopsy, viral hepatitis

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Introduction

In Turkey, viral hepatitis is the leading cause of chronic hepatitis.[1] Liver biopsy is the gold standard to verify clinical diagnosis, to assess the degree of necroinflammation and fibrosis as well as the course of comorbidities, and to make a contribution to therapy regulation.[2,3] Assessment of fibrosis and necroinflammatory changes such as interface hepatitis, portal area (PA) inflammation, intralobular focal lytic and confluent necrotic lesions is of great importance for histopathology of viral hepatitis as was for the other etiological factors of chronic hepatitis.[3,4]

Different scoring systems have been developed for necroinflammation and fibrosis. In these systems, the degree represents necroinflammation, whereas disease activity substantially determines the capacity of response to therapy. Although fibrosis is defined as stage, it provides information about disease course in the long-term.[2,3] It has been reported in the literature that the number of PA should be at least 11 to assess stage and inflammatory score.[3] A PA should include connective tissue with at least two

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embedded lumen configurations and lumens should have been surrounded with this tissue.[5]

The present study aimed to evaluate the adequacy of the number of PAs in the liver biopsy samples referred to our clinic for chronic viral hepatitis considering 11 PAs as the reference and to highlight histopathological characteristics of these biopsy samples.

Materials and Methods

The study included biopsies of 107 cases diagnosed with chronic viral hepatitis in 2011, which had been referred to the Pathology Department of Haseki Training and Research Hospital. Biopsies had been performed in the radiology department from the right lobe of the liver via percutaneous biopsy method and fixed with 10% formaldehyde. After routine procedures in the pathology laboratory, samples have been embedded into paraffin. Sections of 5 μm in thickness, which have been obtained by microtome, had been stained with histochemical stains including hematoxylin and eosin, reticulin, Masson's trichrome, Periodic Acid-Schiff (PAS), and Prussian blue. All preparations were re-evaluated by two observers in reference to Ishak Modified Histological Activity Index (IMHAI) [Tables 1 and 2].[6] Inflammatory score was determined by the presence of periportal or periseptal interface hepatitis, confluent necrosis and portal inflammation, whereas stage was determined by the presence of fibrosis. Age and gender of patients, type of viral hepatitis, histological ground glass change, macro and micro vesicular steatosis, and small and large cellular changes were reviewed. The cases were divided into four groups according to the number of PAs; 2-4, 5-7, 8-10, and 11 and over.

Statistical analyses were done using SPSS package program. *T*-test was used for the comparison of two groups, whereas "one-way ANOVA and Tukey-b" test were used for the comparison of more than two groups.

Results

Of the 107 cases, 72 were male and 35 were female; 97 had chronic hepatitis B (CHB), 5 had chronic hepatitis BD (CHBD), and 5 had chronic hepatitis C (CHC).

The mean age of the patients was 39.9 ± 1.3 years for CHB, 37.8 ± 9.5 years for CHBD and 50.6 ± 2.1 years for CHC.

Number of cases was 8 in the PA 2-4 group, 23 in the PA 5-7 group, 39 in the PA 8-10 group and 37 in the PA 11 and over group.

The mean number of PA was 8.1 ± 0.9 in 97 CHB cases, 7.6 ± 1.14 in CHC cases and 7.4 ± 1.14 in CHBD cases [Tables 3 and 4].

No large or small cell change was detected.

Table 1: Scoring of necroinflammatory activity

Periportal or periseptal interface hepatitis	
Absent	0
Mild (focal, few portal areas)	1
Mild-moderate (focal, most portal areas)	2
Moderate (continuous around <50% of tracts or septa)	3
Severe (continuous around >50% of tracts or septa)	4
Confluent nekrosis	
Absent	0
Focal	1
Zon 3 necrosis in some areas	2
Zon 3 necrosis in most areas	3
Zon 3 necrosis+occasional portal-central bridging	4
Zon 3 necrosis+multiple portal-central bridging	5
Panaciner or multiacinar necrosis	6
Focal lytic necrosis	
Absent	0
One focus or less per×10 objective	1
Two to four foci per×10 objective	2
Five to ten foci per×10 objective	3
More than ten foci per×10 objective	4
Portal inflamation	
Absent	0
Mild, some or all portal areas	1
Moderate, some or all portal areas	2
Moderate/marked all portal areas	3
Marked, all portal areas	4

Table 2: Ishak staging

Ishak staging	
Absent	0
Fibrous expansion of some portal areas	1
Fibrous expansion of most portal areas	2
Fibrous expansion of most portal areas with occasional Portal to portal bridging	3
Fibrous expansion of most portal areas with maked Portal-portal and portal-central bridging	4
Marked bridging with occasional nodules (incomplete cirrhosis)	5
Cirrhosis	6

Table 3: The group with portal area number and viral hepatitis

Chronic hepatitis category	2-4 group	5-7 group	8-10 group	11 and over group	sum
CHB	6	20	36	35	97
CHBD	1	2	1	1	5
CHC	1	1	2	1	5

CHB=Chronic hepatitis B, CHBD=Chronic hepatitis BD, CHC=Chronic hepatitis C

Interface hepatitis was absent in 17 CHB cases and in one CHBD case. Of the interface hepatitis cases with a score of 8, eight had CHB and two had CHBD.

Confluent necrosis was detected in 11 cases; of which 5 were focal necrosis and 6 were in the form of zone 3 necrosis in some areas.

Focal lytic necrosis was absent in two of CHB and one of CHC cases.

Portal inflammation was not detected in two cases. The score was 4 in five cases, 3 in 20 cases, 2 in 34 cases and 1 in 46 cases.

Steatosis was observed in the form of macrovesicular steatosis. Steatosis was not detected in 44 CHB cases, in one CHC case and in 3 of CHBD cases.

Ground glass appearance was detected in 76 CHB and 4 CHBD cases.

With regard to stage, the score was 5 in two CHB cases, 4 in three CHB and one CHC cases, and 3 in seven CHB and one CHBD cases. Cirrhosis was not observed in any of the cases [Figures 1-4].

Statistical analyses performed to compare IMHAI score and PA revealed that inflammatory score is significantly higher in the PA 11 and over group as compared to the PA 2-4 group. No significant difference was found between PA 5-7, 8-10 and PA 11 groups [Table 5].

Comparing IMHAI stage and PA, it was significantly higher in the PA 11 and over group than the PA 2-4 group, whereas no significant difference was observed between the PA 5-7 and 8-10 groups [Table 6].

Table 4: The mean stage and inflammatuar score of viral hepatitis

IMHAI stage and inflammatory scoring	CHB	CHBD	CHC
Stage	1.19	1.2	1
Inflamatuar score	5.39	9.4	4.6

CHB=Chronic hepatitis B, CHBD=Chronic hepatitis BD, CHC=Chronic hepatitis C

Table 5: Inflammatory score is significantly higher in the PA 11 and over group as compared to the PA 2-4 group

PA	*N*	Mean	*P*
2-4 group	8	2.88	
11 and over group	37	6.3	0

PA=Portal area

Table 6: Stage is significantly higher in the PA 11 and over group than the PA 2-4 group

PA	*N*	Mean	*P*
2-4 group	8	0.62	
11 and over group	37	1.41	0.01

PA=Portal area

Discussion

Clinical chronic hepatitis is the persistence of hepatic inflammation for more than 6 months without

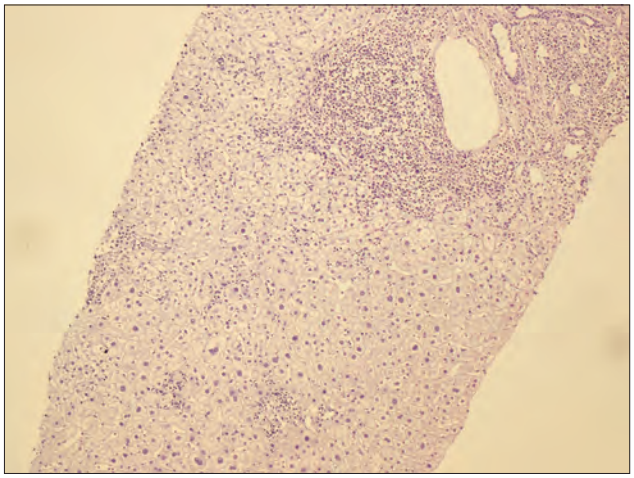

Figure 1: Interface hepatitis- focal lytic necrosis H and E, ×100

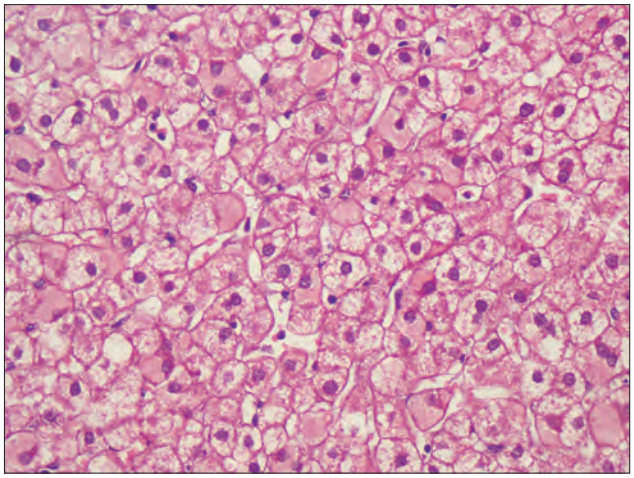

Figure 2: Graund glass apperance H and E, ×100

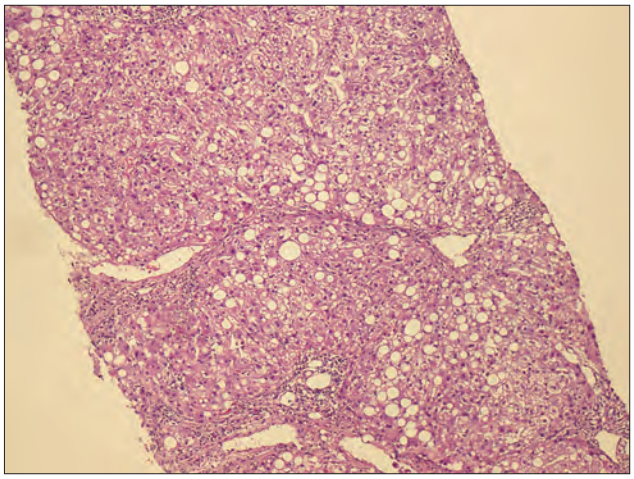

Figure 3: Bridging fibrosis, steatosis H and E, ×100

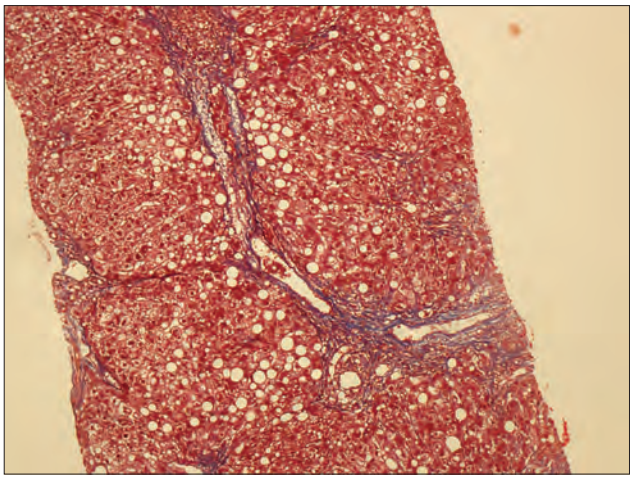

Figure 4: Bridging fibrosis. Masson Trichrome, ×100

improvement.[3,4] Its etiology includes hepatitis B viruses (HBV), hepatitis C viruses (HCV), and hepatitis D viruses, autoimmune hepatitis, α-1-antitripsin deficiency, Wilson's disease and drug-induced hepatotoxicity.[1,3] Drug-induced hepatotoxicity results from the fact that the liver is the main organ for the metabolism of many drugs and chemical substances. However, the rate of drug-induced hepatotoxicity is 10% for acute hepatitis and 1% for chronic hepatitis.[7] HBV and HCV involve approximately 400-500 million people all over the world.[8] While HBV is the most common cause of chronic hepatitis both in Turkey and worldwide, HCV is the second leading cause of chronic hepatitis in Turkey.[1] CHB is seen at a rate of 5-10% in adults. The incidence of becoming chronic is approximately 95% in the neonates that receive HBV infection in the perinatal period from an infected mother. HCV becomes chronic at a rate of 60-80% in adults.[9]

HBV infection leads to acute, fulminant or chronic hepatitis, as well as cirrhosis and hepatocellular carcinoma (HCC). In the long-term studies, incidence of cirrhosis within 5 years after diagnosis was 8-20% in the patients with CHB. HCC is the fifth leading cancer all over the world with higher risk in cirrhosis patients. The incidence of hepatic carcinoma has been decreased along with worldwide immunization.[10] HCV infection is the leading cause of cirrhosis and HCC in the Western countries.[11] Since CHC is usually asymptomatic, it can be diagnosed in the late period. The incidence of cirrhosis due to CHC is about 20% within 20 years from the beginning.[10]

Liver biopsy has begun to be used as the low-risk procedure in 1923 and is indicated for acute or subacute hepatic insufficiency, hepatic disease of unknown origin, post-transplantation cellular rejection, and for the evaluation of inflammatory score and stage of chronic hepatitis. It is the gold standard for the evaluation of hepatic diseases.[5,12] Technically, percutaneous liver biopsy is used at the most. Presence of ascites and coagulopathy make biopsy impossible, and bleeding is the most important complication.[12,13] Biopsies had been performed in the radiology clinic of our hospital through the right hepatic lobe using Toshiba Aplio device with the assistance of 3.5 MHz convex probe. A 16 G semi-automatic needle had been used. Prior to the biopsy procedure, bleeding parameters of the patients had been evaluated and medications that are likely to influence bleeding had been discontinued 3 days before the procedure. The needle is able to take a sample of 22 mm in length. The shrinkage on the biopsy length which occurs due to formalin fixation shall be considered. For patients with viral hepatitis short specimens lead to difficulties in the diagnosis of cirrhosis and reduction at the score of IMHAI.[14] Although there is no current consensus on the number of PA and length of needle for the adequacy of biopsy, it has been reported in the literature that it should include at least 4-6 PA.[2,5] Again, it has been reported that accurate evaluation of inflammatory score and stage requires 11-15 PA and/or 20 mm needle length.[6,12,13]

Histologically, a PA includes portal vein, hepatic artery and interlobular bile duct, which is also defined as portal triad. Sometimes, one of these configurations may be absent and then it is defined as portal dyad.[5] The number of patients having 2-4 PAs in the biopsies was lower than the patients in other groups. This may be associated with statistically significant IMHAI in the other groups. However, considering histological significance of the procedure, it is possible to say that evaluation with the presence of 5 or more PA would be more accurate.

Scoring systems have been developed to determine necroinflammatory activity and fibrosis development in chronic hepatitis. Ishak suggested the modified histological activity index.[15,16] Necroinflammation indicates disease activity, severity and response to therapy and is defined as "grade", whereas fibrosis, which indicates disease prognosis in the long term, is defined as "stage".[2] IMHAI comprises higher number of stages as compared to other scoring systems.[16] In our department, IMHAI scoring system is being used for the evaluation of necroinflammatory activity and fibrosis in chronic hepatitis. As is defined in the literature, descriptive detail in IMHAI is beneficial for pathological evaluation.[6,15] Consultations performed in our department revealed enhanced interpersonal consistency. In this system, confluent necrosis is another category and is important in terms of indicating the severity of disease.[6] While, confluent necrosis involves higher number of hepatocytes (multiple contiguous), spotty lytic necrosis involves less number of hepatocytes and in the form of focal necrosis. Both of them indicate the severity of lobular inflammation.[11,15] Necrosis area including lymphocytes and plasma cells as well as apoptotic corpuscle is conspicuous in focal lytic lesions. This area also includes Kupffer cell hypertrophy and granular debris.[4,7] In severer

cases, confluent necrosis results in bridge necrosis between PAs or between PA and central vein; massive hepatic necrosis may be developed when it becomes widespread.[3,7] Five of the present cases had focal and six had confluent necrosis in some areas in the form of zone 3 necrosis. Two of them had CHBD, one had CHC and remaining had CHB. While focal lytic lesion was absent in two CHB and one CHC cases, score 4 was present in one CHB and one CHBD cases.

Hepatitis leads to clinical and functional changes.[17] Immune cell reaction joins from PA in chronic viral hepatitis. Great majority of inflammatory cells consist of T-lymphocytes but may sometimes be accompanied by plasma cells and one or two eosinophils.[3,17] Inflammation becomes more remarkable in immunoreactive period.[18] Although predominance of plasma cell in PA is characteristic for autoimmune hepatitis, it may be seen also due to CHB, certain drug-induced chronic hepatitis and CHC. Lymphocytic aggregate and lymphoid follicles are typical, but not pathognomonic, for CHC.[4] Portal inflammation score was 4 in five cases, 3 in 20 cases, 2 in 34 cases and 1 in 46 cases; whereas the score was 'zero' in two cases.

Severe hepatocyte injury occurs due to intracellular viral load and as the result, remarkable balloon degeneration (cell swelling), steatosis and cholestasis appear.[3] Cell death occurs via lysis and apoptosis in nucleus and cell membrane. Cytoplasmic density and eosinophilic evidence attract attention during apoptosis. Apoptosis is frequent, but not pathognomonic, in viral hepatitis.[3,4,17] Ground glass appearance occurs in hepatocytes due to accumulation of Hepatitis B surface Antigen (HbsAg) in endoplasmic reticulum.[9,11] Ground glass appearance may also be seen in drug-induced endoplasmic reticulum hypertrophy, cyanamide toxicity and storage diseases.[18] Although steatotic change in hepatocytes is known as the characteristic of CHC, it may be seen in other types of chronic hepatitis and fatty liver disease as well. It has been reported in the literature that steatosis is more common in CHB as compared to the general population.[6,18] It is usually macrovesicular.[4] Hepatitis C-related steatosis is less severe, do not show zonal distribution, but may be a little pericentral as is in fatty liver disease, and may also be seen in the mid-zone or periportal area. The diagnosis should be fatty liver when it is much more widespread and zonal.[6] Steatosis was macrovesicular in the present cases. It was observed in 53 CHB, 2 CHBD and 4 CHC cases; however, zonal distribution could not be determined.

Iron storage is mildly increased in chronic viral hepatitis, particularly in CHC. Again, it is increased also with ribavirin therapy.[6] Chronic hepatitis is rarely encountered in genetic hemochromatosis and almost always occurs due to HCV and HBV infections.[4] Iron storage could not be detected in the present cases during histochemical staining with Prussian blue.

Interface hepatitis (piecemeal necrosis) is the periportal hepatocyte injury caused by very close portal inflammatory cells by passing over limiting plate. It may not be seen in all periportal areas but may occur in a part of, or entire, periportal area.[3,4,15] Bile duct proliferation and fibrosis occur in interface hepatitis. Collagen synthesis in the PA is caused by portal myofibroblasts and hepatic satellite cells. Ductal reaction itself is also profibrogenic.[3,11] Interface hepatitis was not present in 17 CHB and in one CHBD cases. Of the interface hepatitis case with a score of 4, eight were CHB and two were CHBD.

Regenerative changes occur in the hepatocytes along with progression in interface hepatitis and periportal fibrosis. Thickness of cell cords is increased up to the thickness of two or more cells.[3] Nucleus and nucleolus sizes of hepatocytes are morphologically increased and cells including two or three nuclei attract attention.[4,17] Large and small cell changes, previously known as dysplasia, have not been detected in the present cases. While large cell change indicates increased risk for HCC, small cell change is considered to be premalignant. In large cell change, hepatocytes are found in periportal and periseptal areas mixed with other hepatocytes, whereas they are found as groups in small cell change.[6]

As fibrosis progresses, bridging fibrosis occurs between the PAs. In time, central-portal bridging fibrosis may occur in the necrosis including zone 3. Fibrosis is reversible and shows the low-rate progression particularly in CHC patients treated with interferon. Cirrhosis is developed as the consequence of fibrosis.[4] Capsule and subcapsular region should be paid particular attention during evaluation. Capsule shows extensions into the parenchyma and may lead to bridging and nodularity even though the spectrum is normal.[6] Cirrhosis is the leading cause of morbidity and mortality in viral hepatitis.[11] Fibrosis is evaluated by using histochemical staining such as Masson's trichrome and reticulin.[3] Evaluation of staging revealed a score of 5 in two CHB cases, 4 in three CHB and one CHC cases, and 3 in seven CHB and one CHBD cases. Cirrhosis was not found in any of the present cases.

In this paper, a statistical evaluation was performed about the adequacy of liver biopsies based on the number of PAs, and histology of chronic viral hepatitis was highlighted by evaluating existing biopsies in reference to IMHAI. Accordingly, more than 4 portal areas are required in biopsies. As is reported in the literature, a PA number of at least 11 would provide more accurate results to avoid inadequate scoring.[6] Report of liver biopsies should be consistent with the clinic, and scoring system should be mentioned in detail in the report.

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