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## Original research article

# Histopathologic criteria to differentiate autoimmune hepatitis from chronic viral hepatitis (B, C) in liver biopsy



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## ABSTRACT

**Introduction:** Chronic hepatitis is a common disease in the community which is caused mainly by viral agents or immune factors. Differential diagnosis of hepatitis in the treatment is very important due to different therapy.

**Aim:** This study is designed to determine importance of histological findings of autoimmune hepatitis (AIH) to distinguish from other chronic hepatitis.

**Material and methods:** In a cross-sectional study, 112 patients with hepatitis (autoimmune, B and C) were assessed about histopathological criteria on liver biopsy.

**Results:** Among the 12 indicators in histopathologic criteria, 5 indexes of interface hepatitis, lymphoid follicle in port area, emperipolesis, hepatic rosette and ground glass hepatocyte have the ability to differentiate types of hepatitis.

**Discussion:** Clinical and laboratory features of hepatitis B and C can overlap with those of AIH. From therapeutic point of view, distinguishing AIH from viral hepatitis is vital, as their treatment is totally different. Some histopathologic criteria on liver biopsy accompanied with other clinical and paraclinical findings help differentiation of them.

**Conclusions:** Histopathological parameters can differentiate types of hepatitis at an acceptable level which among them, interface hepatitis is used to differentiate between AIH and hepatitis B, lymphoid follicle in port area is used to differentiate hepatitis C and the other two types, emperipolesis to differentiate AIH from the other two types, hepatic rosette to differentiate AIH from the other two types and ground glass hepatocyte to differentiate hepatitis B from the other two types.

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## 1. Introduction

Chronic hepatitis regardless of the causes is defined as a group of inflammatory lesions and necrosis (necro-inflammatory) in liver tissue.<sup>1</sup> The most common cause of this condition is viral hepatitis which hepatitis B and C are at the head of them.<sup>2</sup> Another cause of chronic hepatitis is autoimmune hepatitis (AIH), which is marked with increased immunoglobulins and autoantibodies, and inflammation of the liver and will respond to treatment with immunosuppressive drugs.<sup>3</sup> The main lesions constituent histopathologic features of chronic hepatitis are tissue regeneration including the spotty necrosis (apoptosis and necrosis of individual hepatocytes), confluent lytic necrosis (necrosis of group hepatocytes with the collapse of scaffolding reticulum), portal inflammation (mononuclear inflammatory cell infiltration, mainly lymphocytes and plasma cells and sometimes histiocytic in port areas), interface hepatitis (inflammatory infiltrate extends outside the port area and the adjacent hepatocytes), fibrosis, and cirrhosis.<sup>4</sup>

Depending on the cause of hepatitis, almost special group of lesions have been described, including lesions in chronic hepatitis B that is ground glass hepatocytes (pale hepatocyte with fine granular appearance in part or all of its cytoplasm) and sanded nuclei (hepatocyte nuclei with pale and fine eosinophilic granular appearance in the central part of the core).<sup>5</sup> Hepatitis C is also associated with a number of almost special histopathological findings, including lymphoid aggregates sometimes lymphoid follicles with prominent germinal centers in the port areas (the case can also be chronic hepatitis B or AIH)<sup>6</sup>; lesions of interlobular bile ducts with frequencies ranging from 15% to 91% of biopsies that is characterized by swelling and poly stratification of bile duct lining cells, infiltration by lymphocytes and preservation of the basement membrane of the bile ducts,<sup>7</sup> hepatocytes with oxyphil granular appearance in more than one-third of cases,<sup>8</sup> mild steatosis usually of the macro vesicular type is described in frequencies ranging between 40% and 80%<sup>9</sup>; increased the amount of iron in the liver,<sup>10</sup> epithelioid granulomas in about 5% of biopsies,<sup>11</sup> mild loss of bile ducts in the later stages of chronic hepatitis C<sup>11</sup> and angiogenesis in the port areas, fibrous septa and periportal zones of lobules.<sup>12</sup> AIH is a common disease that affects women 3.6 times more than men.<sup>13</sup> There is no pathognomonic pattern for the diagnosis of AIH and rule out of other causes of liver disease can make accurate diagnosis based on clinical and laboratory findings and histology is suggestive of this condition.<sup>14</sup>

For this purpose, modified diagnostic criteria were developed in 2008 by the international AIH group with the sensitivity of 88% and specificity of 97% for the probable AIH diagnosis and sensitivity of 81% and specificity of 98% for the certain AIH diagnosis. Among histological findings consistent with AIH, dominant infiltration of lymphocytes and plasma cells in the port areas and the occasional presence of eosinophils with and without fibrosis can be pointed. More specific findings on liver biopsy in patients with AIH are emperipolesis, interface hepatitis (infiltration of inflammatory cells into liver cells) and hepatic rosettes (clusters of reactive hepatocytes surrounded by inflammatory cells). The

simultaneous presence of all these three criteria together in liver biopsy is a typical for AIH although these may also be seen in other cases of hepatitis.<sup>15</sup>

Given the importance of distinguishing between AIH and chronic viral hepatitis C, B in liver biopsy samples, study the frequency of histopathologic criteria for these two liver biopsy samples was carried out for distinguishing each and to enhance guideline for better and more accurate differential diagnosis of chronic viral hepatitis, AIH in liver biopsy samples.

## 2. Aim

Considering the high prevalence of hepatitis, AIH study aimed to determine the histopathologic criteria to differentiate it from chronic viral hepatitis in liver biopsy samples.

## 3. Material and methods

This cross-sectional study was conducted in Al-Zahra Hospital pathology laboratory in 2004–2014. The studied population was people who had liver biopsy which was done in Al-Zahra Hospital with a confirmed diagnosis of AIH or chronic viral hepatitis B and C. Inclusion criteria included previous definitive diagnosis of AIH and chronic viral hepatitis in the corresponding slides and second the good quality of hematoxylin and eosin (HE) staining, access to slide sand no other pathologic diagnosis. Poor quality of slides to verify and lack of access to high-quality paraffin embedded blocks for preparing slides were considered as exclusion criteria. The required sample number by using sample size formula for comparing two equally and with respect to 95% confidence level, power of 80%, similar to the prevalence of portal inflammation due to lack of study were considered 0.5 as well as significant differences between the two diseases, were calculated equivalent to at least 0.3 and finally 45 patients in each group were considered.

At first, samples from the liver biopsy pathology archives of Al-Zahra Hospital in 2004–2014 were collected with the definite diagnosis of AIH and chronic viral hepatitis C, B and then based on the ID number of pathology slides from the archives, the slides and paraffin embedded blocks were collected. After collecting samples, slides of both groups (stained by HE) regardless of initial diagnosis and completely random were evaluated in terms of histopathologic criteria based on the presence or absence of relevant parameters and finally concessions related to each criterion was written.

Histopathological criteria included portal inflammation, spotty necrosis, confluent lytic necrosis, interface hepatitis, lymphoid follicle in the port areas; steatosis, emperipolesis, hepatic rosette, ground glass hepatocytes, bile duct injury, epithelioid granuloma and fibrosis were evaluated. After evaluation of all slides in terms of histopathologic criteria; score of each criterion in two groups of AIH and chronic hepatitis B, C, based on early diagnosis of relevant slides were inserted. The data were collected by computer and analyzed with SPSS v. 23, statistical t-test, i.e.  $\chi^2$  and discriminate analysis were carried out.

**Table 1 – Age and sex distribution based on type of hepatitis.**

Variables	Kind of hepatitis			P
	AIH	B	C	
Age, years	33 ± 17.9	41.8 ± 12.5	39.3 ± 13.1	0.03
Sex, N (%)				
Male	15 (33.3)	26 (76.5)	24 (72.7)	<0.001
Female	30 (66.7)	8 (23.5)	9 (27.3)	

#### 4. Results

In this study, 112 patients with hepatitis were assessed, among them 45 cases (40.2%) with AIH, 34 cases (30.4%) hepatitis B and 33 patients (29.4%) hepatitis C. Table 1 shows the distribution of age and sex in each group. According to one-way ANOVA test; there was significant difference between the average ages of the three groups. Also results of analyze with post hoc Tukey test, there was a significant difference between the average age of patients with AIH and patients with hepatitis B ( $P = 0.032$ ) but the mean age of patients with hepatitis C and AIH were not significantly different ( $P = 0.16$ ). The two groups of patients with hepatitis B and C also had no significant difference in age ( $P = 0.79$ ). The gender distribution had also a significant difference between the three groups, so that in AIH, 66.7% were female and 33.3% were male while in patients with hepatitis B, 23.5% were female and 76.5 were male. In the group of hepatitis C, 27.3% were female and 72.7% were male.

In Table 2, the frequency of histopathologic findings in three groups of patients with AIH, hepatitis B and hepatitis C are shown. Based on the results, eight criteria including interface hepatitis, lymphoid follicle in port areas, portal inflammation, emperipolesis, hepatic rosette, ground glass hepatocytes, bile duct injury and confluent lytic necrosis had significant difference between the three groups, as the frequency of Interface hepatitis was considerably lower in hepatitis B than AIH and hepatitis C. Lymphoid follicle in port areas and also the severity of portal inflammation in hepatitis C was significantly higher than the other two groups. The case of emperipolesis in patients with hepatitis B was not observed and the prevalence was low in hepatitis C, however a high prevalence was observed in AIH. The prevalence of hepatic rosette was higher in AIH than the other two groups. In contrast, in patients with hepatitis B frequency of ground glass hepatocytes were higher. In addition, cases of bile duct injury and confluent lytic necrosis were not seen in viral hepatitis but the prevalence in AIH was 13.3%.

Testing and diagnostic analysis (discriminate analysis) on the data obtained showed that among the 12 indicators assessed in patients with hepatitis, 5 indexes i.e. interface hepatitis; lymphoid follicle in port areas, emperipolesis, and hepatic rosette and ground glass hepatocytes has the ability to differentiate between patients with hepatitis. The overall accuracy of the test by five parameters to determine the type of hepatitis was 75.9%. The predictive ability percentage of above parameters for AIH was 80% (36 of 45 patients with AIH), for hepatitis B was 76.5% (26 of 36 patients) and for hepatitis C was 69.7% (23 out of 33 patients). According to this test, 3 cases (6.7%) of AIH as hepatitis B and 6 cases of AIH (13.3%) as

hepatitis C were recorded and sorted. Also of 34 patients with hepatitis B, 2 cases (5.9%) as AIH, and 6 cases (17.6%) as hepatitis C and of 33 patients with hepatitis C, 7 cases (21.2%) as AIH, and 3 cases (9.1%) as hepatitis B have been classified. Results are shown in Tables 2 and 3. Based on the results, interface hepatitis to distinguish AIH from hepatitis B, lymphoid follicle in port areas to differentiate the hepatitis C of two other types, emperipolesis for distinguishing AIH of other two types, hepatic rosette to differentiate AIH of other two types and ground glass hepatocytes to differentiate hepatitis B of other two types are useful.

#### 5. Discussion

Clinical and laboratory features of hepatitis B and C can sometimes be mistaken with those of AIH and makes the diagnosis wrong.<sup>16,17</sup> The differentiation between AIH, viral hepatitis B, C is very important because each have their own specific therapeutic strategies. Viral hepatitis is usually treated with interferon-alpha that can lead to the development of autoimmune reactions. In contrast, AIH treated by immunosuppressive drugs, which can lead to further proliferation of the virus in cases of viral hepatitis.<sup>16-19</sup> On the other hand the differential diagnosis of viral hepatitis and AIH usually done on the basis of lab tests that such tests are usually costly and sometimes due to lack of access to specialized laboratories, diagnosis is delayed, so efforts to discover a solution that can be reasonably identified certain type of hepatitis always been a concern. Considering the high prevalence of hepatitis, AIH study aimed to determine the histopathologic criteria to differentiate it from chronic viral hepatitis in liver biopsy samples.

Based on the results of our study, age and sex of patients with AIH, B and C were distributed widely so that there were a significant difference between mean age of patients with hepatitis B and AIH but there was no significant difference between the mean age of patients with hepatitis C and AIH, and the two groups of patients with hepatitis B and C also had no significant difference in age. The gender distribution was also significantly different between the three groups, so that AIH 66.7% of patients, in hepatitis B 23.5% of patients and in hepatitis C 27.3% of patients were female. Other studies also indicate sex differences of AIH, so that 6.3 times more in women than men.<sup>13</sup> The prevalence of 12 histopathologic criteria in three groups of patients with a confirmed diagnosis of AIH, hepatitis B and hepatitis C were studied and of 12 pathological criteria, 8 parameters in the three groups had significant differences. In terms of diagnostic analysis, 5 parameters namely interface hepatitis, lymphoid follicle in

**Table 2 – Frequency distribution of histopathologic findings in three groups.**

Histopathologic indexes	Kind of hepatitis			P
	AIH	B	C	
Spotty necrosis				
Negative	12 (26.7)	12 (35.3)	9 (27.3)	0.67
Positive	33 (73.3)	22 (64.7)	24 (72.7)	
Interface hepatitis				
Negative	6 (13.3)	25 (73.5)	9 (27.3)	<0.001
Positive	39 (86.7)	9 (26.5)	24 (72.7)	
Lymphoid follicle in port areas				
Negative	35 (77.8)	28 (82.4)	13 (39.4)	<0.001
Positive	10 (22.2)	6 (17.6)	20 (60.6)	
Portal inflammation				
Negative	4 (8.9)	12 (35.3)	5 (15.2)	0.007
Minimal	6 (13.3)	7 (20.6)	2 (6.1)	
Low	24 (53.3)	13 (38.2)	12 (36.4)	
Moderate	9 (20)	2 (5.9)	12 (36.4)	
Sever	2 (4.4)	0 (0)	2 (6.1)	
Emperipolesis				
Negative	32 (71.1)	34 (100)	31 (93.9)	<0.001
Positive	13 (28.9)	0 (0)	2 (6.1)	
Hepatic rosette				
Negative	28 (62.2)	34 (100)	31 (93.9)	<0.001
Positive	17 (37.8)	0 (0)	2 (6.1)	
Ground glass hepatocytes				
Negative	45 (100)	19 (55.9)	32 (97)	<0.001
Positive	0 (0)	15 (44.1)	1 (3)	
Bile duct injury				
Negative	39 (86.7)	34 (100)	33 (100)	0.009
Positive	6 (13.3)	0 (0)	0 (0)	
Epithelioid granuloma				
Negative	42 (93.3)	34 (100)	33 (100)	0.1
Positive	3 (6.7)	0 (0)	0 (0)	
Confluent lytic necrosis				
Negative	39 (86.7)	34 (100)	33 (100)	0.009
Positive	6 (13.3)	0 (0)	0 (0)	
Steatosis				
Negative	44 (97.8)	30 (88.2)	27 (81.8)	0.053
Positive	1 (2.2)	4 (11.8)	6 (18.2)	
Fibrosis score				
0	5 (11.1)	9 (26.5)	7 (21.2)	0.1
1	10 (22.2)	10 (29.4)	7 (21.2)	
2	13 (28.9)	13 (38.2)	11 (33.3)	
3	12 (26.7)	2 (5.9)	7 (21.2)	
4	5 (11.1)	0 (0)	1 (3)	

port areas, emperipolesis, hepatic rosette and ground glass hepatocytes have the ability to differentiate AIH from viral hepatitis in 80% of cases. The diagnostic power for hepatitis B and C were 76.5% and 69.7%, respectively. In this regard, another study by Kumari et al., histopathologic findings of liver biopsy in children with AIH and non- AIH showed that

4 criteria i.e. interface hepatitis, emperipolesis, rosette formation and inflammatory cell infiltration of lymphocytes and plasma cells in the port area have been found together in 56% of AIH cases. Emperipolesis and rosette formation are matched clearly with the diagnosis of AIH, which ultimately recommended that due to their high specificity for the diagnosis of AIH the criteria could be applied for the diagnosis of liver disease of unknown etiology and where no other laboratory findings are available to help for diagnosis of AIH.<sup>20</sup> These findings are matched with the results of our study where interface hepatitis to differentiate AIH from hepatitis B, lymphoid follicle in port areas to differentiate hepatitis C from the other two types, emperipolesis to differentiate AIH from the other two types, hepatic rosette to differentiate AIH from the other two types and ground glass hepatocytes for differentiation of hepatitis B from the other two types are found to be useful for diagnosis. In a study by Hennes et al.,

**Table 3 – Prediction of hepatitis type based on histopathological characteristics, N (%).**

Original	Predicted Group membership		
	AIH	B	C
AIH	36 (80.0)	3 (6.7)	6 (13.3)
B	2 (5.9)	26 (76.5)	6 (17.6)
C	7 (21.2)	3 (9.1)	23 (69.7)

histological protests consistent with AIH were infiltration of lymphocytes and plasma cells in the port areas and the occasional eosinophils with and without fibrosis and more specific findings in liver biopsy of patients with AIH include emperipolesis, interface hepatitis and hepatic rosettes. According to the findings, the simultaneous presence of all three criteria together in liver biopsy is typical for AIH although these may also be seen in other cases of hepatitis. In this study, histopathologic criteria had a sensitivity of 88% and specificity of 97% for the diagnosis of AIH.<sup>15</sup>

## 6. Conclusions

Based on the results, histopathological parameters at an acceptable level can determine which type of hepatitis is in patients that among them interface hepatitis to differentiate between AIH and hepatitis B, lymphoid follicle in port areas to differentiate hepatitis C from the other two types, emperipolesis to distinguish AIH from other two types, hepatic rosette to differentiate AIH from the other two types and ground glass hepatocytes to differentiate hepatitis B from the other two types are most useful and can benefit from these parameters to determine the type of hepatitis.

## Conflict of interest

None declared.

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