Immunopathologia Persa
http www.immunopathol.com
DOI:10.34172/ipp.2022.12

Correlation between viral load, histopathologic necroinflammatory grade and fibrotic stage in viral hepatitis

Hamid Reza Ghasemi Basir[1], Peyman Eini[2*], Mohammad Mahdi Majzoobi[2], Abbas Moradi[3], Somayeh Tayebi[4]

[1]Department of Pathology, Hamadan University of Medical Sciences, Hamadan, Iran
[2]Department of Infectious Diseases, Hamadan University of Medical Sciences, Hamadan, Iran
[3]Department of Community Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
[4]General Practitioner, Hamadan University of Medical Sciences, Hamadan, Iran

***Correspondence to**
Peyman Eini,
Email: eini@umsha.ac.ir

Received 12 Oct. 2020
Accepted 19 Dec. 2020
Published online 13 June 2021

Keywords: Viral load, Necroinflammatory grade, Fibrotic stage, Hepatitis B, Hepatitis C

Abstract

Introduction: Liver needle biopsy and histopathology of liver parenchyma are one of the most accurate methods for assessing the severity of inflammation and fibrosis in these patients, which is based on Knodell's standard system.
Objectives: The aim of this study was to investigate the relationship between viral load of hepatitis B and C in polymerase chain reaction (PCR) with pathologic necroinflammatory grade and fibrotic stage of hepatitis in liver biopsy.
Patients and Methods: Pathologic evaluation performed to determine the grade and stage of viral hepatitis by using 5µ sections stained with hematoxylin-eosin, Masson trichrome and reticulin in unique center. Data were analyzed using SPSS 16 software.
Results: There was a significant relationship between viral load and grade of hepatitis B and C. No association was found between viral load of hepatitis B and C and fibrotic stage of hepatitis. Additionally, relationship between spotty necrosis and portal inflammation with viral load of hepatitis B and relationship between periportal interface hepatitis and viral load of hepatitis C were statistically significant.
Conclusion: Despite the association between viral load in PCR and necroinflammatory grade in the histopathology of liver needle biopsy, the relationship between viral load and fibrotic stage is rejected, which can be affected by the duration of the disease.

Citation: Ghasemi Basir HR, Eini P, Majzoobi MM, Moradi A, Tayebi S. Correlation between viral load, histopathologic necroinflammatory grade and fibrotic stage in viral hepatitis. Immunopathol Persa. 2022;8(1):e12. DOI:10.34172/ipp.2022.12.

Introduction

Chronic hepatitis is referred to a set of liver complications with different causes and severities in which inflammation and liver necrosis last at least six months. The chronic hepatitis is classified based on the causing agent into viral hepatitis- especially the types B and C, autoimmune hepatitis, drug-related hepatitis, and uncertain hepatitis (1).

Hepatitis B virus (HBV) is one of the most common causes of chronic hepatitis. It is estimated that one third of the world's population has serologic reasons in favor of a previous involvement with HBV infection. More than 400 million people worldwide have the disease, which 15%-40% of them finally develop long-term complications including liver failure, cirrhosis, and even hepatocellular carcinoma (HCC), while one million people die of the disease every year. HBV belongs to the Hepadnaviridae family. It is a partially double-stranded DNA virus, which its genome is surrounded by a nucleocapsid or core antigen (HBcAg), which itself is also surrounded by a spherical shape, lipid envelope containing hepatitis B surface antigen (HBsAg). The HBV genome also produces a peptide called e-antigen (HBeAg) released into the bloodstream that is an indicator of active viral replication. To determine the patient's status, the serum level of several antigens and antibodies, including HBsAg, HBc Ab, HBeAg, and HBe Ab, as

Key point

There are several methods for assessing the extent of damage to liver tissue due to viral hepatitis. In this study, several methods of histological examination of the liver following viral hepatitis have been compared with each other. The sensitivity and specificity of each method were discussed and compared.

well as liver enzymes (AST, ALT) and, if necessary, HBV-PCR are required to measure serum viral load.

About 130-170 million people are infected with hepatitis C virus (HCV) worldwide, which develops into chronic disease in 85% of cases and can progress to cirrhosis and sometimes HCC. The prevalence of cirrhosis in HCV infection is over 50%, and 10%-30% of people who have HCV infection more than 30 years, may develop cirrhosis. The HCV is a small, enveloped, single-stranded RNA virus of the Flaviviridae family. HCV has seven major genotypes and genotype 1 is the most common cause of the disease in the United States and Europe. The basis for diagnosis is the detection of HCV Ab in serum, followed by HCV-PCR to measure serum viral load; nevertheless, histopathology of liver is probably the best prognostic indicator of chronic HCV infection (7-9).

In various studies, such as those of Guido et al in Italy and Theise et al in the United States, the importance of liver biopsy has been evaluated to determine the prognosis of viral hepatitis and the relationship between the severity of the disease and the findings of the biopsy. Using a scoring system causes an improvement in the accuracy of biopsy to assess the severity of inflammation and necrosis. Scoring system can be applied to detect the degree of fibrosis and to reconcile the reports and make the results repeatable too (10). Several scoring systems are designed based on the histopathological examination of tissue samples obtained by core needle biopsy of the liver to evaluate necroinflammatory activity and the progression toward cirrhosis that the most popular of which is the Knodell system with a histological activity index (HAI), which classifies chronic hepatitis into mild, moderate, and severe degrees.

In this histological scoring, grade is an indicator of necroinflammatory activity, which includes the sum of the scores of the four following main items ranging from 0 to 18;

1. The degree of periportal inflammatory activity depends on the severity and the extent of necrosis around the portal areas and destruction of limiting plate of hepatocytes adjacent to the portal tract by inflammatory cells called piecemeal necrosis or peri-septal interface hepatitis scored 0 to 4.
2. The extent of confluent necrosis in the centrilobular areas, which sometimes causes connection between the portal tract and the central vein called portal-central bridging necrosis to pan-lobular necrosis scored 0 to 6.
3. The degree of focal degradation and necrosis of hepatocytes in the liver lobule based on the number of foci in the low power field of microscope called spotty necrosis scored 0 to 4.
4. The inflammation degree of the portal is based on the severity of inflammation and the extent of involved portal tracts scored 0 to 4.

In this assessment, stage represents the classification of the disease progression toward cirrhosis based on the severity and the extent of fibrosis in the liver parenchyma by forming various forms of fibrosis as follows; fibrous expansion of portal areas with or without short fibrous septa, portal-portal bridging fibrosis, portal-central bridging fibrosis, and cirrhotic nodule scored 0 to 6. If the intensity of fibrosis is increased to such an extent that can change the normal structure of the liver lobule, it is referred to as cirrhosis (1-6).

Several studies were published regarding the relationship between viral load and histopathological findings in recent years with various results. In a study by Shafaei et al, 72 patients with chronic HBV infection were evaluated from 2006 to 2011 in Babol University, Iran. The patients were divided into three groups of $<10^3$, 10^3-10^5, and $>10^5$ copy/mL based on viral load, and the groups were compared in terms of total score of grading and score of piecemeal necrosis. The results indicated a significant relationship between these two obtained scores and viral load; however there was no significant relationship between viral load and the stage of disease or the degree of fibrosis. In addition, the presence of confluent necrosis was indicative of severe disease (8). In a study by Rodriguez-Inigo et al, in the United States, 27 patients with chronic HCV infection were examined for viral load in peripheral blood by polymerase chain reaction (PCR); the percentage of involved cells was also investigated in a liver biopsy by in situ hybridization. Then their association with pathologic grading was evaluated based on biopsy findings. In their study, a direct correlation was seen between viral load in the blood and the rate of infected hepatocytes, while no relationship was observed between viral load and rate of infected cells with the degree of pathologic grade (11). In the study by Pie et al, in China, the biochemical findings (alanine aminotransferase, ALT), virology data (viral load), and histopathology score (grading and staging) were investigated in 132 patients with HCV infection. In their study, despite the relationship between ALT and necroinflammatory grade, the association between ALT and fibrotic stage was absent. It was also suggested that patients with advanced hepatitis can have normal or near normal ALT level. In addition, no relationship was observed between the viral load and histopathological grade and stage of the disease (12). In the study by Xu et al, in China, 233 patients with chronic HBV infection were evaluated; the study showed, viral load has no correlation with the grade or stage of the disease in a pathologic exam. They also showed that the ALT level can indicate the grade of the disease; however it has no correlation with the stage of the disease (13).

Objectives

According to the presence of standard grading system that was agreed by pathologists to increase repeatability of the results of grading and staging in viral hepatitis and the availability of a quantitative PCR to determine the

viral load before onset of treatment, the current study aimed at determining the relationship between these two findings as well as the relationship between viral load and the details of histopathologic grading and staging in liver biopsies.

Patients and Methods

Study design

The current descriptive-analytical study was conducted at Sina hospital in Hamadan, Northwest of Iran in 2017. A total of 122 new patients who had positive serology including HBS-Ag or HCV-Ab by electrochemiluminescence (ECL) method and were willing to conduct liver biopsy and give blood samples, were enrolled in the study after obtaining written consent. A 5-mL blood sample was taken from each subject to test the biochemical parameters of AST and ALT, and another 5-mL blood sample was also obtained for quantitative PCR in the same laboratory. The AST and ALT tests were performed by Mindray-480 autoanalyzer using Pars-Azmoon kit (Iran). Quantitative-PCR was performed to detect HBV and HCV by Cobas TaqMan-48 analyzer using Roche viral N/A purification real time kit (USA) with 25 IU/ml sensitivity, 95% specificity and detection range of 25-11×10^7 IU/mL.

The liver core needle biopsy was transferred to the laboratory in 10% buffered formalin. After performing fixation and 24-hours tissue processing, the paraffin block was prepared and accordingly, three 5-µm sections were cut for hematoxylin and eosin, Masson trichrome and reticulin staining. Histopathological assessments were performed by an expert pathologist who was blind to the study and revealed the grade and stage of chronic hepatitis based on the Knodell histology activity index (HAI) (Tables 1 and 2). In addition, owing to the wide range of histopathological grading scale in the Knodell system, from 0 to 18, another variable was defined as modified grade with a smaller range as follows; total grade 1 to 4 as the 1st rank, total grade 5 to 8 as the 2nd rank, total grade 9 to 12 as the 3rd rank, and total grade 13 to 18 as the 4th rank.

The census method was employed in the current study to set the sample size and all patients with HBV or HCV infection referring to Sina hospital willing to participate in the study were selected within a five-year interval from 2012 to 2017 after obtaining written consent.

Data analysis

The data were then transferred into SPSS software (version 16) for statistical analysis. To express the quantitative data, the mean and standard deviation (±SD) were used,

Table 1. Details of the Knodell's modified histological activity index-grading

Knodell's modified histological activity index-grading (necroinflammatory score)	Score
A- Periportal or periseptal interface hepatitis (piecemeal necrosis)	
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
B- Confluent necrosis	
Absent	0
Focal confluent necrosis	1
Zone 3 necrosis in some areas	2
Zone 3 necrosis in most areas	3
Zone 3 necrosis occasional portal-central (P-C) bridging	4
Zone 3 necrosis multiple P-C bridging	5
Panacinar or multiacinar necrosis	6
C-Focal (spotty) lytic necrosis, apoptosis and focal inflammation	
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
D-Portal inflammation	
None	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
Total	
Maximum possible score for grading	18

Table 2. Details of the Knodell's Modified Histological Activity Index-staging

Modified Histological Activity Index-staging (Architectural Changes, Fibrosis, and Cirrhosis Score)	Score
No fibrosis	0
Fibrous expansion of some portal areas, with or without short fibrous septa	1
Fibrous expansion of most portal areas, with or without short fibrous septa	2
Fibrous expansion of most portal areas with occasional portal to portal (P-P) bridging	3
Fibrous expansion of portal areas with marked bridging (P-P) as well as portal-central (P-C)	4
Marked bridging (P-P and/or P-C) with occasional nodules (incomplete cirrhosis)	5
Cirrhosis, probable or definite	6
Total	
Maximum possible score	6

and in terms of the qualitative data the percentage and ratio were applied. Student *t* test was used to evaluate normal distribution of quantitative variables, and in case of non-normal distribution, the Mann-Whitney U test was employed. To test the qualitative variables, the χ^2 test was used. A *P* value of <0.05 was considered as the level of significance. Independent *t* test was used to determine the relationship between viral load, and grade and stage of hepatitis.

Tukey post hoc test was also used. To determine the correlation between viral load and other quantitative variables, Spearman's correlation coefficient was used.

Results

Of 122 patients, 71 had HCV and 51 HBV infections; 85.2% of the subjects were male. The mean age of patients in the HCV and HBV groups was 40.59 ± 12.54 and 35.16 ± 11.15 years, respectively. The mean viral load in the HBV and HCV groups was 13 076 151.66 ± 57 587 802.53 and 27 793 360.14 ± 87 925 501.63 IU/mL, respectively.

Based on the results of one-way ANOVA, the relationship between viral load and different necroinflammatory grades was evaluated in the HBV and HCV groups as well as the total population and the results indicated a significant relationship between, the severity of grades – based on histopathological findings – and viral load – based on PCR results – in the HBV group ($P=0.004$). In addition, using the Tukey post hoc test, a significant difference was observed in the HBV group in terms of viral load between grade 4 and the rest of grades, except grades 8 and 9 ($P=0.007$). However, the same relationship was not statistically significant in the HCV group (Table 3).

Based on the results of the one-way ANOVA, no significant relationship between viral load and different fibrotic stages in the HBV and HCV groups as well as the total population was seen (Table 4).

The relationship between viral load and different scores of spotty necrosis in the HBV and HCV groups was evaluated using one-way ANOVA and the results indicated a statistically significant correlation between the severity of spotty necrosis and viral load in the HBV group ($P=0.013$). In addition, a significant difference was observed in viral load between spotty necrosis grades 0 and 1 in the HBV group using Tukey post hoc test ($P=0.009$). In the HCV group, the same relationship was not statistically significant (Table 5).

In the HCV group, the Spearman's Rho test showed a statistically significant correlation between viral load and the severity of periportal interface hepatitis, in one direction ($P=0.028$). Additionally, the relationship between viral load and the modified grade was consistently significant ($P=0.020$). In addition, the relationship between grade and viral load was near the significant level.

In the HBV group, there was a consistently significant correlation between viral load with the severity of portal inflammation and serum ALT levels, according to the Spearman's Rho test ($P=0.004$ and 0.034 respectively).

In the total study population, a statistically significant correlation was observed between the severity of portal inflammation-based on histopathological findings, and viral load-based on PCR results, in the same direction ($P=0.002$). Furthermore, this relationship was nearly significant in terms of periportal interface hepatitis. However, the same correlation was statistically insignificant in terms of the other variables (Table 6).

Discussion

The current study aimed at determining the association between serum viral load of HBV and HCV infections and the grade and stage of hepatitis in histopathologic evaluation of the liver biopsy that was performed on 122 patients.

The results of our study showed a significant relationship between viral load and grade of hepatitis in the HBV group. There was also a statistically significant correlation between viral load and modified grade in the HCV group; in other words, by increasing the viral load, the necroinflammatory grade also increased.

In the study by Shafaei et al, (8) the relationship between three different levels of viral load and total necroinflammatory grade was statistically significant in patients with HBV infection, which was consistent with the results of the current study; however in the studies by Xu et al (13) and Martinot-Peignoux et al (14) on patients with HBV infection, the association between viral load and grade of the disease was statistically insignificant,

Table 3. Correlation of histological activity index-grading and serum viral loud in chronic viral hepatitis

Type of hepatitis	Histological activity index grading	Number	Mean of viral loud	Standard deviation	P value
HCV	2	5	2577719.80	5624634.315	0.404
	3	2	883197.50	997953.235	
	4	16	5084534.06	13685102.771	
	5	12	59275073.25	133326333.263	
	6	13	1986331.23	1997678.588	
	7	9	1410821.00	2291088.950	
	8	7	11126677.86	19008382.842	
	9	3	414456.33	403571.038	
	10	3	231513.67	34686.194	
	13	1	2754000.00	.	
	Total	71	13076151.66	57587802.539	
HBV	2	5	206.40	8.764	**0.004**
	3	8	12614433.00	10396470.754	
	4	7	161309828.57	196456372.223	
	5	7	218445.14	1491.371	
	6	8	22698.12	29658.471	
	7	4	143778.50	59838.314	
	8	3	233452.00	404042.420	
	9	2	71346543.87	17340.491	
	10	7	6341414.29	4320943.958	
	Total	51	27793360.14	87925501.631	
Total; HCV & HBV	2	10	1288963.10	3988246.385	0.360
	3	10	10268185.90	10423249.428	
	4	23	52631362.83	126711581.037	
	5	19	37517368.16	108257476.761	
	6	21	1238280.52	1830170.169	
	7	13	1020961.77	1967425.654	
	8	10	7858710.10	16389119.650	
	9	5	28248673.80	38114640.245	
	10	10	4508444.10	4599765.768	
	13	1	2754000.00	.	
	Total	122	19228427.34	71876741.886	

Table 4. Correlation of histological activity index-staging and serum viral loud in chronic viral hepatitis

Type of hepatitis	Histological activity index staging	Number	Mean of viral loud	Standard deviation	P value
HCV	Zero	20	11982862.50	41675655.380	0.945
	One	28	21545889.25	84586441.136	
	Two	12	5348129.83	12696769.890	
	Three	2	341186.50	481032.722	
	Four	3	457207.33	356263.290	
	Five	6	3205511.00	3271280.057	
	Total	71	13076151.66	57587802.539	
HBV	Zero	16	7261904.94	9104785.358	0.459
	One	19	58702995.16	139081214.450	
	Two	2	195600.00	281321.381	
	Three	2	71115897.80	29113.217	
	Four	4	4543881.00	4997103.989	
	Five	8	3418407.00	4521646.514	
	Total	51	27793360.14	87925501.631	
Total; HCV & HBV	Zero	36	9884659.14	31369645.065	0.400
	One	47	36566846.96	110038895.035	
	Two	14	4612054.14	11828256.408	
	Three	4	35170593.25	40218493.635	
	Four	7	2792449.43	4159268.947	
	Five	14	3327165.86	3890607.777	
	Total	122	19228427.34	71876741.886	

Table 5. Correlation of spotty necrosis score and serum viral loud in chronic viral hepatitis

Type of hepatitis	Spotty necrosis score	Number	Mean of viral loud	Standard deviation	*P* value
HCV	Zero	8	1915894.50	3288191.270	0.667
	One	29	25843041.38	87994591.784	
	Two	31	5172563.77	15458843.242	
	Three	2	263967.50	19753.028	
	Four	1	2754000.00		
	Total	71	13076151.66	57587802.539	
HBV	Zero	10	10073747.20	10618444.344	0.013
	One	11	103309828.63	172554166.804	
	Two	28	6688609.79	18198277.143	
	Three	2	178542.10	38267.572	
	Total	51	27793360.14	87925501.631	
Total: HCV & HBV	Zero	18	6448034.89	9030106.263	0.061
	One	40	46972266.62	120003666.595	
	Two	59	5892043.24	16684121.902	
	Three	4	132072.75	152725.333	
	Four	1	2754000.00		
	Total	122	19228427.34	71876741.886	

which may be due to the high percentage of mild grade and lower viral load in their populations. In addition, in the study by Rodriguez-Inigo et al (11) and Pie et al (12), the same relationship was not statistically significant in patients with HCV infection. Moreover, in our study the association between viral load and total grade was statistically insignificant in the HCV group.

In the current study, no relationship between the viral load and the fibrotic stage of disease both in HBV and HCV groups was seen. It should be noted that in the studies by Shafaei et al (8), Xu et al (13), Martinot-Peignoux et al (14), and Chi-Jen Chu et al (15) on patients with HBV as well as the study by Pie et al (12) on patients with HCV infection, the same relationship was statistically insignificant, which was in agreement with the results of the current study. Perhaps, the stage of liver fibrosis is more affected by the duration of the disease than the level of viral load; however further comprehensive studies are required to obtain more conclusive results.

In the current study, in relationship between the components of grade – based on histopathological findings – and viral load – based on PCR results – the association of spotty necrosis and portal inflammation with HBV viral load, and the relationship between periportal interface hepatitis and HCV viral load were statistically significant. In addition, a significant association between viral load and the degree of portal inflammation in all patients with viral hepatitis was seen, which indicates the increase in portal inflammation following the increase in viral load. However, the association with confluent necrosis was insignificant, which can be attributed to the very small number of subjects with confluent necrosis in the current study. In the study by Shafaei et al (8), a significant relationship was observed between HBV viral load and periportal interface hepatitis, but this relationship was insignificant in terms of other components of grade. The results of his study, despite the similarity in the relationship between total grade and HBV viral load, were inconsistent with the current study findings regarding the grade components in the HBV group. In addition, in the study by Shafaei et al, the presence of confluent necrosis indicated a more severe disease, which was inconsistent with the results of the current study due to the low number of patients with confluent necrosis. Moreover, in the study by Rodriguez et al (11), a direct relationship was observed between the viral load of HCV and the percentage of virus-infected hepatocytes, however no association was observed with the grade in histopathology. In the recent studies, the relationship between grade components and viral load was less considered, especially in HCV infection, since further extensive studies are required in this regard.

In the current study, the relationship between viral load and ALT in the HBV group was significant, but the same relationship was not observed in the HCV group. In the studies by Martinot-Peignoux et al (14) and Chi-Jen Chu et al (15), this relationship was not significant, which was inconsistent with our findings. This difference can be attributed to the higher number of patients with HBV infection in the two former studies compared with that of the current study. In addition, in the study by Martinot-Peignoux et al, most of the subjects had a viral load of $<10^5$ and a mild histopathological grade that might affect the statistical findings. Nevertheless, in the study by Pie et al (12) on patients with HCV as well as the study by Xu et al (13) on subjects with HBV infection, the relationship between ALT and grade of viral hepatitis was statistically significant. In other words, ALT increases with more activation of liver inflammation, but its association with fibrotic stage was rejected in both studies and their results showed that in patients with advanced hepatitis and a high stage, ALT can even be normal or close to normal.

Table 6. The relationship between viral load and histopathology findings related to grading and staging, as well as ALT and AST in patients with viral hepatitis

Type of hepatitis	Correlations with:	Spearman's Rho	P value
HCV	Stage	+0.208	0.082
	Grade	+0.218	0.068
	Portal inflammation	+0.164	0.173
	Periportal interface hepatitis	+0.261	**0.028**
	Confluent necrosis	-0.044	0.713
	Spotty necrosis	-0.016	0.898
	ALT	+0.221	0.064
	AST	+0.154	0.200
	Modified grade	+0.276	**0.020**
HBV	Stage	+0.011	0.938
	Grade	+0.084	0.557
	Portal inflammation	+0.401	**0.004**
	Periportal interface hepatitis	+0.030	0.834
	Confluent necrosis	-0.089	0.533
	Spotty necrosis	-0.150	0.294
	ALT	+0.298	**0.034**
	AST	-0.069	0.628
	Modified grade	+0.022	0.878
Total: HCV & HBV	Stage	+0.116	0.202
	Grade	+0.137	0.133
	Portal inflammation	+0.277	**0.002**
	Periportal interface hepatitis	+0.165	0.069
	Confluent necrosis	-0.077	0.402
	Spotty necrosis	-0.079	0.387
	ALT	-0.009	0.917
	AST	+0.068	0.459
	Modified grade	+0.144	0.114

Conclusion

Despite the association between the viral load – based on PCR results – and necroinflammatory grade in the histopathologic evaluation of liver core needle biopsy, shown in various studies, the association between viral load and fibrotic stage was rejected in all studies, which can indicate more influence of other factors such as the duration of the disease, compared with viral load, in the development of liver fibrosis, which is practically unattainable since the onset of the disease is unclear in most cases.

The controversy in the relationship between necroinflammatory grade components and the viral load that was observed in the various studies, indicate the existence of other influential factors such as immune-system parameters of the infected individuals in the degree of necroinflammatory changes of liver, which apart from viral load, can influence the severity of necrosis and inflammation of the liver parenchyma and contribute as a confounding factor to the relationship between viral load and the grade components.

Overall, due to the significant relationship between viral load and histopathologic grade of hepatitis, the quantitative-PCR can be used to estimate the necroinflammatory grade; however to estimate the fibrotic stage, viral load is not reliable and other non-invasive methods such as liver FibroScan can be employed. Besides, for estimation of the necroinflammatory grade based on viral load, larger studies with adequate sample size for each necroinflammatory grade are needed to calculate cut-off value with ROC curve analysis.

Limitations of the study

Our study requires further investigation by larger samples in multi-centric studies.

Acknowledgments

The authors would like to thank all personnel of the medical records office in Sina hospital.

Authors' contribution

Study concept and design; HRGB and PE. Analysis and interpretation of data; AM. Literature review; MMM and ST. Drafting of the manuscript; HRGB. Critical revision of the manuscript for important intellectual content; PE. Review of the final version of the manuscript before submission: MMM.

Conflicts of interest

The authors declare that there is no conflict of interest in this study.

Ethical issues

The study was conducted based on the Helsinki Declaration, and it was approved by the Ethics Committee of the Deputy of Research and Technology, Hamadan University of Medical Sciences, Hamadan, Iran (No: IR.UMSHA.REC.1396.608). Written consent forms were obtained from all patients. Moreover, ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

Funding/Support

This study was supported by a grant from the Hamadan University of Medical Sciences (Grant# 9609145658).

References

1. Cobb BR, Valsamakis A. Chronic Hepatitis B, C, and D. Microbiol Spectr. 2016;4(4). doi: 10.1128/microbiolspec.DMIH2-0025-2015.
2. Kawano Y, Miyanishi K, Takahashi S, Kubo T, Ishikawa K, Sugita S, et al. Hepatitis C virus reactivation due to antiemetic steroid therapy during treatment of hepatocellular carcinoma. J Infect Chemother. 2017;23:323-5. doi: 10.1016/j.jiac.2016.12.008
3. Li L, Lan X. Association between hepatitis B virus/hepatitis C virus infection and primary hepatocellular carcinoma risk: A meta-analysis based on Chinese population. J Cancer Res Ther. 2016;12:C284-C7. doi: 10.4103/0973-1482.200763.
4. Mahale P, Torres HA, Kramer JR, Hwang LY, Li R, Brown EL, et al. Hepatitis C virus infection and the risk of cancer among elderly US adults: A registry-based case-control study. Cancer. 2017;123:1202-11. doi: 10.1002/cncr.3055.
5. Rosai J. Non-neoplastic disease. In: Desmet VJ, Rosai J, editors. Rosai and Ackermans surgical pathology. 10th ed. Edinburgh: Elsevier; 2011. p. 867.
6. Jameson JL, Kasper DL, Longo DL, Fauci AS, Hauser SL, Loscalzo J. editors. Harrison's Principles of Internal Medicine. 20th ed. New York: McGraw-Hill; 2018. p. 2375-96.
7. Jarmay K, Karacsony G, Ozsvar Z, Nagy I, Schaff Z, Lonovics J. [Histological characteristics of chronic hepatitis C in biopsy

material]. Orv Hetil. 1998;139:1955-60.
8. Shafaei S, Soleimani Amiri S, Hajiahmadi M, Sadeghi-Haddad-Zavareh M, Bayani M. Histological grading and staging of liver and its relation to viral loads in chronic anti-HBe positive hepatitis. Caspian J Intern Med. 2013;4:681-5.
9. Kim HJ, Oh EJ, Yoon JH, Kim YJ, Lee HS, Hwang ES, et al. Analysis of hepatitis B virus quasispecies distribution in a Korean chronic patient based on the full genome sequences. J Med Virol. 2007;79:212-9. doi: 10.1002/jmv.20789.
10. Guido M, Mangia A, Faa G. Chronic viral hepatitis: the histology report. Dig Liver Dis. 2011;43 Suppl 4:S331-43. doi: 10.1016/S1590-8658(11)60589-6.
11. Rodriguez-Inigo E, Bartolome J, de Lucas S, Manzarbeitia F, Pardo M, Arocena C, et al. Histological damage in chronic hepatitis C is not related to the extent of infection in the liver. Am J Pathol. 1999;154:1877-81. doi: 10.1016/S0002-9440(10)65445-4.
12. Pei L, Ying Li, Cui-ming Sun. Correlations of serum hepatitis C virus RNA and alanine transaminase with liver histopathological changes in patients with chronic hepatitis C. Lab Medicine. 2009;40:167-9. doi:10.1309/LM5XQBWG0QMLNNPC.
13. Xu QH, Shu X, Chen LB, Huang HH, Zhang K, Li G. [Relationships between liver function test, serum HBeAg or HBV DNA level and liver pathological changes in patients with chronic hepatitis B]. Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi. 2008;22:422-4.
14. Martinot-Peignoux M, Boyer N, Colombat M, Akremi R, Pham BN, Ollivier S, et al. Serum hepatitis B virus DNA levels and liver histology in inactive HBsAg carriers. J Hepatol, 2002;36:543-6. doi: 10.1016/s0168-8278(02)00004-1.
15. Chu C, J, Hussain M, Lok AS. Quantitative serum HBV DNA levels during different stages of chronic hepatitis B infection. Hepatology. 2002;36:1408-15. doi:10.1053/jhep.2002.36949.