

HISTOPATHOLOGICAL GRADING AND STAGING IN LIVER BIOPSIES OF HEPATITIS-C PATIENTS AND THEIR ASSOCIATION WITH ALT LEVELS

Shuja Izhar Syed¹, Saleem Sadiq²

ABSTRACT

Objective: To perform the histological evaluation of liver biopsies and see its association with the serological levels of ALT.

Methodology: This was a prospective study based on liver biopsies of fifty hepatitis C patients, whose serum ALT levels were also estimated. H and E stained slides were examined to determine the histological activity and fibrosis score.

Results: Grade 1 activity was found in 44% of patients; whereas 42% had an activity of grade 3. About 44% cases having grade 1 activity had a mean ALT of 52.3 U/L while 42% cases having severe activity had a mean ALT of 76.14 U/L. The present study revealed a significant relationship of ALT with fibrosis ($p < 0.005$). A steady and proportional rise in mean age with increasing score of fibrosis stage was also evident.

Conclusion: Higher ALT levels are associated with more pronounced forms of necroinflammatory activity; however normal ALT level does not rule out severity of the disease suggesting that all such patients require liver biopsy to unearth clinically subtle but advanced liver disease.

KEY WORDS: Liver Biopsy, Hepatitis C, Necroinflammatory Activity, Grade, Stage, ALT.

Pak J Med Sci July - September 2010 Vol. 26 No. 3 644-648

How to cite this article:

Syed SI, Sadiq S. Histopathological grading and staging in liver biopsies of Hepatitis-C patients and their association with ALT levels. Pak J Med Sci 2010;26(3):644-648

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- * Received for Publication: August 19th, 2009
- * Revision Received: April 22nd, 2010
- * Revision Accepted: April 25th, 2010

INTRODUCTION

Patients with chronic hepatitis C typically undergo liver biopsy to determine the severity of disease and thereby assess the urgency of treatment. Liver histology provides direct evidence of hepatic necroinflammatory activity, fibrosis, and progression to cirrhosis, and is a surrogate endpoint of the long term efficacy of interferon treatment.¹

Before the discovery of HCV, elevation of ALT levels served as the cornerstone for the definition of non-A, non-B hepatitis. HCV possibly influences ALT levels.²

Most patients with hepatitis C will have elevations of the ALT, but these do not indicate how severe the disease is.³ Furthermore, a normal ALT does not guarantee that the liver

disease is inactive.⁴ The liver biopsy is the gold standard for the grading and staging of chronic hepatitis C.⁵

Grading: Necroinflammation is not only a measure of severity but also of ongoing disease activity and the parameter most potentially responsive to therapy. This is referred to as 'grade'.⁶ Grading the degree of disease activity is evaluated by considering the main components in the histopathologic picture.⁷

Staging: The lesions of fibrosis and parenchymal or vascular remodeling are referred to as 'stage' and indicate long-term disease progression.⁶ Its histologic evaluation is based on the extent of fibrosis and the development of cirrhosis.⁷ Several systems for semiquantitative scoring for grading and staging have been proposed for liver lesions in chronic hepatitis.⁶ In METAVIR scoring system, the score is composed of a two letter and two number coding system. It has the advantage of simplicity, reproducibility, and application to a large number of biopsies.⁸

Not many studies regarding the histopathological features of chronic hepatitis C patients in Pakistan are available. It was therefore decided to carry out this study on histological evaluation of liver biopsies of chronic hepatitis C patients and to find association of histological findings with the serological levels of ALT.

METHODOLOGY

This was a prospective study based on liver biopsies of hepatitis C patients received at the Department of Pathology at Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Center, Karachi during the period from September 2007 to July 2008 and was carried out in collaboration with the Department of Medicine Ward 07 and the Department of Radiology, Jinnah Postgraduate Medical Center. Fifty (50) patients who were serologically positive for hepatitis C were included in the study. These patients underwent estimation of pretreatment serum ALT levels and then after consent ultrasound guided liver biopsies at the department of Radiology.

These biopsies were received at the Department of Pathology, BMSI for histopathological evaluation. The formalin fixed specimens were embedded in paraffin after tissue processing. Sections were taken and stained with H&E, PAS, Trichrome and Perl's stain. The stained slides were examined under the microscope. METAVIRS' system was followed.⁸ The following parameters were recorded in the proformas:

1. Histological activity or the necroinflammatory score
2. Fibrosis score

These above mentioned parameters were evaluated as under:

Histological = activity score (grade)	Piecemeal + necrosis	Lobular necrosis
0 (none)	0	0
1 (mild)	0	1
2 (moderate)	0	2
1	1	0,1
2	1	2
2	2	0,1
3 (severe)	2	2
3	3	0,1,2

Fibrosis score	Description
0	No fibrosis
1	Stellate enlargement of portal tract but no septa formation
2	Enlargement of portal tract with rare septa
3	Numerous septa without cirrhosis
4	Cirrhosis (8)

Statistical Analysis: Chi-square test was applied to the distribution of histological features according to ALT levels and their association with each other. 'P' values of < 0.005 were considered significant.

RESULTS

Grade one activity or mild necroinflammatory score was found in 22 (44%) cases whereas the second highest number of cases i.e. 21 (42%) had an activity of grade three corresponding with severe necroinflammatory score. Only seven

Table-I: Distribution of hepatitis C patients according to age groups and gender.

Age groups	No. of patients (%)	Gender	
		Male (%)	Female (%)
10-20 yrs	3 (6%)	1 (2%)	2 (4%)
21-30 yrs	15 (30%)	7 (14%)	8 (16%)
31-40 yrs	18 (36%)	7 (14%)	11(22%)
41-60 yrs	14 (28%)	7 (14%)	7 (14%)
Total	50	22 (44%)	28 (56%)

Mean age: 35 years Range: 16-55 years.

(14%) cases fell in the category of grade two activity. Majority of the patients i.e. 29 (58%) had their ALT levels more than 40 U/L while only 21 (42%) had the ALT of <40 U/L. Out of the total 50 patients, only four (8%) had their ALT more than 121U/L. The mean ALT level was found out to be 63.4 U/L, with a range of 12 – 292. Out of the 21 (42%) cases with ALT levels <40 U/L, 12 (24 %) cases had an at least grade 1 activity. On the other hand 7(14%) cases each in ALT category of < 40 U/L and 41- 80 U/L had severe necroinflammatory scores (Table-II) Twenty two (44%) cases who had grade one activity had a mean age of 31.3 years and mean ALT of 52.3 U/L, whereas the mean age and mean ALT level increased to 39.8 years and 76.14 U/L respectively in the 21 (42%) cases who had severe necroinflammatory score. Interestingly no case was found showing grade 0 activity (Table-III). Thirty five patients (70%) had some degree of fibrosis, present in the biopsies while the remaining 15 (30%) cases had revealed no fibrosis. Minimal fibrosis i.e. stage

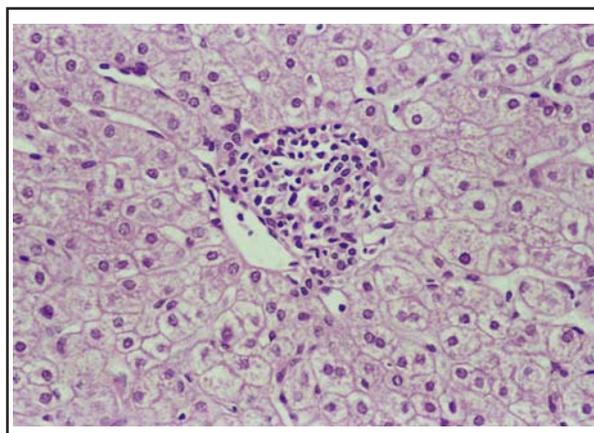


Fig-1: Activity grade 1, fibrosis stage 0, H&E, x200

one was found in 18 (36%) cases, whereas 9 (18%) cases had fibrosis to the extent of cirrhosis. The maximum number of cases without fibrosis i.e. 12 out of 15 cases had their ALT levels in category of < 40 U/L, whereas the maximum number of cases with minimal fibrosis i.e. 9 out of 18 cases had their ALT levels in category of 41-80 U/L (Table-IV). The patients having no fibrosis i.e. 15 (30%) patients had a mean age of 33.2 years with a mean ALT of 38.9 U/L. 18 (36%) cases with minimal fibrosis had a mean ALT of 51.3 U/L, while 9 (18%) patients with cirrhosis i.e. stage 4 fibrosis had a mean age of 46.3 years and a mean ALT of 74 U/L (Table-V).

DISCUSSION

Majority of the patients in our series 47 (94%) cases are more than 20 years old with a maximum number of patients i.e.18 (36%) cases being seen in their 4th decade of life (Table-I).

Table-II: Distribution of Hepatitis C patients according to ALT levels and necroinflammatory scores (grade).

ALT levels U/L	Number of patients (%)	Necroinflammatory score (grade)		
		1 (mild)	2 (moderate)	3 (severe)
< 40	21 (42%)	12 (24%)	2 (4%)	7 (14%)
41-80	18 (36%)	7 (14%)	4 (8%)	7 (14%)
81-120	7 (14%)	1 (2%)	1 (2%)	5 (10%)
>121	4 (8%)	2 (4%)	-	2 (4%)
Total	50	22 (44%)	7 (14%)	21 (42%)

Mean ALT level: 63.4 U/L Range :12-292

Table-III: Correlation of necroinflammatory scores (grade) with mean age and mean ALT levels

Grade	No. of patients (%)	Mean age in years	Mean ALT U/L
0 (absent)	-	-	-
1 (mild)	22 (44%)	31.3	52.3
2 (moderate)	7 (14%)	32.2	60
3 (severe)	21 (42%)	39.8	76.14

This is in accordance with the third National Health and Nutrition Examination Survey (NHANES), a national survey of a representative sample of non institutionalized civilian Americans conducted between 1988 and 1994. They also found 76% of cases older than 20 years.⁵

The mean age of our patients (35 years) is less than the mean ages found by Mushtaq et al⁹, Umar et al¹⁰ and Khokhar et al,¹¹ all from Pakistan who had found the mean ages to be 40 years, 46 years and 44.5 years respectively. This could be due to the better awareness and early detection by efficient screening programmes evolved in the last few years. The female preponderance in our study i.e. 56%, was against the findings of Mushtaq et al⁹, Mihm et al¹², Umar et al¹⁰ and Khokhar et al¹¹, who had shown male preponderance i.e. 72 %, 63%, 57%, and 56.8% respectively, however large cross sectional studies like the NHANES study have not demonstrated gender differences in the rate of chronicity in hepatitis C infection; they had reported similar rates of HCV chronicity among both men and women.⁵ In our series , we have found that 22(44%) cases having grade one activity had a mean ALT of 52.3 U/L while 21 (42%) cases having severe necroinflammatory

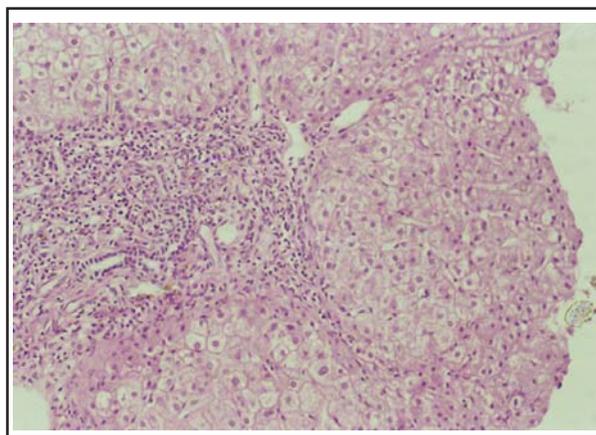


Fig-2: Activity grade 3, fibrosis stage 2, H&E, x100

score had a mean ALT of 76.14 U/L (Table-III). This is in accordance with Mihm et al¹² who had found 56% cases with mild activity having mean ALT of 45 U/L and 44% cases with severe activity having a mean ALT of 90 U /L, suggesting that higher ALT levels are associated with more pronounced forms of necroinflammatory activity. Elevation of ALT levels have been the hallmark of hepatocyte necrosis and had long been used as best diagnostic marker for hepatitis but we have found 21 (42%) cases with ALT levels <40 U/L, out of which 12 cases have an at least grade 1 activity and seven cases have grade three activity. (Table-II). This is in accordance with Jamal et al¹³, who had found histologically significant liver disease in 27 % patients with normal ALT levels. It shows that a normal ALT level does not rule out severity of the disease. According to Dienstag¹⁴ some of the patients having normal ALT levels, had histologically very severe or advanced liver disease suggesting that all such patients require liver biopsy to unearth clinically subtle but advanced

Table-IV: Distribution of hepatitis C patients according to ALT levels and fibrosis scores (stage).

ALT levels U/L	No. of patients (%)	Fibrosis score (stage)				
		0	1	2	3	4
< 40	21 (42%)	12 (24%)	7 (14%)	-	-	2 (4%)
41-80	18 (36%)	1 (2%)	9 (18%)	3 (6%)	2 (4%)	3 (6%)
81-120	7 (14%)	1 (2%)	1 (2%)	-	1 (2%)	4 (8%)
>121	4 (8%)	1 (2%)	1 (2%)	1 (2%)	1 (2%)	-
Total	50	15 (30%)	18 (36%)	4 (8%)	4(8%)	9 (18%)

Chi. Sq.: 28.73, Chi. Sq. tab.:28.3 (p<0.005)

Table-V: Association of fibrosis score (stage) with age and average ALT levels.

Stage	No. of patients (%)	Mean age in years	Mean ALT U/L
0	15 (30%)	33.2	38.9
1	18 (36%)	30.5	51.3
2	4 (8%)	39	89
3	4 (8%)	42.7	130
4	9 (18%)	46.3	74

liver disease. It is important to note that there were no healthy hepatitis C carriers in our study i.e. no normal biopsy specimen (Table-III). This was also reported by Mushtaq et al⁹ and Jamal et al.¹³

Fibrosis to any extent whether minimal or maximal up to cirrhosis has been found in 35 (70%) cases in our study (Table-V) which is lower than that found by Mushtaq et al⁹ and Khokhar et al¹¹, who had reported 92 % and 95% cases having fibrosis respectively.

We have found a significant relationship of ALT with fibrosis ($p < 0.005$) evident from Table-IV, showing 12 cases without fibrosis who had ALT < 40 U/L; and revealing a somewhat proportional rise in mean ALT levels with increasing fibrosis stage from 1 to 3. This is in accordance with Forns et al¹⁵ who had found mean ALT of 92U/L in patients with no fibrosis and mean ALT of 111 U/L in patients with significant fibrosis. In the same pattern Wai et al¹⁶ had found mean ALT of 76 U/L in patients with no fibrosis and mean ALT of 134 U/L in patients with significant fibrosis. The present study also shows a steady and proportional rise in mean age with increasing score of fibrosis stage. (Table-V). Adinolfi et al¹⁷ had found a significant relationship between age of patient and fibrosis stage ($p < 0.001$).

It can be concluded that higher ALT levels are associated with more pronounced forms of necroinflammatory activity and fibrosis; however it was also confirmed that a normal ALT level does not rule out severity of the disease suggesting that all such patients require liver biopsy to unearth clinically subtle but advanced liver disease.

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