

QUANTITATIVE ASSESSMENT OF LIVER FIBROSIS IN PATIENTS WITH CHRONIC HEPATITIS C USING SPECIAL STAINS

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ABSTRACT

Background: Chronic hepatitis C has a prevalence of at least 1% world wide. It is highly co-related with development of cirrhosis. The aim of this study was to make a quantitative assessment of fibrosis by using special staining methods.

Material and methods: This study was conducted at Khyber Teaching Hospital Peshawar, Pakistan, from October 2002 to March 2003. Fourteen male patients suffering from chronic hepatitis C were included in the study. They were divided into two groups, pre and post treatment. Percutaneous liver biopsy was performed, processed, stained with special stains and examined under light microscope. Observations were made according to the Knodell Histology Activity Index numerical scoring system of liver biopsy specimen.

Results: Nodule formation and fatty changes were observed and graded. Connective tissue (fibrosis) was noted and staged. All observations were scored according to the Knodell Histology Activity Index.

Conclusion: Morphological study of liver in chronic hepatitis C provides a much broader and clear field for making the diagnosis and following the response to therapy.

Key words: Liver, Liver fibrosis, Hepatitis C.

INTRODUCTION

Chronic hepatitis C is a viral disease with a prevalence of at least 1% world wide.¹ In Pakistan, up to 10% adults carry hepatitis C virus (HCV).² Nearly 4 million Americans and 100 million people worldwide are infected with the HCV.³ At least 70-80% of infected patients progress to chronic hepatitis, which is highly co-related with the development of cirrhosis and hepatocellular carcinoma.⁴ In chronic liver disease, development of fibrosis is the first step towards progression to cirrhosis and its complications (such as organ failure, esophageal variceal bleeding and hepatocellular carcinoma), independent of the underlying etiology.⁵ Cirrhosis is the result of long-term damage caused by chronic inflammation and liver cell death.⁶ The wound healing or a scarring response in response to liver damage leads to hepatic fibrosis.³ Liver fibrosis results from chronic damage to the liver in conjunction with the accumulation of extra-cellular matrix proteins, which is a characteristic of most types of chronic liver diseases.⁷ As liver becomes fibrotic, significant changes occur both qualitatively and quantitatively in the interstitial extra cellular matrix. The total content of collagenous and

non-collagenous components increases 3-5 fold, which is characterized by transformation from normal extra cellular matrix to a reticulated and dense matrix, which is much more resistant to enzyme degradation.^{8,9}

Histologic assessment of the liver biopsy specimens remains the "gold standard" for quantifying fibrosis and is the only way to determine the amount of fibrosis in a liver biopsy.¹⁰ Accurate assessment of the extent of fibrosis is essential to guide management and predict prognosis in patients with chronic liver injury. No other available test is as yet able to provide this important piece of information.⁶ Most studies of chronic hepatitis C defined liver lesions according to the so called activity grades, which is classification of necrosis and inflammation without identification of fibrosis stages. In such studies patients were classified as having chronic persistent or active hepatitis but the development of fibrosis itself was not assessed.^{11,12} The Knodell Histology Activity Index (HAI) was the first system of its type and is widely regarded as benchmark for objective, semi-quantitative, reproducible description of various morphological lesions of chronic hepatitis.¹³

Review of literature regarding the treatment of patients suffering from chronic hepatitis C reveals very few morphological studies in quantifying fibrosis. The few studies conducted so far are also deficient due to non-application of special stains. Although the liver histology in patients with chronic hepatitis C shows both inflammation and fibrosis, it is the latter which is the factor of greater concern. It is important to assess the stage of liver fibrosis because the presently available therapies have an inverse correlation of the response to the treatment and the quantity (stage) of the fibrosis present in the liver. Most of the studies so far conducted are based only on biochemical analysis or the grades and stages of microscopic observations with no morphological details of hepatocytes and stroma.

The present study was undertaken to make a quantitative assessment of fibrosis by differentiating between the parenchyma and stroma at light microscopic level using special stains.

MATERIAL AND METHODS

Adult males, 18 to 60 years of age, suffering from chronic hepatitis C with the following hematologic, biochemical and histological criteria were included in the present study.

- Hemoglobin level > 13g/dL for males, > 12g/dL for females
- WBC count >3000/mm³
- Granulocyte count >1,500/mm³
- Platelets count >100,000/mm³
- Direct and Indirect serum Bilirubin levels within normal limits
- Serum albumin levels within normal limits
- Elevated ALT levels
- Hepatitis C Antibody positive (MEIA/4th generation ELISA)
- HCV-RNA positive by PCR

Exclusion criteria included;

- Patients unwilling for liver biopsy
- Co-infection with HBV or HIV
- Wilson's disease
- Autoimmune hepatitis
- Alcoholic liver disease
- Obesity induced liver disease
- Drug related liver disease
- Evidence of advanced liver disease such as

history or presence of ascites, bleeding varices or spontaneous encephalopathy

A pre and post-treatment percutaneous liver biopsy was taken from the liver with a Tru-cut disposable liver biopsy needle of 1.5 cm notch size. The size of biopsy specimen, varied between 1 and 3 mm in length and between 1.2 and 2 mm in diameter. For evaluation of diffuse liver disease such as chronic hepatitis C, a specimen of 1.5cm in length is adequate for a diagnosis to be made.^{14,15}

Fourteen male patients suffering from chronic hepatitis C were included in the present study. They were grouped into two, Group-I and Group-II. Group-I contained six patients treated with Interferon and Ribavirin. Group-II contained eight patients treated with Interferon, Ribavirin and vitamin E.

Biopsy specimens were fixed in neutral buffered formalin (pH 7.0) for 24 hours, embedded in paraffin and sectioned at 4-5 micron thickness.¹⁶ Three slides of each case were prepared in a serial order and stained by the following methods:

- Hematoxylin and eosin for routine microscopy.¹⁶
- Periodic Acid Schiff (PAS) for demonstration of carbohydrates.¹⁶
- Masson's Trichrome for demonstration of collagen fibers.¹⁷

The measurements were made by means of oculometer at magnification of 10x and 40x. Stromal connective tissue and infiltration with inflammatory cells was labeled as minimum (\pm), mild (+), moderate (++) and marked (+++).

RESULTS

In Group-I an incomplete nodule formation with fatty change was seen in one patient before treatment, while no such alteration could be observed after treatment. (Figure-1 & 2)

In Group-II well-marked nodule formation with fatty change was seen in two cases before treatment while atypical or incomplete nodule was seen in one patient after treatment. (Fig-3 & 4)

The connective tissue component was moderately increased (++) in Group A before treatment (Figure-1) and remained unchanged in most of the patients, while it was markedly increased (+++) in one patient after treatment. (Figure-2)

Similarly a moderate increase (++) was observed in Group-II before treatment (Figure-3), which was reduced after treatment to a minimum (\pm) level after treatment. (Figure-4)

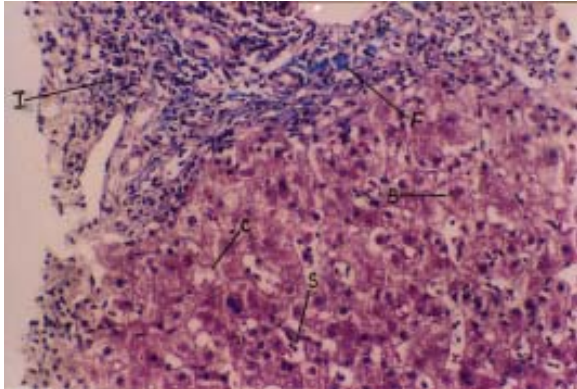


Fig. 1: Photomicrograph of a patient from Group-I before treatment, illustrating (C) fatty change in hepatocytes, (S) congested sinusoids, (B) necrotic cell, (I) inflammatory cells in portal triad stroma and (F) fibrous tissue in triad.

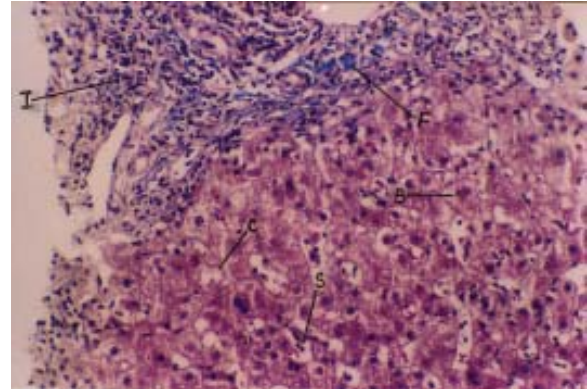


Fig. 3: Photomicrograph of a patient from Group-I before treatment, illustrating (C) fatty change in hepatocytes, (S) congested sinusoids, (B) necrotic cell, (I) inflammatory cells in portal triad stroma and (F) fibrous tissue in triad.

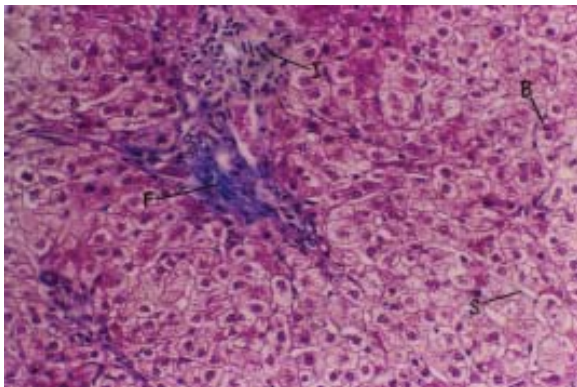


Fig. 2: Photomicrograph of a patient from Group-I after treatment showing a portal triad with hepatic cellular plates of the surrounding lobules around it with: (I) inflammatory cells in the triad, (F) fibrous tissue, (B) a normal binucleated cell and (S) sinusoid. Masson's Trichrome x 150

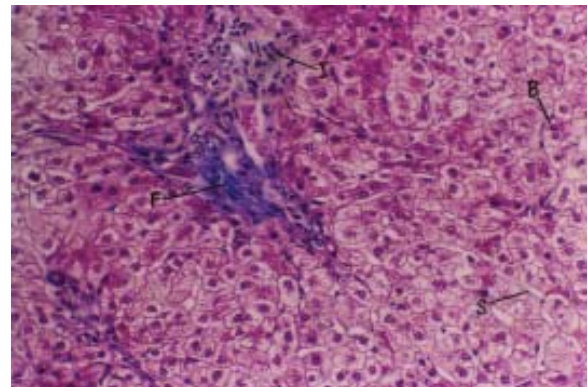


Fig. 4: Photomicrograph of a patient from Group-I after treatment showing a portal triad with hepatic cellular plates of the surrounding lobules around it with: (I) inflammatory cells in the triad, (F) fibrous tissue, (B) a normal binucleated cell and (S) sinusoid. Masson's Trichrome x 150

Infiltration by inflammatory cells was marked (+++) in both groups before treatment. (Figure-1 & 3) and mild (+) in both after treatment. (Figure-2 & 4)

Both groups were also observed according to the Knodell Histology Activity Index (HAI) numerical scoring system of liver biopsy specimens. The slides were scored for grades and stages according to which there was a significant decrease in activity grade in both the groups after the treatment. (Table-1 & 2)

While in the fibrosis stage the change was less significant in Group-I as compared to the change in Group-II after treatment. In addition, in

Group-I there was no change in fibrosis score in three patients rather an increase in a patient from stage 1 to 3 was recorded, while in Group-II there was no increase in the score in any case after the treatment.

DISCUSSION

For the last few years chronic Hepatitis C has been on the top of the list of liver diseases world wide and has become a burning issue for discussion and research among the hepatologists. The most favorite site of attack for hepatitis C virus is the hepatocytes followed by a reactionary (inflammatory) change in the surrounding stroma. The results of present study suggest that in an

Table-1: Comparison of Grades, Stages and Knodell Score in Group-I.

Groups	Patient number	1	2	3	4	5	6
Before Treatment	Grade / Necrosis	07	05	09	09	13	11
	Stage /Fibrosis	01	01	03	01	03	03
	Knodell score	08	06	12	10	16	14
After Treatment	Grade /Necrosis	05	03	05	03	05	03
	Stage /Fibrosis	01	01	01	03	03	01
	Knodell Score	06	04	06	06	08	04

Table-2: Comparison of Grades, Stages & Knodell Score in Group-II.

Groups	Patient number	1	2	3	4	5	6	7	8
Before Treatment	Grade / necrosis	07	14	09	05	07	12	05	09
	Stage / fibrosis	01	04	01	03	01	04	01	01
	Knodell score	08	18	10	08	08	16	06	10
After Treatment	Grade / necrosis	03	09	03	03	03	05	03	03
	Stage / fibrosis	01	03	01	01	01	03	00	01
	Knodell score	04	12	04	04	04	08	03	04

endemic area like Pakistan, chronic HCV infection contributes mainly to chronic liver disease, a finding consistent with the observations made by Umar et al.² Chronic hepatic inflammation is tightly linked to fibrosis in virtually all individuals with liver disease and in experimental models of fibrogenesis. Also, chronic activation of inflammatory pathways has been shown to promote hepatocarcinogenesis.¹⁸

The majority of studies so far conducted to assess the severity of liver disease are mainly based on biochemical analysis, very few or no attempts have been made to observe the hepatocellular and stromal morphology in detail. Keeping the above mentioned deficiency, we carried out a study that was mainly focused on the morphological hepatocellular and stromal alterations caused by HCV infection. This study is supported by the observations of Jurgens, according to which appropriate morphologic descriptions generally provide the information pertinent to grading and staging.¹⁹

It was also noted that the necro-inflammatory process was closely associated with fibrogenesis. With high necro-inflammatory activity, there was increased fibrosis before treatment in both groups. Similar observations were made by Baroni et al, according to whom necro-inflam-

matory process was implicated in fibrogenesis around the necro-inflammatory lesions.²⁰

Our morphological study also provides the necessary basic information for grading and staging. We graded chronic hepatitis morphologically to quantify inflammation and the accompanying tissue damage and staged the condition to determine its position on an imaginary time line from the onset of disease to cirrhosis, which actually meant degree of fibrosis. For this purpose we used the most popular Knodell scoring system.¹⁴

CONCLUSION

Morphological study of liver in chronic hepatitis C provides a much broader and clear field for making a diagnosis and following the response to therapy.

Because of lack of sensitive serum markers for the assessment of liver fibrosis, research efforts must go on to the development of appropriate quantitative methods for the measurement of liver fibrosis.

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