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Ultrasound based evaluation of hepatic steatosis and fibrosis in hepatitis C non-responders

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ORIGINAL ARTICLE

Ultrasound Based Evaluation of Hepatic Steatosis and Fibrosis in Hepatitis C Non-Responders

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ABSTRACT

Objective: To determine the accuracy of ultrasound in the diagnosis and grading of steatosis and fibrosis in Hepatitis C (HCV) patients not responding to ribavarin-interferon therapy.

Study Design: A cross-sectional, analytical study.

Place and Duration of Study: Radiology Department, Civil Hospital, Karachi, from March 2008 to August 2010.

Methodology: Patients with positive HCV RNA despite 24 weeks ribavarin-interferon therapy (non-responders) were subjected to ultrasound and biopsy prior to institution of pegylated interferon therapy for detection and grading of steatosis and fibrosis. Using histopathology as the gold standard, sensitivity, specificity, negative and positive predictive values for ultrasound were determined.

Results: The sensitivity of ultrasound for hepatic steatosis was 90.9% for no steatosis (NS), 100% for moderate and gross steatosis and 84.4% for mild steatosis with 100% specificity. The senitivity for fibrosis was 25% for no fibrosis, 100% for mild fibrosis, 89.74% for moderate fibrosis and 100% for gross fibrosis. The overall accuracy for detection of steatosis was 95.39% and that for fibrosis was 98.02%. Hepatic vein showed increased dampening of flow with advancing grades of steatosis and fibrosis.

Conclusion: Ultrasound has a high accuracy in the diagnosis and grading of steatosis and fibrosis in HCV non-responders. Mild fibrosis may confound the diagnosis of mild steatosis.

Key Words: *Chronic hepatitis C. Fibrosis. Hepatitis C virus (HCV). Non-responders. Steatosis. Ultrasound.*

INTRODUCTION

Hepatitis C virus infection causes severe morbidity with development of hepatic fibrosis leading to chronic liver disease, cirrhosis and hepatocellular carcinoma. Epidemiologically, Pakistan falls in the intermediate prevalence zone for HCV infection, where infection rate is between 4 – 6% and approximately 10 million populations is affected.[1] It is usually treated in Pakistan with ribavarin-interferon therapy due to cost and the prevalent genotype (type-3). Pegylated interferon is only used when there is persistent positive RNA despite 24 weeks of therapy.[2] With this approach, the reported frequency of this subset of patients called the non-responders, is about 16.8 – 17% in Pakistan.[3,4] However, these patients who are not responding to therapy are the ones more likely to develop complications such as those arising from fibrosis and steatosis.

Presence of steatosis has been frequently reported in the HCV affected patients.[5,6] It is even said to affect the treatment outcome such as non-response, and the natural history and progression of disease with onset of complications.[7] On the other hand, it may cause confusion with ultrasound features of fibrosis. Ultrasound is an important noninvasive means of evaluating hepatic morphology particularly in the presence of coagulopathy and ascites when biopsy is contraindicated.[8] To the best of authors' knowledge, no published local (Pakistan-based) data exist on ultrasound evaluation of hepatic fibrosis and steatosis; there is a similar paucity of literature on the ultrasound evaluation of liver in the HCV patients who did not respond to ribavarin-interferon therapy while these are the group of patients requiring regular morphologic evaluation. The hitherto unexplored accuracy of ultrasound in HCV non-responders constituted the rationale of this study.

The precise objective of the study was to evaluate the accuracy of ultrasound in diagnosis and grading of steatosis and fibrosis in patients with HCV who had a positive HCV RNA despite 24 weeks of ribavarin-interferon therapy.

METHODOLOGY

It was a cross-sectional, analytical study carried out in the Department of Radiology, Dow Medical College and Civil Hospital, Karachi, between March 2008 and August 2010. The study was approved by the Ethical Review Board, Dow University of Health Sciences. Adult

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Pakistani patients regardless of gender, with positive HCV RNA after 24 weeks of therapy with ribavirin and conventional interferon (non-responders) and being considered for pegylated interferon therapy and liver biopsy were included. The selected patients were further divided into a steatosis positive (s+) group and a steatosis negative (s-) group on the basis of ultrasound findings. The former comprised HCV non-responders showing steatosis of liver on u/s and the latter comprised those who did not show hepatic steatosis on u/s. Using WHO sample size calculator software, 80% study power, and assuming a difference in accuracy (100% for biopsy and 95% for ultrasound), the calculated sample size was 152 with equal number in both groups i.e. HCV in the hepatic steatosis and HCV without hepatic steatosis.

Patients aged less than 15 years, those with history of alcohol intake, other positive viral hepatitis markers such as hepatitis-B, auto-immune hepatitis or drug induced hepatitis, deposition disorder, concomitant HIV, and a hepatic mass lesion (s) or ascites were excluded.

Selected patients underwent ultrasound of hepatobiliary system or upper abdomen using convex probe followed by ultrasound guided liver biopsy after written informed consent. Presence or absence of fibrosis was documented on ultrasound in all patients prior to biopsy; and graded into nil (absent), mild, moderate and gross as per criteria laid down in Table I, modified from Nishiura *et al*.[9]

The ultrasound was conducted by the senior teaching faculty member and principal researcher. The images were reviewed by another radiologist of similar standing and experience blinded to the grading given by the first researcher. Grading of steatosis was in the steatotic group only according to criteria led by Scatarige *et al*.[10] Mild steatosis was defined as increased echogenicity of hepatic parenchyma (equal as or greater than the body of pancreas) with normal visualization of posterior diaphragm and intra hepatic vessels' borders (IHVB's). Moderate steatosis-increased echogenicity of hepatic parenchyma with impaired visualization of posterior diaphragm IHVB's. Severe/gross steatosis-increased echogenecity of hepatic parenchyma with poor or no visualization of posterior diaphragm, intra hepatic vessels' borders and posterior portions of the right lobe of liver. Nil meant absence of steatosis on ultrasound. Final grades were assigned with consensus. Portal vein congestion index (CI) and hepatic vein damping index (DI) were also determined on Doppler ultrasound.

Ultrasound guided liver biopsy was then performed by the principal researcher using standard technique and precautions. A sample of at least 1 cm^3 was considered adequate. The specimen was then sent for histopathology and findings were recorded with respect to grade of steatosis - nil, mild, moderate and gross; fibrosis stage, activity grade and Ishak score. Histopathology based final diagnosis of liver status was made as minimal hepatitis, mild hepatitis, chronic hepatitis and chronic active hepatitis with impending cirrhosis.

Statistical analysis was carried out on Statistical Package for Social Sciences (SPSS) version 19. Relevant descriptive statistics were computed for measures of central tendency and dispersion. Accuracy of ultrasound for diagnosis and grading of steatosis and fibrosis was calculated using histopathology as the gold standard. The sensitivity, specificity and the negative and positive predictive values were calculated for the overall and the separate subgroups of the steatosis and fibrosis. Mean values for continuous variables were compared between the two main groups using t-test with significance at $p < 0.05$.

RESULTS

There were a total of 152 subjects, 76 in each steatosis and the non-steatosis groups. The overall female subjects were 81 (53.3%) and males were 71 (46.7%) in number. The overall mean age was 38.73 ± 9.24 years ranging from 22 to 69 years. The mean overall BMI was 23.4 ± 3.02 kg/m^2. The overall daily mean dietary fat intake of the studied group was 15 ± 0.43 grams. The NS group had 03 (3.9%) cases of type-1 and 73 (96.1%) cases of HCV type-3; corresponding numbers were 04 (5.3%) and 72 (94.7%) in the steatotic group ($p = 0.5$).

Regarding the ultrasound findings, there was no significant difference in the spleen size, portal vein size and congestion index among the two groups, however, the Damping index was significantly different among the subgroups as described later. Overall, the liver was mildly fibrotic (n = 50, 32.89%) and mildly steatotic in the majority (n = 42, 55.26%) of the studied subjects.

Table I: Ultrasound scoring criteria used for staging fibrosis in this study modified from Nishiuri *et al.*[9]

Stage	Criteria-parenchyma	Criteria-inferior edge	Criteria-liver surface
Mild fibrosis	Heterogeneous coarsening scattered in central segments-may go undetected by low frequency probe (3.5 MHz) and visible by high frequency probe (5MHz) only	Sharp to blunt -the latter seen with high frequency probe	Smooth to rough
Moderate fibrosis	Coarse, echogenic parenchyma in diffuse distribution	Blunt to rounded-the latter seen with high frequency probe, the former with low frequency probe	Irregular
Advanced or gross fibrosis	Diffuse coarse echogenic irregular heterogeneous to nodular readily visible with low frequency probe	Rounded seen with low frequency probe	Irregular to nodular

Among the steatosis positive group on ultrasound, 38 had mild steatosis, 33 had moderate steatosis and 05 had gross steatosis. On histopathologic confirmation, 42 of these cases had mild steatosis, 29 had moderate steatosis and 05 had gross steatosis (Table II). There were 07 cases among the non-steatotic group who turned out to have mild steatosis on histopathology which gave an overall accuracy of 90.9% for the exclusion of steatosis. All these cases had mild fibrosis seen on both the histopathology and the ultrasound. The accuracy for sonographic grading for gross steatosis was 100%, 100% for moderate steatosis and 95.9% for mild steatosis (Table III).

Table II: Distribution of steatosis and histopathological confirmation. (Please note that those assigned a particular steatosis grade on ultrasound but found to have another grade on histopathology acted as false positive and false negative for ultrasound assigned grades as applicable).

	Histopathology steatosis grade				Total
	Gross	Mild	Moderate	Nil	
Us steatosis grade					
Gross	5 (TP)	0 (TN)	0 (TN)	0 (TN)	5
Mild	0 (TN)	38 (TP)	0 (TN)	4 (FP)	42
Moderate	0 (TN)	0 (TN)	29 (TP)	0 (TN)	29
Nil	0 (TN)	7 (FN)	0 (TN)	69 (TP)	76
Total	5	45	29	73	152

Table III: Accuracy analysis for the sonographic diagnosis of steatosis.

	No steatosis	Mild steatosis	Moderate steatosis	Gross steatosis
Sensitivity	90.9%	84.4%	100%	100%
Specificity	100%	100%	100%	100%
Positive predictive value	100%	100%	100%	100%
Negative predictive value	91.4%	93.69%	100%	100%

Table IV: Distribution of fibrosis and histopathological confirmation. (Please note that those assigned a particular fibrosis grade on ultrasound but found to have another grade on histopathology acted as false positive and false negative for ultrasound assigned grades as applicable).

	Histopathology fibrosis stage				Total
	Gross	Mild	Moderate	Nil	
Us fibrosis grade					
Gross	16 (TP)	0 (TN)	4 (FP)	0 (TN)	20
Mild	0 (TN)	50 (TP)	4 (FP)	0 (TN)	54
Moderate	0 (TN)	4 (FN)	70 (TP)	0 (TN)	74
Nil	0 (TN)	3 (FN)	0 (TN)	1 (TP)	4
Total	16	57	78	1	152

Table V: Accuracy analysis for the diagnosis of fibrosis.

	No fibrosis	Mild fibrosis	Moderate fibrosis	Gross fibrosis
Sensitivity	25%	100%	89.74%	100%
Specificity	100%	96.07%	89.18%	97.05%
Positive predictive value	100%	92.59%	89.74%	80%
Negative predictive value	98.01%	100%	89.18%	100%

Overall, sonographically, there were 04 cases of no fibrosis, 54 cases of mild fibrosis, 74 cases of moderate fibrosis and 20 cases of gross fibrosis (Table IV). The accuracy is given in Table V. Among the steatotic group, 11 (14.5%) had gross fibrosis, 27 (35.5%) had mild fibrosis, 38 had moderate fibrosis and none had no fibrosis. Conversely among the non-steatotic group, 09 had gross fibrosis, 27 had mild fibrosis, 36 had moderate fibrosis and 04 had no fibrosis. Despite a relatively less advanced stage of fibrosis in the non-steatotic group, the difference was not statistically significant with p value of 0.234 on chi-square test.

The mean fibrosis (Ishak) score was 2.3 in the non-steatotic group and 2.4 in the steatotic group (p = 0.4). While the portal vein CI was not significantly different among the sub-groups, there was significant difference towards a higher DI in both steatosis and fibrosis as their respective grades advanced. The mean DI in both groups was 0.5.

DISCUSSION

This study evaluated a very important group of Chronic Hepatitis C (CHC) patients i.e. non-responders to ribavarin-conventional interferon therapy. The main finding was a very high frequency of some degree of fibrosis in these patients. In fact only 04 out of the 152 i.e. 2.63% were spared from fibrosis. Slow and progressive fibrosis is a known feature of the CHC infection particularly in the non-responders, and its evaluation has an important bearing on the outcome.[5,11] Steatosis, on the other hand, is a recognized predictor to an unfavourable response to therapy and exert an adverse effect on the natural progression of HCV infection.[3-8,11,12]

The studied group of patients was a predominantly non- obese cohort bordering on being overweight as per Asian standards taking an overwhelmingly carbohydrate-based diet. The liver was mildly fibrotic and mildly steatotic in the majority of this group of non-responders.

As there were only a very few cases of no fibrosis, this caused a marked difference in the sensitivity and specificity for the detection of mild fibrosis. Except for this outlier, the values of sensitivity, specificity and prediction of presence or absence of various grades of fibrosis was fairly accurate ranging from 89 – 100%.

Iliopaulos *et al*. found a sensitivity of 71.1%, specificity of 72.9%, PPV of 58.7% and 82.3% NPV for ultrasound based diagnosis of mild steatosis; corresponding values for higher grades of steatosis, were 85.7%, 60.4%, 13% and 98.4%.[13] They found a much lower sensitivity (13.6 – 27.4%) and specificity (62.5 – 66.3%) for the ultrasound detection of fibrosis.[13] Their NPV (exclusion capability) for lower stages of fibrosis was low (19.5%) and somewhat better for higher stage of fibrosis (75%).

Conversely, the positive predictive value was 71.9% for lower stages of fibrosis and only 9.4% for higher stages of fibrosis.[13] This wide variation can only be explained by the higher BMI of their selected population (25 kg/m^2). Higher BMI values considerably affect the sonographic diagnosis of steatosis.[14] Moreover, Iliopaulos *et al*. found no correlation between the sonographic grade and histologic score.[13] The present results are different, likely to be due to lower BMI and more importantly adherence to a standardized criteria for grading of steatosis and fibrosis instead of using the 'bright liver pattern' only. Another report from Iacobillis *et al*. corroborated that u/s parameters had a specificity rate of 90% or greater in excluding bridging fibrosis.[15] They had used the Metavir system for histopathological evaluation.[15]

While the portal vein CI was not significantly different among the sub-groups, there was significant difference towards a higher DI in both steatosis and fibrosis as their respective grades advanced. The mean DI in both groups was 0.5. A DI of 0.6 is said to be an important cut off limit for monitoring the response to therapy.[16]

A systematic review by Smith and Sterling showed that Doppler findings alone cannot differentiate between grades of fibrosis and ultrasound sensitivity and specificity do tend to vary between studies.[17] However, it improves upon combining various parameters be it liver surface or portal vein size or splenic size or Doppler indices or Doppler spectra in many possible combinations.[17] Same can be true for ultrasound detection and grading of steatosis.[18] The difficulty or limitation of using Doppler as single criteria is likely to be due to difficulty in reliable reproduction of Doppler measurements.[19] O'Donohue *et al*. evaluated multiple Doppler parameters along with spleen size and found that splenomegaly and hepatic vein waveform not only reliably predicts significant fibrosis but these are reproducible parameters as well.[20] Hepatic vein waveform provides good evaluation of cardiac and hepatic physiology by tracing the direction, regularity and phasicity of the spectral tracing.[21] In this study, majority of the patients had an abnormal waveform with 57.2% having biphasic and 12.5% having monophasic waveforms. No significant difference was seen in the proportion between groups and sub-groups. This can be due to the presence of some degree of fibrosis in an overwhelming majority of the patients. Non-triphasic hepatic flow was found to be a helpful Doppler criterion of differentiating between cirrhosis and CHC by Haktamir *et al*.[22]

Many previous studies have reported a low accuracy and low reliability of ultrasound regarding the evaluation of steatosis and fibrosis in HCV infection; most were retrospective in nature and strict grading criteria as suggested by Nishiura *et al*.[9] and Scatarige *et al*.[10], were not used. The authors used these criteria prospectively in combination and utilized ultrasound probes of multiple frequencies, changing the depth for focused study of liver parenchyma and its gross morphology. This may be responsible for the present improved results. There have been reports of good reliability and accuracy of ultrasound for diagnosing and grading both fibrosis and steatosis when semi-quantitative measures, multiple parameters and multi-frequency probes have been used.[9]

Adopting a number of criteria as used in this study, may avoid unnecessary biopsies,[23] and help when liver biopsy is contraindicated by ascites.

The limitations of this study were the highly skewed absence of mild fibrosis and lack of obese controls. The first was what was found and reported what we found in this cohort. The second was based on ethical grounds. It was a histopathology-confirmed study and biopsy for histopathology in obese patients without liver disease can not be taken only for the sake of obtaining controls.

CONCLUSION

Based on the present results, using objective criteria including grayscale ultrasound and Doppler indices, ultrasound can be utilized for differentiation, detection and grading of fibrosis and steatosis in CHC non-responders.

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