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Etiology and Impact of Hepatic Steatosis in Pakistan: Role of Hepatitis C Virus Genotype 3 Infection

Shahid Rasool*, Saeed Hamid, Wasim Jafri, Hasnain A Shah, Shahab Abid and Zubair Ahmed

Department of Medicine and Pathology, The Aga Khan University Hospital, Karachi, Pakistan

**Corresponding e-mail: drshrasool@yahoo.com*

ABSTRACT

Introduction: *Hepatic steatosis has emerged as an important histological finding in patients with deranged liver function. It may be an important factor for the progression of hepatitis C virus-associated liver disease, particularly in genotype 3 infections.* ***Aims:*** *To determine the etiology and impact of hepatic steatosis in our patients presenting with chronic hepatitis.* ***Methods:*** *All liver biopsies performed at our hospital during 2010-2014 were analyzed by a single pathologist using histological activity index (HAI) scores and Brunt's classification for steatosis. Patients were evaluated for factors reported to be associated with steatosis, including the prevalence of HCV.* ***Results:*** *Biopsies of 439 patients (284 male, mean ages 38.5 ± 11.2 years) were studied. Hepatic steatosis was present in 324 (73.8%) biopsies. It was mild in 190/439 (43.3%), moderate in 88/439 (20%) and severe in 46/439 (10.5%) cases. On univariate analysis, steatosis was associated with HCV infection (p=0.023), BMI >25 (p=0.008) and raised ALT (p=0.003), but not with diabetes, hypertriglyceridemia, HBV infection or alcohol intake. On multiple logistic regression HCV and BMI >25 were independent risk factors for steatosis. There was a linear ascending association of hepatic steatosis with grade and stage of liver disease (p ≤ 0.001). Among 369 HCV patients, 280 (76%) had steatosis. It was mild in 159/369 (43%), moderate in 82/369 (22.2%) and severe in 39/369 (10.6%) cases. There were only 32 non-alcoholic, non-viral hepatitis patients and 8/32 (25%) had moderate or severe steatosis.* ***Conclusions:*** *Significant hepatic steatosis is present in 30.5% of our patients with chronic hepatitis. HCV genotype 3 infection is the predominant factor for hepatic steatosis in Pakistan. Steatosis has a linear ascending correlation with hepatic inflammation and fibrosis.*

Keywords: Hepatic steatosis, Hepatitis C virus, Body mass index

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) and Hepatitis C virus (HCV) infection are the most common form of liver diseases worldwide. NAFLD is a clinicopathological syndrome with a wide spectrum of histopathological abnormalities and clinical outcomes ranging from simple steatosis to steatohepatitis, advanced fibrosis and cirrhosis [1]. Major etiological factors associated with hepatic steatosis are alcohol intake, metabolic syndrome, and HCV infection. Epidemiologic studies have shown HCV-associated steatosis to correlate with both patient factors such as obesity and viral factors like HCV genotype 3a. Furthermore, the degree of steatosis has been linked to the extent of hepatic fibrosis in several studies, implying that steatosis may be contributing to disease progression in chronic HCV infection [2].

Recent studies suggest that liver steatosis in chronic hepatitis C (CHC) may be the expression of a direct cytopathic effect of hepatitis C virus, particularly in patients infected with genotype 3 [3]. The association between hepatic steatosis in HCV patients and known risk factors of hepatic steatosis such as increased BMI, hyperlipidemia, alcohol intake and diabetes is not well understood. In the past, some studies have shown significant improvement in steatosis in HCV genotype 3 patients, who achieved sustained viral clearance after HCV treatment [3]. This provides further evidence for direct involvement of HCV genotype 3 in the pathogenesis of hepatic steatosis. Few recent studies with direct-acting antiviral drugs for the treatment of CHC have shown significant resolution of hepatic steatosis after

sustained virological response [4,5]. The effect of HCV eradication on hepatic steatosis and progression to fibrosis warrants further study in the era of direct-acting antivirals.

The objective of our analysis was to determine the etiology of hepatic steatosis in our patients with chronic hepatitis. The second objective was to look for comorbid conditions that may be associated with steatosis in HCV and to establish whether the severity of steatosis leads to an increase in hepatic necroinflammatory changes and fibrosis.

MATERIALS AND METHODS

Patients and Data Collection

All liver biopsies (n=439) performed at the Aga Khan University Hospital between January 2010 to December 2014 were evaluated. Patient's demographic information such as height, weight, risk factors for steatosis e.g. diabetes, alcohol intake, serum cholesterol and triglyceride, viral serology, HCV genotype were collected. A single histopathologist, who was blinded from laboratory and clinical data, re-evaluated the liver biopsies. Biopsy specimens were fixed, embedded in paraffin wax and stained with hematoxylin and eosin. In addition, special stains including PAS and reticulin (for collagen) were performed on each specimen. Grading and staging were performed using the Scheuer system [6].

Fibrosis was scored on a scale of 0-4 with stage 0 being no fibrosis, stage 1 was minimal portal fibrosis, stage 2 was periportal fibrosis with intact architecture, stage 3 was septal fibrosis with architectural distortion but no cirrhosis and stage 4 was possible or definite cirrhosis.

Grading was scored from 0-4 with grade 0 being no inflammation, grade 1 portal inflammation without necrosis, grade 2 mild periportal inflammation with focal necrosis, grade 3 moderate periportal inflammation with more extensive necrosis and grade 4 severe periportal inflammation and bridging necrosis [6].

Hepatic steatosis was recorded as the percentage of affected hepatocytes and was graded according to Brunt classification [7]. Grade 0 (0-2%), grade 1 (3-29%), grade 2 (30-59%), and grade 3 (>60%) hepatocytes affected.

BMI was calculated by the formula, bodyweight/height^2 (kg/m^2). According to the proposed classification of weight by body mass index in adult Asians, Body mass index (BMI) of less than 25 was considered normal while BMI of 25 or more was considered as obese [8].

Statistical Methods

A descriptive analysis was done for demographic, clinical and radiographic features and results are presented as the mean ± standard deviation for continuous variables and number (percentage) for categorical variables. In univariate analysis, differences in proportions for steatosis were assessed using the Chi-square test or Fisher exact test where were appropriated. For contrasts of continuous variables, independent sample t-test was used to assess the difference of means. Multiple logistic regression analysis was used to identify the independent risk factors associated with steatosis. Initially, all the variables were selected for a regression model with significant level ≤ 0.25 in univariate analysis or clinical impermanence, but the best model was derived using purpose full selection (enter) method 9 importance of the predictors was assessed by the likelihood ratio test. The goodness of fit test for the final model was examined by using Pearson Chi-square test. The analysis was conducted by using the Statistical package for social science SPSS version 18. All the p-values were two-sided and were considered statistically significant at $p<0.05$.

RESULTS

Biopsies of 439 adult patients performed at our hospital during 2010-2014 were studied. Demographic characteristics, relevant biochemical and serological features of the study group are shown in Table 1.

Table 1 Demographic and virological characteristics of patients studied

Factors	Results n (%)
Gender	
Male	284 (64.6%)
Female	155 (35.4%)
Age (in years)	38.5 ± 10.8

BMI (kg/m^2)	26.2 ± 4.9
Type II Diabetes Mellitus	59 (13.4%)
H/O Alcohol Intake	18 (0.4%)
Biochemistry	
Triglycerides (mg/dl)	177.0 ± 133.5
Cholesterol (mg/dl)	161.3 ± 43.6
ALT	103.7 ± 84.7
Hepatitis Serology	
Hepatitis C	359 (81.8%)
Hepatitis B	34 (7.7%)
Hepatitis B and C	10 (2.3%)
Non-B Non-C	36 (8.2%)
HCV Genotypes (n=160)	
Genotype 3	141 (88.1%)
Genotype non-3	19 (11.9%)

BMI was available in all patients with a mean value of 26.15 kg/m^2 which falls in the obese range for adult Asian population as a whole [8]. Among HCV infected patients, genotype was available in 160 patients, 88% having genotype 3.

Histological evaluation of biopsy specimens reveals that hepatic steatosis was present in 324 (74%) biopsies. It was mild in 190/439 (43%), moderate in 88/439 (20%) and severe in 46/439 (10.5%) cases. Histological characteristics of the patients are shown in Table 2.

Table 2 Histological characteristics of patients studied

Variables	Score	Results n (%)
Inflammation	Grade 0	25 (5.7%)
	Grade 1	112 (25.5%)
	Grade 2	178 (40.5%)
	Grade 3	124 (28.3%)
Fibrosis	Stage 0	94 (21.4%)
	Stage 1	113 (25.7%)
	Stage 2	104 (23.7%)
	Stage 3	70 (16.0%)
	Stage 4	57 (13.2%)
Steatosis	No steatosis	115 (26.2%)
	Mild steatosis	190 (43.3%)
	Moderate steatosis	88 (20.0%)
	Severe steatosis	46 (10.5%)

On univariate analysis HS was associated with HCV infection (p=0.023), BMI >25 (p=0.008) and raised ALT (p=0.003). Association was not statistically significant with gender (p=0.71), diabetes (0.14), hypertriglyceridemia (p=0.09), HBV infection or alcohol intake (p=0.68).

On multiple proportional odds logistic regression, HCV and BMI >25 were independent risk factors for HS. (Table 3). There was a linear ascending association between hepatic steatosis, the grade of inflammation and stage of fibrosis (p<0.001).

Table 3 Independent risk factors of steatosis identified by multiple logistic regression analysis

Factors	Adjusted Odds Ratio	95% CI for Adjusted Odds ratio	p-value
Body mass index			
<25 kg/m^2	1.0	1.2-2.8	0.009
≥ 25 kg/m^2	1.8		
Hepatitis-C			

Negative	1.0	1.0-2.9	0.049
Positive	1.7		

To determine the impact of different factors on steatosis in chronic HCV infection we performed a sub-analysis of HCV infected patients (369). Total 280 (76%) had steatosis. It was mild in 159/369 (43%), moderate in 82/369 (22%) and severe in 39/369 (11%) cases. Genotype 3 was the only independent risk factor for steatosis in HCV infected patients.

Gender (p=0.81), diabetes (p=0.06), alcohol intake (p=0.79), hypertriglyceridemia and BMI >25 (p=0.20) were not statistically significant factors for steatosis in HCV infected patients. There was a linear ascending association of steatosis in chronic HCV with necroinflammation and stage of fibrosis ($p \leq 0.001$) (Figures 1 and 2).

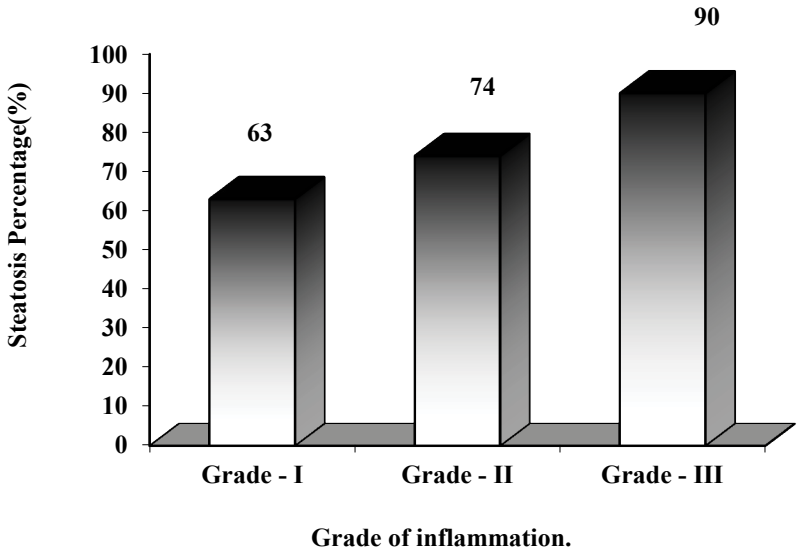

Figure 1 Association between HS and necroinflammation in HCV infected patients

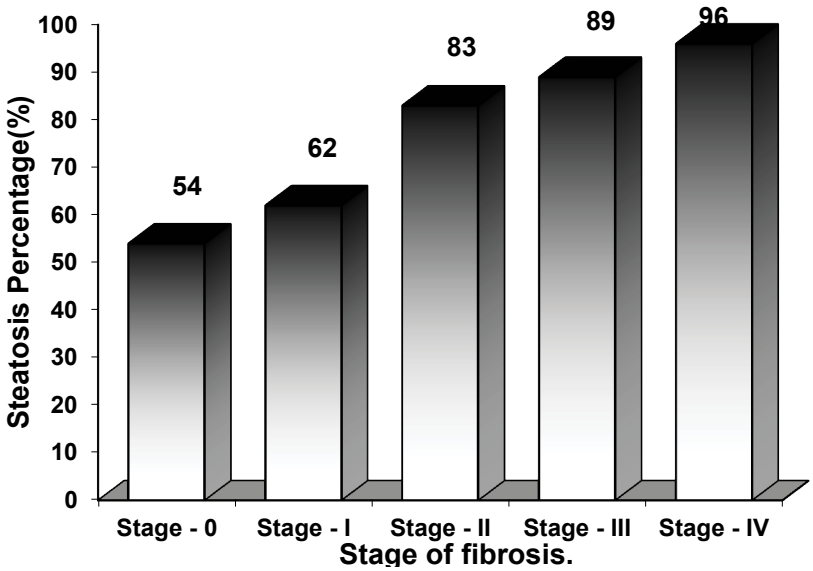

Figure 2 Association between HS and fibrosis in HCV infected patients

Among non-HCV patients (n=70), only 13/70 (18.5%) have moderate or severe steatosis and BMI >25 is the only risk factor for steatosis in this sub-group of patients. These include 34 patients with hepatitis B virus infection and 4

alcoholic patients. There were only 32 non-alcoholic, non-viral hepatitis patients and 8/32 (25%) have moderate or severe steatosis.

DISCUSSION

Hepatic steatosis is a frequent histopathological abnormality which may progress to steatohepatitis and cirrhosis. Major etiological factors include ethanol consumption, metabolic syndrome and HCV infection. Steatosis is an important histological feature of chronic hepatitis C which may affect treatment response. It is also presumed to contribute to the progression of fibrosis in patients with chronic hepatitis C [9,10]. This study was planned to determine the etiology and impact of hepatic steatosis in our patients, especially in chronic hepatic C which constitutes 369/439 (84%) of our study patients.

There is scanty data about the prevalence of hepatic steatosis in the general population as well in patients with metabolic syndrome. Hepatic steatosis has been reported in 5% of some populations and up to 75% in patients with obesity and type II diabetes mellitus [11]. The population-based study is difficult to conduct. In our study of patients with chronic hepatitis, HS was present in 324 (73.8%) biopsies. It was mild in 190/439 (43.3%) and moderate in 88/439 (20%) and severe in 46/439 (10.5%) cases.

Hepatic steatosis is encountered with great frequency among people who consume large amounts of ethanol (more than 6 drinks per day) [12]. History of alcohol intake was not a statistically significant factor for HS in our study. This is most probably due to a very small number of alcoholics in our study. Alcohol intake is not a major problem in our part of the world, so we had a few patients with a history of alcohol intake and most of them were not heavy drinkers.

Obesity is a major health problem in industrialized societies, and hepatic steatosis is common in obese individuals [13]. Oxidative stress originating from increased intracellular levels of fatty acids has been implicated as a cause of hepatocellular injury in steatosis, although the precise mechanisms remain to be elucidated. Bellentani, et al., documented that the prevalence of steatosis in obese patients is 75.8% and obesity increases risk of HS 4.6 fold as compared with control. They also concluded that steatosis is more strongly associated with obesity than with heavy drinking [11]. In our study, BMI >25 were significantly associated with HS on both univariate and multivariate analysis. This fact has been validated by other authors in patients with deranged liver function and normal healthy transplant donors [14,15].

Hepatic steatosis is a well-recognized feature of chronic HCV infection, especially in genotype 3 though the relative importance of host and viral factors is controversial. Prevalence of HS in patients with chronic HCV infection has been reported at around 50%, with a range of 30-70% [2,16]. In our study 75% with chronic HCV have steatosis, 44% of them have moderate to severe steatosis. Hwang and his colleagues reported that 52% of HCV infected patients have significant steatosis [16]. Other studies from Pakistan have also shown a prevalence of 62-65% steatosis in chronic hepatic C [17,18]. In our study, HCV infection is significantly associated with HS on both univariate and multivariate analysis.

Some studies have reported a correlation of hepatic steatosis with body mass index in patients with CHC [13,19]. In our study BMI is not a statistically significant factor for steatosis in chronic hepatitis C. Sharma, et al., in their recent study also concluded that there was no statistical association between the grade of steatosis and increased body mass index in CHC [20]. Herald, et al., did not find any correlation between steatosis and BMI in HCV genotype 3 patients though there was a correlation of hepatic steatosis with BMI in HCV genotype [1,21].

In our study genotype, 3 is the only independent risk factor associated with steatosis in HCV infected patients. Hofer, et al., documented in their study that marked steatosis was found in 74.5% of patients infected with HCV-3a compared with 21.7% in HCV non-3a infected patients ($p<0.01$) [21]. Many other studies have concluded that infection with genotype 3 is the most important risk factor for steatosis in chronic hepatic C [20-22]. On the basis of cross-sectional studies, it has been proposed that hepatic steatosis is a cytopathic effect of hepatitis C virus genotype 3 but not genotype 1 infections. This hypothesis is strengthened by the observations that antiviral treatment altered hepatic steatosis in genotype 3 patients. Kumar, et al., documented that in patients with HCV genotype 1, there was no change in hepatic steatosis after treatment, irrespective of the treatment response [23]. Among those infected with genotype 3, Sustained virological response (SVR) significantly reduced steatosis ($p<0.001$), but there was no change in steatosis among those without an SVR [23]. Castera, et al., in a recent study documented a significant improvement

in steatosis in patients infected with HCV genotype 3 who achieved sustained viral clearance [24]. This provides further evidence for direct involvement of HCV genotype 3 in the pathogenesis of hepatic steatosis. This has been validated by the treatment data of direct-acting viral drugs which have shown improvement in steatosis after SVR in chronic hepatitis patients [4,5].

The association between hepatic steatosis and insulin resistance is well known and type II diabetes may play a role in HCV associated steatosis as has been proposed in NASH [25]. Though steatosis was seen more in HCV infected with diabetes in our study it was not a statistically significant factor. This may be because we have data regarding diabetes in a small number of patients and, fasting serum insulin levels and glucose tolerance test was not available. Several studies have shown that fibrosis and necroinflammation correlate with steatosis in CHC and it may play a role in disease progression [26,27]. In our study, there was a linear ascending correlation of steatosis with necroinflammatory score and stages of fibrosis but Sharma, et al., did not find a correlation between steatosis and fibrosis, though there was a correlation between steatosis and necroinflammation in chronic hepatitis C [20] (Figures 1 and 2).

There are certain limitations of this study including its retrospective design, lack of important data like glucose tolerance test, fasting insulin levels and effect of HCV treatment on steatosis.

CONCLUSION AND RECOMMENDATIONS

In conclusion, hepatic steatosis is present in a significant number of patients with chronic hepatitis. HCV genotype 3 is the major cause of steatosis in our part of the world while NASH is uncommon. Hepatic steatosis is associated with disease progression in chronic hepatic C.

DECLARATIONS

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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