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EFFECT AND ETIOLOGY OF HEPATIC STEATOSIS IN PAKISTAN: IMPORTANCE OF GENOTYPE 3 HEPATITIS C VIRUS INFECTION

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Abstract:

Introduction: *Fatty liver has become an important histological symptom in patients with hepatic dysfunction. It may be an important factor in 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 with chronic hepatitis.*

Place and Duration: *In the Pathology and Gastroenterology department of Jinnah Hospital Lahore for one-year duration from May 2019 to May 2020.*

Methods: *All liver biopsies performed in our hospital were analyzed by one pathologist using the results of the histological activity index (HAI) and Brunt's grading for steatosis. Patients were assessed for factors related to steatosis, including the presence of HCV.*

Results: *The biopsies of 439 patients (284 men, mean age 38.5 ± 11.2 years) were examined. Fatty liver was found 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. In a one-way analysis, steatosis was associated with HCV infection ($p = 0.023$), BMI> 25 ($p = 0.008$), and elevated ALT ($p = 0.003$), but not with diabetes mellitus, hypertriglyceridemia, HBV infection, or alcohol consumption. In multiple logistic regression, HCV and BMI> 25 were independent risk factors for steatosis. There was a linearly increasing relationship between fatty liver disease and the degree and severity of liver disease ($p \leq 0.001$). Of the 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 patients without viral hepatitis, and 8/32 (25%) had moderate to severe steatosis.*

Conclusions: *Significant fatty liver occurs in 30.5% of our patients with chronic hepatitis. HCV infection with genotype 3 is the main causative agent of fatty liver in Pakistan. Steatosis has a linearly increasing correlation with hepatitis and fibrosis.*

Key words: *fatty liver, hepatitis C virus, body mass index*

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INTRODUCTION:

Non-alcoholic fatty liver disease (NAFLD) and hepatitis C virus (HCV) infection are the most common forms of liver disease 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[1-2]. The major etiological factors associated with fatty liver are alcohol consumption, metabolic syndrome, and HCV infection. Epidemiological studies have shown that HCV-associated steatosis correlates with both patient factors such as obesity and viral factors such as HCV genotype 3a. Moreover, in several studies, the degree of steatosis has been associated with the degree of liver fibrosis, suggesting that steatosis may contribute to disease progression in chronic HCV infection[3-4]. Recent studies suggest that fatty liver in chronic hepatitis C (CHC) may be an expression of the direct cytopathic effect of the hepatitis C virus, especially in patients infected with genotype 3[5-6]. The relationship between fatty liver in HCV patients and known risk factors for fatty liver such as increased BMI, hyperlipidemia, alcohol consumption, and diabetes mellitus is not well understood. In the past, some studies have shown significant improvement in steatosis in patients with HCV genotype 3 who achieved sustained viral clearance after HCV treatment [3]. This provides further evidence of a direct involvement of HCV genotype 3 in the pathogenesis of fatty liver. Several recent studies with direct-acting antiviral drugs used in the treatment of CHC have shown a significant resolution of fatty liver after a sustained virological response[7-8]. The impact of HCV eradication on fatty liver and progression to fibrosis requires further research in the era of direct-acting antiviral drugs. The aim of our analysis was to determine the etiology of fatty liver in our patients with chronic hepatitis. The second goal was to search for comorbidities that may be associated with steatosis in HCV and to determine whether the severity of steatosis leads to an increase in hepatic necro-inflammatory lesions and fibrosis.

MATERIALS AND METHODS:

All liver biopsies (n = 439) were assessed in the Pathology and Gastroenterology department of Jinnah Hospital Lahore for one-year duration from May 2019 to May 2020. Patient demographics such as height, weight, risk factors for steatosis, e.g. diabetes taken, alcohol consumption, serum cholesterol and triglyceride levels, viral serology, HCV genotype. One histopathologist, who was blinded by laboratory and clinical data, re-evaluated the liver biopsies. Biopsy specimens were fixed, embedded in paraffin and stained with hematoxylin and eosin. In addition, special staining was performed on each sample, including PAS and reticulin (for collagen). Classification and staging were performed using the Scheuer system. Fibrosis was rated on a scale of 0 to 4, with grade 0 being no fibrosis, grade 1 minimal portal fibrosis, grade 2 was periportal fibrosis with intact architecture, grade 3 was septal fibrosis with architectural distortion but no cirrhosis, and stage 4 was possible or pronounced cirrhosis of the liver. Grade 0 to 4 with 0 being no inflammation, Grade 1 non-necrotic portal inflammation, Grade 2 mild periportal inflammation with focal necrosis, Grade 3 moderate periportal inflammation with more extensive necrosis and Grade 4 severe periportal inflammation and sternal necrosis [6]. Fatty liver was recorded as a percentage of diseased hepatocytes and assessed according to the Brunt classification [7]. Grade 0 (0-2%), Grade 1 (3-29%), Grade 2 (30-59%) and Grade 3 (> 60%) hepatocytes involved. BMI was calculated according to the formula: body weight / height2 (kg / m2). According to the proposed classification of body mass by body mass index in adult Asians, a body mass index (BMI) of less than 25 was considered normal, and a BMI of 25 or more was considered obese [8]. Statistical methods A descriptive analysis of demographic, clinical and radiographic characteristics was performed and the results were presented as mean ± standard deviation for continuous variables and number (percent) for categorical variables. In the univariate analysis, the differences in proportions for steatosis were assessed using the Chi-square test or the Fisher's exact test where they were applied. For the contrasts of the continuous variables, the t-test for the independent sample was used to evaluate the mean difference. Multiple logistic regression analysis was used to identify independent risk factors associated with steatosis. Initially, all variables were selected for a regression model with a significance level ≤ 0.25 in univariate or clinical instability analysis, but the best model was derived using the full-selective (enter) method 9. The importance of the predictors was assessed by the likelihood-ratio test. The concordance test for the final model was tested using the Pearson Chi-square test. The analysis was performed using the SPSS version 18 social science statistical package. All p-values were two-sided and were considered statistically significant with p less than 0.05.

RESULTS:

The biopsies of 439 adult patients performed were examined. The demographic characteristics, important biochemical and serological features of the study group are presented 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 / m2, which is within the obesity range for the entire Asian adult population. Among HCV infected patients, genotype was available in 160 patients, 88% of whom were genotype 3. Histological evaluation of biopsy specimens showed that fatty liver 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.

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%)

The histological characteristics of the patients are presented in Table 2.

In one-way analysis, HS was associated with HCV infection (p = 0.023), BMI> 25 (p = 0.008), and elevated ALT (p = 0.003). The association was not statistically significant with gender (p = 0.71), diabetes mellitus (0.14), hypertriglyceridemia (p = 0.09), HBV infection, or alcohol consumption (p = 0.68). In logistic regression of multiple proportional odds, HCV and BMI> 25 were independent risk factors for HS. (Table 3). There was a linearly increasing association between fatty liver, degree of inflammation, and degree of fibrosis (p <0.001). To determine the influence of various factors on steatosis in chronic HCV infection, we sub-analyzed HCV infected patients

(369). A total of 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 mellitus (p = 0.06), alcohol consumption (p = 0.79), hypertriglyceridemia and BMI> 25 (p = 0.20) were not statistically significant factors in steatosis in HCV infected patients.

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		

There was a linearly increasing association of steatosis in chronic HCV with necrotic inflammation and the fibrosis stage (p ≤ 0.001). Among patients without HCV (n = 70), only 13/70 (18.5%) have moderate or severe steatosis, and a BMI> 25 is the only risk factor for steatosis in this subgroup of patients. Among them there are 34 patients with hepatitis B infection and 4 alcoholics. There were only 32 non-alcoholic patients without viral hepatitis, and 8/32 (25%) had moderate to severe steatosis.

DISCUSSION:

Fatty liver disease is a common histopathological abnormality that can lead to steatohepatitis and cirrhosis. The main etiological factors include ethanol consumption, metabolic syndrome and HCV infection. Steatosis is an important histological feature of chronic hepatitis C that can affect treatment response. It is also believed to contribute to the progression of fibrosis in patients with chronic hepatitis C[9-10]. The aim of this study was to determine the etiology and impact of fatty liver in our patients, especially in chronic C, which is 369/439 (84%) of our subjects' patients. There is little data on the prevalence of fatty liver in the general population as well as in patients with metabolic syndrome. Fatty liver has been reported in 5% of some populations and up to 75% in obese patients with type II diabetes. A population 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[11]. Fatty liver disease occurs with high frequency in people who consume large amounts of ethanol (more than 6 drinks a day). History of alcohol consumption was not a statistically significant factor for HS in our study. This is most likely due to the very low number of alcoholics in our study. Alcohol consumption is not a major problem in our part of the world, so we have had several patients who have consumed alcohol in the past, and most of them did not drink much. Obesity is a major health problem in industrialized societies, and fatty liver disease is common in obese people. Oxidative stress from increased intracellular fatty acid levels has been recognized as a cause of liver damage in steatosis, although the exact mechanisms remain to be elucidated. Bellentani et al. Have documented that the prevalence of steatosis in obese patients is 75.8%, and obesity increases the risk of HS by 4.6 times compared to the control group[11]. They also concluded that fatty tissue is more strongly associated with obesity than with heavy drinking. In our study, BMI> 25 was significantly associated with HS in both univariate and multivariate analyzes. This fact has been confirmed by other authors in patients with impaired liver function and healthy transplant donors. Fatty liver is a well-recognized feature of chronic HCV infection, especially in genotype 3, although the relative importance of the host and viral factors is controversial. The incidence of HS in patients with chronic HCV infection is approximately 50%, with a range of 30-70%. In our study, 75% of those with chronic HCV had steatosis, 44% of them had moderate to severe steatosis. Hwang and his colleagues reported that 52% of HCV-infected patients had significant steatosis[12-13]. Other studies from Pakistan also found the presence of 62-65% of steatosis in chronic liver C. In our study, HCV infection is significantly associated with HS in both univariate and multivariate analysis. Some studies have shown a correlation between fatty liver and body mass index in patients with CHC. In our study, BMI is not a statistically significant factor for steatosis in chronic hepatitis C. Sharma et al. In their recent study, they also found that there is no statistical significance. relationship between the degree of steatosis and increased body mass index in CHC. Herald et al. Found no correlation between steatosis and BMI in patients with HCV genotype

3, although there was a correlation between fatty liver and BMI in HCV genotype. In our study, genotype 3 is the only independent risk factor for steatosis in HCV-infected patients. Hofer et al. Documented in their studies that significant steatosis was found in 74.5% of HCV3a infected patients compared with 21.7% of HCV 3a uninfected patients (p <0.01). Many other studies have found that genotype 3 infection is the most important risk factor for steatosis in chronic hepatitis C. Based on cross-sectional studies, it has been proposed that fatty liver is a cytopathic effect of hepatitis C virus genotype 3, but not genotype 1 infection. confirm the observations that antiviral treatment modifies steatosis in patients with genotype 3. Kumar et al. Documented that in patients with genotype 1 HCV there was no change in fatty liver after treatment, regardless of treatment response[14]. Among genotype 3 infected subjects, sustained viral response (SVR) significantly reduced steatosis (p <0.001), but there was no change in steatosis among those without SVR. Castera et al. In a recent study, they documented significant improvement in steatosis in genotype 3 HCV infected patients who achieved sustained viral clearance. This provides further evidence of a direct involvement of HCV genotype 3 in the pathogenesis of fatty liver. This was supported by data on treatment of direct acting viral drugs that showed improvement in steatosis after SVR in patients with chronic hepatitis. The relationship between fatty liver disease and insulin resistance is well known, and type II diabetes may play a role in fatty liver disease as proposed in NASH. Although steatosis was observed more frequently in HCV infected individuals with diabetes in our study, it was not a statistically significant factor. This may be due to the fact that we have data on diabetes in a small number of patients and that fasting serum insulin and glucose tolerance test were not available[15].

CONCLUSION:

In summary, fatty liver occurs in a significant number of patients with chronic hepatitis. HCV genotype 3 is the leading cause of steatosis in our part of the world, while NASH is rare. Fatty liver is associated with disease progression in chronic hepatitis C.

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