

FREQUENCY OF STEATOSIS AND ITS RELATION WITH THE GRADE OF FIBROSIS IN PATIENTS WITH HEPATITIS C

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ABSTRACT

Objective: To study the frequency of steatosis and observe the relation between steatosis and grade of fibrosis in patients with hepatitis C.

Methodology: This descriptive case series study was undertaken at Liaquat University of Medical & Health Sciences hospital from July 2005 to November 2007. It included 158 PCR-positive hepatitis C cases with genotype 3. Patients demographic data was enrolled in well designed proforma BMI was calculated and history of diabetes mellitus was obtained. Liver biopsy was done after written consent and was sent for grading of fibrosis and steatosis. T-test was applied for Continuous variables whereas stage of fibrosis was compared with grade of steatosis, BMI and age by chi-square test. 0.05 was made a level of Significance.

Results: This study included 158 patients out of which 109 (69%) were male and 49(31%) were female. The mean age of the patient was 36.8± 9.8. The BMI was <25 in 86(54.4%) whereas BMI 25-30 was present in 53 (33.5%) and BMI >30 in 19 (12%) of cases. The steatosis was found in 71(45%) of cases. Mild (<30% of hepatocytes involved) 33(21%), moderate (30-60% hepatocytes involved) in 26 (16.5%) and severe (>60% hepatocytes involved) steatosis in 12(7.5%) cases. A strong correlation between steatosis score and fibrosis stage was observed in our study (P= < 0.001) whereas no relationship was observed between BMI (P = 0.67) or age (P =0.39) with stage of steatosis.

Conclusion: This study showed that increased steatosis is associated with worsening fibrosis suggesting a possible role for steatosis in the acceleration of liver disease in HCV Patients and efforts to control steatosis may therefore have an important role in halting HCV liver disease progression.

KEYWORDS: Steatosis, Fibrosis, Hepatitis C, PCR, BMI.

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INTRODUCTION

Hepatitis C virus is a major cause of chronic liver disease with about 170 million people infected worldwide.¹

The severity of disease varies widely from asymptomatic chronic infection to cirrhosis and hepatocellular carcinoma.¹ Although most HCV associated liver damage is immunomediated,² some histopathological features, such as liver steatosis, suggest a viral cytopathic effect.³

Several observations indicate that steatosis may be directly due to HCV: its association with genotype 3,⁴ correlation between its severity and level of HCV replication,⁵ and its disappearance on response to antiviral therapy.⁶ However, some data suggest that the pathogenesis of mild steatosis of most HCV infected patients may be metabolic as its severity correlates with body mass index (BMI)⁷ whereas only the moderate to severe steatosis typically found in patients with genotype 3 may be HCV related.⁷ Thus steatosis observed in chronic hepatitis C is not always virally related as other factors may coexist. This is not surprising considering the frequency of liver steatosis in the general population (15%).⁸ A major question concerns the impact of steatosis on liver disease progression, as suggested by some authors.⁹

Cohort studies on patients with non-alcoholic fatty liver disease show that simple steatosis runs a benign nonprogressive clinical course.¹⁰ However, steatosis in chronic hepatitis C is almost invariably accompanied by some degree of necroinflammation. Thus steatosis may contribute to liver disease progression either directly or via a synergistic effect with inflammation or other cofactors.

Our aim in this study was to determine the frequency of steatosis in patients with hepatitis C and to explore the relation between steatosis and other risk factors for steatosis such as BMI and age with steatosis in patients with hepatitis C.

METHODOLOGY

Study population: This study included 158 consecutive, Anti HCV ,HCV RNA positive, and genotype 3 patients admitted in Liaquat University Hospital Jamshoro/Hyderabad between July 2005 and March 2007. Patients with HCV genotypes except genotype3, active Liver disease related to hepatitis B virus, stigmata of autoimmune liver disease (as defined by international criteria),¹¹ patients with hyperlipidemia: total cholesterol and/or triglycerides >200 mg/dL; alcoholism (ingestion of >40g ethanol per day for men and >20g ethanol per

day for women);diabetes mellitus: fasting glycemia >126mg/dL⁸ and/or use of oral hypoglycemics or insulin; and use of potential steatosis-inducing drugs such as corticosteroids, estrogen, amiodarone, nifedipine or diltiazem during the 6 months preceding the liver biopsy were excluded from the study.¹²

This study was conducted in conformity with the Helsinki declaration and all patients consented to participate.

All patients fulfilling the above criteria were studied to assess the relationship between steatosis and fibrosis. The following data were entered on a clinical database: age, gender, height, weight, presence or absence of diabetes, and BMI (kg/height in meters²). Blood samples for liver functions test, prothrombin time, Protein profile, blood glucose, lipid profile, blood complete blood picture with platelet count were collected before the biopsy. Liver biopsy was performed under local anesthesia by a well trained person. A Tru-cut needle (14-gauge) was used and the procedure was conducted under ultrasound guidance. An adequate biopsy sample defined as specimen size greater than 10mm and more than 5 portal tracts was obtained in all patients.¹³ No major complications such as requirement of blood transfusion, hypotension or biliary peritonitis were observed. A single well qualified histopathologist who was unaware about the clinical data assessed the biopsy slides.

The degree of hepatic fibrosis was staged according to Metavir fibrosis score as: F0= no fibrosis; F1= fibrous portal expansion; F2= fibrous bridging fibrosis (portal-portal or portal-central linkage); F3= bridging fibrosis with lobular distortion(disorganization); and F4= cirrhosis.¹⁴ The severity of steatosis was graded as 0 or absent (<1% of total hepatocytes), 1 or mild (between 1% and 30% of hepatocytes), 2 or moderate (between 30% and 60% of hepatocytes), and 3 or severe (>60% of hepatocytes).¹⁵ The patients were divided into three categories with category one having BMI <25(kg/m²) ,category two with BMI 25-30(kg/m²) and category three with BMI >30(kg/m²).¹⁶

Statistical procedure: Descriptive statistics are provided as means \pm 1 SD. The t-test was used to compare quantitative data, and the chi-square test was used for categorical data. P-values <0.05 were considered significant. All analyses were carried out using SPSS version 16 software (SPSS, Inc, Chicago, IL).

RESULTS

This study included 158 patients out of which 109 (69%) were male and 49(31%) were female. The mean age of the patients was 36.8 ± 9.8 . The Body mass index (BMI) of 86 (54.4%) patients was $<25 \text{kg/m}^2$ whereas BMI of 53(33.5%) was between $25\text{-}30 \text{kg/m}^2$ and 19 (12%) patients had $\text{BMI} >30 \text{kg/m}^2$. Liver biopsy showed stage 0 fibrosis (F0) in 24(15.2%) stage one fibrosis (F1) in 50(31.6%), stage two (F2) in 50(31.6%), stage three (F3) in 28 (17.7%) and stage four (F4) in 6 (3.9%) patients. On histological assessment steatosis was present in 71/158 (45%) of cases of which mild Steatosis was found in 33(20.9%), moderate steatosis in 26 (16.5%) and severe steatosis in 12(7.6%) cases. Table-I shows the characteristics of all patients. A strong correlation between

steatosis score and fibrosis stage was observed in our study ($P = <0.001$) as shown in Fig.1 which describe that as the fibrosis progressed so as the steatosis. The patients with $\text{BMI} <25 \text{kg/m}^2$ 37/71 had mild to severe steatosis, 23 / 71 overweight patients with $\text{BMI} 25\text{-}30 \text{kg/m}^2$ had mild to severe steatosis whereas 11/71 obese patients with $\text{BMI} >30 \text{kg/m}^2$ had mild to severe steatosis. A poor relationship was observed between steatosis and BMI ($P = 0.67$) as shown in Fig-2. A nonsignificant relationship was found between age ($P = 0.39$) with grade of steatosis.

DISCUSSION

In this study, steatosis was present in 45% liver biopsies of patients with hepatitis C. This figure is near to the results of the J Wyatt¹¹ who has seen steatosis in 50% of biopsies. According to Zahid et al. who studied 76 patients with Hepatitis-C and found steatosis in 67.5% of cases.¹⁷ In a recently published study by Alia Zubair steatosis was present in 46% of biopsies in 100 patients with Hepatitis-C.¹⁸ We found a highly significant association between steatosis and stage of fibrosis, as has previously been demonstrated in several studies. L Rubbia-Brandt et al. have shown that in

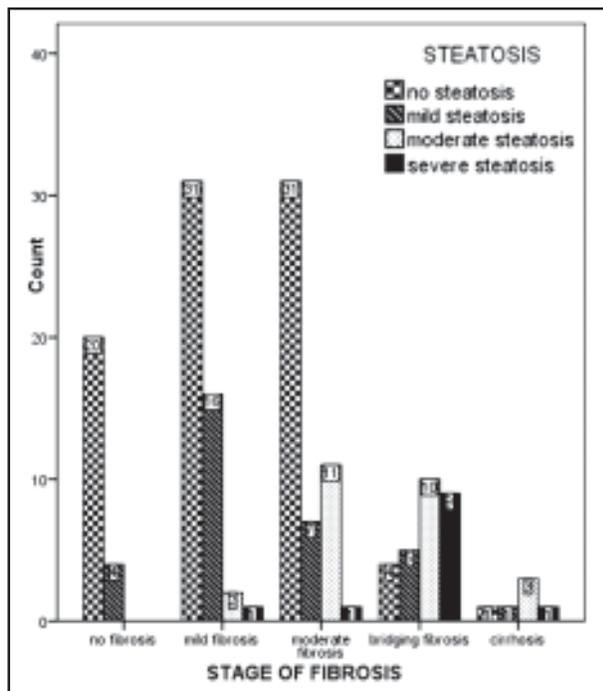


Fig-1: Relationship of steatosis with fibrosis ($p < 0.001$)

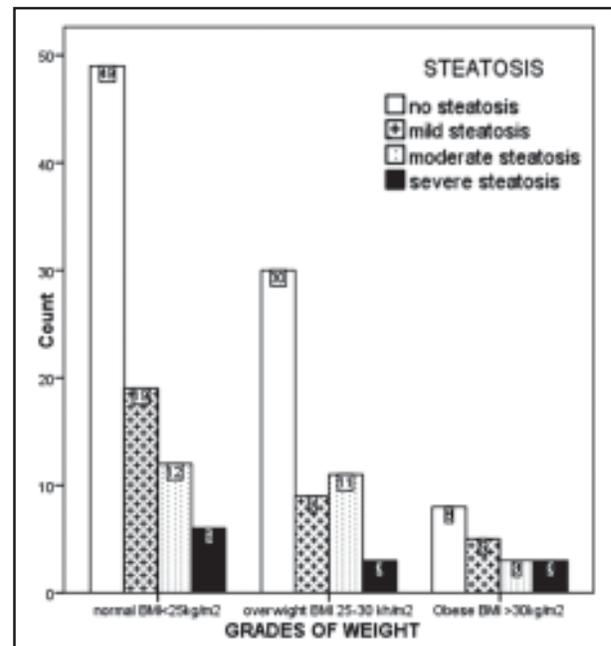


Fig-2: Relationship of steatosis with BMI ($p = 0.638$)

Table-I: Characteristics of all Patients

Quantitative Variables	No. of Pts	Mean Difference	Std. Deviation	95% Confidence Interval of the Difference	
AGE	158	36.81013	9.81624	35.2676	38.3526
WEIGHT	158	64.94531	12.46937	62.7644	67.1263
BMI (kg/m) ²	158	26.13291	4.20639	25.4719	26.7939
INR	158	1.03259	.06842	1.0218	1.0433
PALELET COUNT 10 ⁹	158	236.81646	82.22091	223.8965	249.7365
SGPT IU/L	158	77.75633	60.86619	68.1920	87.3207
SGOT IU/L	158	56.05696	38.84974	49.9522	62.1617
Qualitative Variables	Frequency	percentage			
male	109	69			
female	49	31			
Normal	86	54.4			
BMI < 25 kg/m ²					
overweight	53	33.5			
BMI 25-30 kg/m ²					
Obese	19	12.0			
BMI > 30 kg/m ²					
non-diabetic	132	83.5			
no fibrosis	24	15.2			
mild fibrosis	50	31.6			
moderate fibrosis	50	31.6			
bridging fibrosis	28	17.7			
cirrhosis	6	3.8			
no steatosis	87	55.1			
mild steatosis	33	20.9			
moderate steatosis	26	16.5			
severe steatosis	12	7.6			

Abbreviations: SGPT = aspartate aminotransferase

SGOT = alanine aminotransferase

BMI = Body mass index

chronic Hepatitis C, steatosis may influence liver fibrosis progression in a genotype specific way. In patients with genotype 3, the presence of steatosis, which is due to HCV replication and is frequently moderate to severe, correlates with the liver fibrosis sc.¹⁹

Wyatt, et al had also found a highly significant association between steatosis and stage of fibrosis in non-cirrhotic biopsies.¹¹ Adinolfi et al further strengthened the idea that presence of a significant amount of steatosis (i.e. >20%) in chronic Hepatitis-C patients will increase the hepatic fibrosis with a rate two times

faster than those without.⁶ Castera et al in a recent follow-up study, centered on serial liver biopsies obtained over time, confirmed that worsening of steatosis was the only independent factor associated with hepatic fibrosis progression.²¹ Leandro G et al in a meta-analysis, which included individual patient data of more than 3000 subjects with Chronic Hepatitis-C has demonstrated that liver steatosis is strictly associated with increased liver inflammatory activity and accelerates the progression of liver fibrosis.²²

The presence of steatosis in patients with Hepatitis-C is dependant on a complex interaction of viral and host related Factors.²³ Steatosis in patients without Hepatitis-C is related to alcohol consumption, obesity, high BMI, type II diabetes, and hyperlipidaemia.²⁴ These factors are also important in patients with Hepatitis-C, but a proportion of patients with Hepatitis-C has no other risk factor for steatosis. In particular, this has been reported to be a feature of genotype 3 infection, so that patients with moderate to severe steatosis without other risk factors are probably infected with genotype 3.²⁵ It has been suggested that steatosis acts by fuelling the free radical production associated with expression of the HCV core protein, amplifying the cytopathic effect of HCV.²⁶

In our study no relationship of degree of steatosis with age was found. A recent study by Poynard, not showing any genotype-dependent risk of cirrhosis, proposed that the main part of the fibrosis progression in HCV infec-

tion occurs in patients older than 50 years.²⁷ Wong *et al.* observed that older age was independently associated with more advanced stages of fibrosis in a group of 140 patients with chronic HCV infection.²⁸ No patients in our study was more than 45 years old. The reason for this nonsignificant relation between age and steatosis is possibly due to the fact that patients in our study were younger and possibly had a shorter duration of disease.

No direct relationship of BMI with fibrosis was found in our study. According to Adinolfi LE the correlation between BMI and the grade of steatosis did not reach statistical significance ($P=0.068$) when all patients were included in the evaluation. When the analysis was done for each genotype, the grade of steatosis in genotype one infection correlated with the BMI ($P = 0.001$), and a value close to statistical significance was found in patients infected with genotype 2a/c ($P = 0.078$), whereas no correlation was observed in those with 3a infection.⁶ Sharma *et al.* stated that the BMI correlated with steatosis in patients with genotype and after adjusting for the confounded factors, only genotype 3 correlated independently with steatosis. These results support the hypothesis that mild steatosis seen in non genotype 3 HCV patients may be metabolic in origin and the fact that steatosis did not correlate with BMI in HCV genotype 3 infection may point toward the direct role of virus in the pathogenesis of steatosis.²⁹ Recently, Hourigan *et al.* suggested that the connection between increased BMI and liver steatosis may contribute to the development of fibrosis in CHC.³⁰ Future metabolic studies should help clarify whether certain distributions of fat are more pertinent to steatosis than others.

CONCLUSION

This study shows that steatosis is strongly associated with increased fibrosis in liver biopsies. There is increasing evidence that steatosis reflects an interaction of viral and host factors important in the generation of fibrosis in the liver. Therefore, patients with steatosis in early stage disease may represent a group

at increased risk of progressive fibrosis. Future studies will be required to resolve the issue of age and BMI with the fibrosis.

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