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Non Alcoholic Fatty Liver Diseases in Chronic Hepatitis C: Impact on End Treatment Virologic Response

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Abstract

Background: Both nonalcoholic fatty liver disease (NAFLD) and chronic hepatitis C (CHC) are frequent causes of chronic liver disease. Questions remain regarding the etiology of steatosis and steatohepatitis in hepatitis C, and its impact on disease progression and treatment outcomes.

Objectives: To estimate the association of NAFLD with CHC and to evaluate the effect of steatosis and non alcoholic steatohepatitis (NASH) on the end treatment virologic response of combined anti-viral therapy in such patients.

Subjects and Methods: Eighty-nine naive PCR and biopsy proven CHC patients were included. Clinical, demographic and laboratory data at the time of liver biopsy were obtained. One pathologist reviewed all pathologic specimens using the modified histological activity index score and the Ishak staging for fibrosis and NAFLD pathologic protocol. All patients received combined antiviral therapy in the form of Pegylated Interferon α 2a plus Ribavirin.

Results: Forty-five (50.6%) out of 89 CHC patients had associated NAFLD among which 11 patients encounter superimposed steatohepatitis (NASH). The overall end treatment virologic response was achieved in 61 cases (68.54%). End treatment virologic response was significantly lower (51%) in NAFLD group compared with 86.36% in group not affected by NAFLD (P=0.000). Variables associated with the NAFLD group were: higher serum levels of Aspartate transaminase AST (P<0.001), Alanine transaminase ALT (P<0.001), gamma glutamyle transferase γ-GT (P=0.003), severity of histolopathologic activity (grading, P=0.018) and extent of fibrosis (staging, P=0.020). In the responder group, body mass index (BMI) was significantly lower compared to nonresponder group (P=0.011). Applying the model of logistic regression analysis revealed that NAFLD can be considered as independent risk factor for poor response to combined pegylated interferon α 2a plus ribavirin therapy (odds ratio 0.039, P=0.002).

Conclusion: Overall end treatment virologic response for patients with HCV and significant

steatosis or steatohepatitis is considerably lower than that for HCV in absence of NAFLD.

Keywords: Nonalcoholic fatty liver disease (NAFLD), Chronic hepatitis C (CHC), Non alcoholic steatohepatitis (NASH), Interferon α 2

Background

It is estimated that, worldwide, the number of people with positive hepatitis C antibody is 170 million with a prevalence of 3% (Violante and Nunez-Nateras, 2007), its progression leads to chronic hepatitis, cirrhosis and eventually to hepatocellular carcinoma; and endstage liver disease (Murray and Carithers, 2005). There is a significant regional variation in prevalence, with the highest occurring in North Africa and the Middle East (more than 3%) (Armstrong et al., 2002).

A number of concomitant viral and host-related factors such as HCV genotype, viral load, age, gender, alcohol consumption, obesity, iron overload, stage of fibrosis and coinfection with hepatitis B virus are considered to have an impact on disease progression and response to antiviral treatment (Afdhal, 2004; Guidi et al., 2005; Marcellin et al., 2002; Poynard et al., 2001; Seeff, 2002; Shiffman, 2003; Sulkowski et al., 2000).

Nonalcoholic fatty liver disease (NAFLD) encompasses a continuum that ranges from simple hepatic steatosis to nonalcoholic steatohepatitis (NASH). NAFLD are common features in patients with chronic hepatitis C, detected in 30–70% of patients *(*Lonardo, 2004; Ramesh & Sanyal, 2004). However, HCV infection may also play a direct pathogenic role, influencing lipoprotein metabolism and insulin sensitivity (Petit et al., 2003; Rubbia-et al., 2000; Serfaty et al., 2001; Shintani, 2004).

Both host and viral factors contribute to the complex link between NAFLD and chronic hepatitis C (CHC) infection and appear to have genotypic influences. Host dependent factors include alcohol use, visceral obesity, body mass index (BMI), hyperlipidaemia and genetic background (Adinolfi et al., 2005).

NASH describes a subtype of NAFLD that shows hepatic steatosis associated with hepatocyte injury (ballooning degeneration, parenchymal inflammation, Mallory bodies and fibrosis). Some patients with NASH can progress to cirrhosis or end stage liver disease (Hubscher ,2006).Current estimates make the prevalence of NASH 23% in the general population (*Falck Ytter et al.*,2001).

While there is growing evidence that steatosis contributes to the progression of fibrosis (Adinolfi et al., 2005; Rubbia-Brandt et al., 2000). Its impact on the response to antiviral treatment remains controversial (Adinolfi et al., 2001; Hourigan et al., 1999; Patton et al., 2004; Rubbia-Brandt et al., 2000; Westin et al., 2002).

Aim of the study

To estimate the association of NAFLD with CHC and to evaluate the effect of steatosis and NASH on the end treatment virologic response of combined antiviral therapy in CHC.

Patients and methods

One hundred and twenty naive (no history of previous antiviral therapy) HCV patients referred to tropical medicine outpatient clinic, Mansoura University Hospital, were initially enrolled in this study during the period from January 2006 to January 2008.

Inclusion criteria included anti-HCV and HCV-RNA positivity, elevated ALT and AST levels at least twice for 6 months, chronic inflammation on liver histology, no alcohol consumption and no contraindications to interferon and ribavirin administration.

Exclusion criteria included patients below 20 or above 60 years old, patients with liver cirrhosis (fibrosis stage V and VI in liver biopsy), patients with Diabetes Mellitus (DM), patients with concomitant chronic hepatitis B infection or obese patients with BMI more than 35. Thirty one such patients (19 male and 12 female) fulfilling the above mentioned criteria

were excluded. Finally the study was conducted on only 89 patients.
Clinical and demographic information, including age, gender, weight, height, BMI (calculated as weight in kilograms divided by height in meters squared), laboratory data (complete blood picture, bilirubin, alanine transferase (ALT), aspartate transferase (AST), albumin, alkaline phosphatase, gamma glutamyl transferase (γ-GT), serum glucose, serum cholesterol, serum triglyceride and HCV-RNA level) were recorded at the time of liver biopsy. Informed consent was obtained from each patient and this study was approved from Mansoura Ethical Committee.
Every patient in this study was subjected to history taking, physical examination, abdominal ultrasound and histological assessment. Laboratory data were periodically monitored for all studied patients during the course of the antiviral combination therapy.
The presence of HCV infection was diagnosed by the use of enzyme linked immunosorbent assay (ELISA) (Murex anti-HCV kit, version 3.0) to detect HCV antibodies, which was confirmed by the use of reverse transcription polymerase chain reaction (PCR) to detect HCV-RNA. HCV-RNA levels were done before treatment and at 12, 24 and 48 weeks after initiation of the antiviral combination therapy.
HCV genotyping was not performed for these patients as multiple previous studies revealed that HCV genotype 4 is the most dominant genotype in Egypt (Chamberlain et al., 1997; Ray et al., 2000; Simmonds et al., 1993).
Blood sample was withdrawn from every subject in this study. The separated serum was divided into two aliquots. The first aliquot was used for estimation of liver functions (serum bilirubin, albumin, ALT, AST, alkaline phosphatase and γ-GT) using automatic autoanalyzer Hitachi 902, Roche Diagnostics. The second aliquot was stored at –70°C until time of assay of RTPCR.

Detection and quantification of HCV-RNA in serum: Detection of HCV-RNA in serum was performed by automated RT-PCR assay (COBAS AMPLICOR HCV Test, version 2.0; Roche Diagnostics, Molecular Division). The amplicor HCV monitor test V 2.0 is based on five major processes (Ming-Lung et al., 2000); specimen preparation, reverse transcriptase of target RNA to generate complementary DNA, PCR amplification of target DNA using HCV complementary primers, hybridization of the amplified products to oligonucleotide probes specific to the targets and detection of the probe – bound amplified products by colorimetric determination.

Histopathological assessment: Liver biopsy specimens were reviewed by a single pathologist, who was blinded to the patients' clinical information. For each liver biopsy specimen, hematoxylin and eosin, Masson's trichrome, reticuline stains were available. The histological activity (grade) and degree of fibrosis (stage) of the viral hepatitis were assessed according to the modified histological activity index (HAI) of Ishak (Ishak et al., 1995).
The histopathological diagnosis of associated NAFLD was determined by the presence of steatosis which was graded according to the Brunt grading system, based on percentage of hepatocytes involved: grade 0 none involved, grade 1(mild) up to 33%, grade 2 (moderate) up to 66% and grade 3 (severe) more than 66% (Brunt et al., 1999). NASH was diagnosed according to previous reports by presence of pericentral steatosis, hepatocellular ballooning degeneration and Mallory hyaline, mixed neutrophilic and lymphocytic intralobular infiltrate and pericellular fibrosis in zone 3 (Younossi et al., 1998).

Treatment regimens: All patients were treated with a combination therapy and underwent subcutaneous pegylated interferon α 2a (180 μg weekly) and ribavirin, administered orally from 800 to 1200 mg/day, according to body weight (less than 65 kg, 800 mg/day; 65–85 kg, 1000 mg/day; more than 85 kg, 1200 mg/day). The duration of treatment was 48 weeks. Optimal adherence to treatment regimens were monitored during the course of treatment.
Patients were classified as nonresponders, if there is failure for 2 log. reduction of the viral load after 12 weeks of treatment or if HCV RNA was detectable at end of therapy and end of treatment responders if there is undetectable HCV RNA at the end of therapy.

Analysis of data: The statistical analysis of data was used by using SPSS program (Statistical Package for Social Science) Version 10. The parametric data was summarized using mean ± standard deviation; nonparametric data was summarized using median (minimum-maximum) and number& percentage for qualitative data.

To test statistical significant difference between groups, independent sample t-test was used to compare between two groups for parametric data, Mann-Whitney test was used in non-parametric data. Chi-Square test was used for qualitative data (number & percentage), Multivarate regression analysis was used using significant data in univarate analysis to know the predictors of the response to treatment, Odds ratios and 95% of confidence intervals were calculated. Probability (P) value < 0.05 was considered significant.

Results

Eighty-nine patients with chronic HCV infection were included in this study after exclusion of patients with fibrosis stage V and VI in liver biopsy, patients with DM or BMI more than 35.
Table 1 summarizes the clinical and histopathologic characteristics of the patients. Forty-four patients (49.4%) had no hepatic steatosis (grade 0) on the liver biopsy, while NAFLD was seen in 45 patients (50.6%) among which 19 (21.4%) had grade 1 steatosis, 13 (14.6%) grade 2, two (2.2%) grade 3 (Fig. 1) and 11(12.4%) had NASH. The histological diagnosis of superimposed steatohepatitis was reported by the presence of hepatocellular ballooning degeneration and Mallory hyaline, mixed neutrophilic and lymphocytic intralobular infiltrate in addition to steatosis by hematoxylin and eosin (Fig. 2) and pericellular fibrosis demonstrated by Masson's trichrome stain (Fig. 3). The patients with superimposed NASH (11 cases) were 5 males and 6 females, with a mean BMI of 30.6 ± 2.1 kg/m2. The histological activity in liver biopsy of these cases were moderate and severe activity (7,2 cases) vs 2cases showing mild activity Out of patients with superimposed NASH, 8 patients showed stage III and IV fibrosis.
The clinical and histopathologic parameters are summarized in Table 2, according to the presence of NAFLD. Patients with NAFLD differed from those without NAFLD by having significant higher serum levels of AST (61.4 ± 35.1 vs. 40.2 ± 24.3, P<0.001) and ALT (98.5 ± 31.4 vs. 47.7 ± 16.6, P <0.001), with an AST/ALT ratio <1 in both groups. In addition, Gamma glutamyl transferase was higher in patients with NAFLD (87.0 vs. 34.0, P=0.003). Moreover, the rate of higher histological activity (moderate activity 31 vs. 23, severe activity 5 vs. 1, P=0.018) and more advanced fibrosis stage (stage III 14 vs. 9, stage IV 7 vs. 2, P=0.020) in patients with NAFLD were higher than in those without NAFLD. Patients with or without hepatic steatosis had insignificant difference in levels of serum cholesterol, serum triglyceride, serum albumin, serum alkaline phosphatase and bilirubin (Table 2).

Sixty six cases showed two or more log. reduction of HCV RNA after 12 weeks with no detectable level after 24 week of therapy, but there were breakthrough in 5 cases with reappearance of HCV RNA in serum just after the end of treatment (48 weeks). The overall end treatment virologic response was achieved in 61 cases (68.54%) while 28 cases (31.46%) were nonresponders. Table 3 demonstrated the presence of the end treatment virologic response in relation to different clinical and histopathologic parameters. Cases with BMI more than 30 kg/m2 were significantly associated with lower chances for end treatment virologic response (46.2%) compared with those with BMI less than 30 kg/m2 (77.8%, P=0.011).

End treatment virologic response was significantly higher (86.36%) in patient not affected by NAFLD compared with 51% in group affected by NAFLD (P=0.000). In patients presented with associated NASH, only 3 out of 11 (27.3%) showed undetectable HCV-RNA at the end of therapy (Fig. 4).

A significant decreases in end treatment virologic response to antiviral therapy is noticed with the increase in the extent of fibrosis (stage) although advanced stages (Stage V and VI) were excluded from this study (P= 0.012).

No significant effect on response to therapy was exerted by demographic data (age and gender) and histopathologic activity (Table 3).

Applying the model of logistic regression analysis in all variables in relation to end treatment virologic response revealed that NAFLD can be considered as independent risk factor for poor response to combined pegylated Interferon α 2a plus ribavirin treatment (odds ratio 3.81(95%CI{0.72-22.38}), P=0.002).

Discussion

Chronic HCV infection is commonly associated with hepatic steatosis (Adinolfi et al., 2001; Fiore et al., 1996; Giannini et al., 1999; Hourigan et al., 1999; Hwang et al., 2001; Sharma et al., 2004). In this study the association of hepatic steatosis among patients with chronic active hepatitis C was 50.6%, this data confirms the high prevalence of steatosis in chronic HCV infection in the previous studies, especially in active forms of chronic disease.

Several investigators suggested a pathogenic link between HCV infection and this metabolic disorder, two distinct forms of hepatocellular steatosis can be seen in patients with CHC infection (Castera et al., 2005; Hui et al., 2002) Classical metabolic risk factors of hepatocellular steatosis account for the vast majority of cases. In contrast a direct cytopathic and steatogenic effect of HCV genotype 3 has been clearly demonstrated (Rubbia-Brandt et al., 2000). There is a growing body of observations indicating that HCV core and NS5A proteins influence intracellular lipid metabolism, increasing triglycerides synthesis and impairing very low density lipoprotein secretion (Shi et al., 2002; Tsutsumi et al., 2002). In addition, there is experimental and clinical evidence that HCV enhances reactive oxygen species production and lipid peroxidation (Farinati et al., 1995; Okuda et al., 2002), and promotes insulin resistance (Shintani et al., 2004). The assignment of either of the two types of steatosis (viral and metabolic) to a specific viral genotype is not so clear cut *(Negro,* 2006).

The impact of NAFLD on natural history and response to treatment of CHC has been extensively studied. Steatosis is a well known independent factor enhancing the rate of fibrosis progression (Adinolfi et al., 2001; Hourigan et al., 1999; Patton et al., 2004; Westin et al., 2002), whereas its impact on the response rate to antiviral therapy is a matter of debate (Bressler et al., 2003; Fabris et al., 2005; Harrison et al., 2005; Lam et al., 1994; Patton et al., 2004; Poynard et al., 2003; Zeuzem et al., 2004; Ziol et al., 1996).

In the present study, we selected a cohort of patients with no confounding factors (morbid obesity, diabetes mellitus, alcohol consumption and advanced fibrosis) with homogeneous therapeutic schedules; pegylated interferon α 2a was given in association with ribavirin. The overall end treatment virologic response to combined antiviral treatment was achieved in 61 cases (68.54%). Previous studies reported lower response rates that range between 41% and 62.8% as they assess the sustained virologic response (SVR) with exclusion of relapsers (Fabris et al., 2005; Harrison et al., 2005; Patton et al., 2004; Zeuzem et al., 2004; Ziol et al., 1996). On the other hand, we assess the end treatment virologic response in patients with mild to moderate stage of fibrosis (I-IV).

Our study confirms the previously reported association between the extent of fibrosis, higher index of pathohistological activity and the severity of steatosis and steatohepatitis. The degree of hepatic steatosis may be an important cofactor in both accelerating fibrosis and increasing liver necroinflammatory activity in CHC (Adinolfi et al., 2001). The severity of fat accumulation correlates with activation of hepatic stellate cells, thus steatosis per se may activate fibrogenesis (Poynard et al., 2003; Ramesh and Sanyal, 2004).

The overall end treatment virologic response was significantly higher (86.36%) in patients not affected by NAFLD compared with 51% in group affected by NAFLD (P=0.000) moreover, most of CHC patients with associated NASH were nonresponders 72.72%.

In accordance with previous reports, patients with HCV and significant steatosis or steatohepatitis (NASH) have a significantly reduced SVR to interferon (IFN) monotherapy or combination therapy with IFN and ribavirin (Fabris et al., 2005; Patton et al., 2004; Zeuzem et al., 2004; Ziol et al., 1996). In a large cohort of CHC patients, steatosis was found to be an independent factor that reduces early virological response, and lack of steatosis was associated with higher likelihood of SVR (Fujie et al., 1999).

Appling the model of logistic regression analysis in this study, NAFLD can be considered as independent risk factor for poor response to combined pegylated interferon α 2a plus ribavirin treatment (odds ratio 0.039, P=0.002).

At variance with findings of the present study, other authors reported the presence of steatosis did not alter the response rate to standard antiviral therapy in patients with hepatitis C, irrespective of its severity (Bressler et al., 2003; Lam et al., 1994; Poynard et al., 2001; Poynard et al., 2003).

In this study, patients with hepatitis C and NAFLD have significantly higher levels of aminotransferases (with an AST/ALT ratio <1), some previous studies reported similar results (Adinolfi et al., 2001; Fujie et al., 1999; Giannini et al., 1999).

Cases with BMI more than 30 kg/m2 were significantly associated with lower chances for end treatment virologic response (46.2%) compared with those with BMI less than 30 kg/m2 (77.8%, P=0.011). It has been hypothesized that the two intertwined factors obesity and steatosis act independently on viral clearance. Recent data indicates that both fat within hepatocytes and BMI, better still, visceral obesity appear to influence treatment response (Bressler et al., 2003; Lonardo et al.,2004; Okuda et al., 2002).

CHC and steatosis are common entities that can have interactive synergistic effect on the liver. Steatosis is frequently observed in CHC and seems to have a significant impact on the natural history of the disease with respect to development of fibrosis and reduction of virologic response to current therapy (Patel et al., 2005).

In summary, hepatic steatosis is detected in nearly one half of studied patients with CHC, even when confounding factors such as overweight, diabetes mellitus or alcohol intake were excluded. Irrespective of its grade, NFALD in hepatitis C is associated with a more severe disease and reduced response rate to combined antiviral therapy. Moreover, most of CHC patients with associated NASH were nonresponders: raising a question about the value of screening NASH prior to initiation of antiviral therapy.

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Table 1: Clinical and histopathologic characteristics of studied cases

	Mean±SD
Age (years)	39.5 ± 12.4(21-57)
Gender (male:female)	56/33
BMI (Kg/M^2)	27.8 ± 4.1 (21.4-34.5)
AST (U/L)	53 ± 34.6 (16-112)
ALT (U/L)	97 ± 30.7 (16-103)
Alkaline Phosphatase AP (U/L)	170 ± 67.2 (91–282)
γGT (U/L)	Median(min-max) 74 (9–231)
Bilirubin (mg/dL)	0.78 ± 0.3 (0.4–2.0)
Albumin (g/L)	43.6 ± 6.9 (36.7–53.2)
Cholesterol (mg/dL)	168.2 ± 29.6 (109-234)
Triglyceride (mg/dL)	132.3 ± 45.4 (94-204)
HCV- RNA (IU/mL)	4.95×104 ± 987.4 (103–4.53×106)
NAFLD (No of cases) No steatosis Mild steatosis Moderate steatosis Severe steatosis NASH	N(%) 44(49.4%) 19(21.4%) 13(14.6%) 2(2.2%) 11(12.4%)
Histological activity (No of cases) -mild -moderate -severe	 29(32.6%) 54(60.7%) 6(6.7%)
Histological Staging (No of cases) I II III IV	 16(18%) 41(46.1%) 23(25.8%) 9(10.1%)

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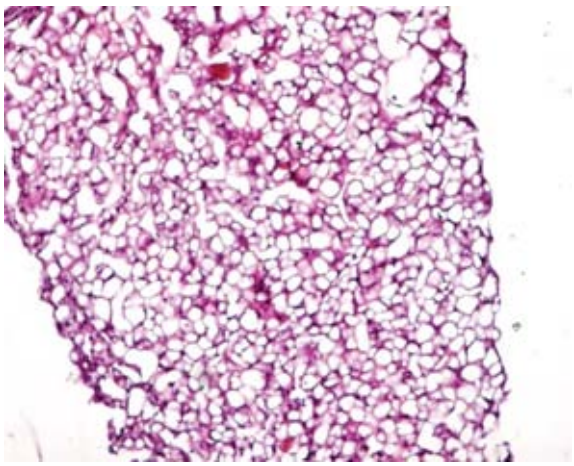

Figure 1: Liver core showing marked macro- and microvesicular steatosis 85%. HX&E X100

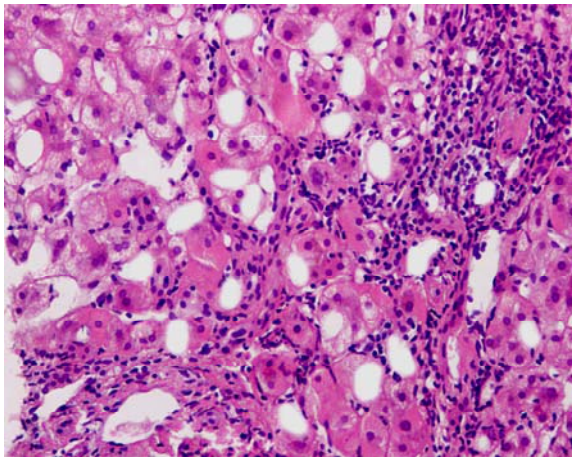

Figure 2: liver tissue showing pericellular lymphocytic and neurophilic infiltrate, hepatocellular ballooning degeneration and Mallory hyaline bodies. HX&E X200

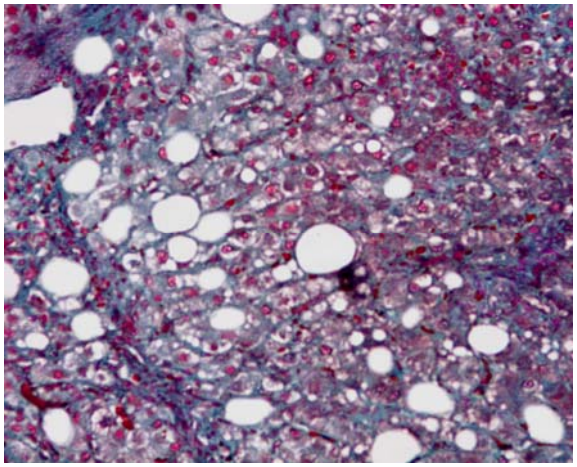

Figure 3: Hepatocytes with pericellular fibrosis. Masson's trichrome. X200.

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Table 2: Clinical, biochemical and histological features in relation to NAFLD

	HCV without NAFLD (N=44)	**HCV with NAFLD (N=45)**	**P value**
Age (years)	39.6 ± 9.5 (21-56)	40.1 ± 13.1 (22-57)	0.89
Gender (male:female)	28/16	28/17	0.83
BMI (Kg/M^2)	27.3 ± 5.3(21.4-31.3)	29.1 ± 4.9 (22.1-34.5)	0.73
AST (U/L)	**40.2 ± 24.3 (16-88)**	**61.4 ± 35.1 (23-112)**	<0.001***
ALT (U/L)	**47.7 ± 16.6 (16-65)**	**98.5 ± 31.4 (26-103)**	**<0.001*** **
AP (U/L)	**167.2 ± 79.3 (91–282)**	**175.1 ± 69.5 (97–261)**	**0.61**
γGT(U/L)#	34 (9–211)	87 (19–231)	0.003**
Bilirubin (mg/dL)	0.78 ± 0.3 (0.4–2)	0.77 ± 0.3 (0.4–1.8)	0.78
Albumin (g/L)	43.6 ± 6.3 (36.7–53.2)	43.6 ± 5.9 (37.0–50.8)	0.81
Cholesterol	163.4 ± 28.2 (109-211)	169.1 ± 27.2 (119-234)	0.56
Triglyceride	130.8 ± 45.4 (94-190)	135.6 ± 51.8 (98-204)	0.35
HCV RNA (iu/mL)	4.9×10^4 ± 863.8 (10^3–4.1×10^6)	5.1×10^4 ± 1075.6 (10^3–4.53×10^6)	<0.001***
Histological activity **-mild** **-moderate** **-severe**	 20 23 1	 9 31 5	 0.018*
Histological Staging **I** **II** **III** **IV**	 13 20 9 2	 3 21 14 7	 0.020*

Mann-Whitney test is used (Non-parametric data)

For number and percentage Chi-Square test is used

For Parametric data Independent sample t-test is used

Table 3: clinical and histopathologic features in relation to response to treatment

Variable	No of cases	Responder (N=61)	Non responder (N=28)	P value
Age (years)				
< 40	39	28	11	0.55
➢ 40	50	33	17	
Gender				
Males	56	40	16	44
Females	33	21	12	
BMI (Kg/M^2)				
21 - 25	2	2	0	
>25-30	61	47	14	0.011*
>30-35	26	12	14	
NAFLD				
No	44	38	6	
Mild	19	15	4	<0.00***
Moderate/severe	15	5	10	
NASH	11	3	8	
Activity (grade) by modified HAI				
Mild	29	24	5	0.21
Moderate	54	33	21	
Severe	6	4	2	
Fibrosis (Stage) by modified HAI				
I	16	15	1	
II	41	30	11	0.012*
III	23	11	11	
IV	9	4	5	

For number and percentage Chi-Square test is used

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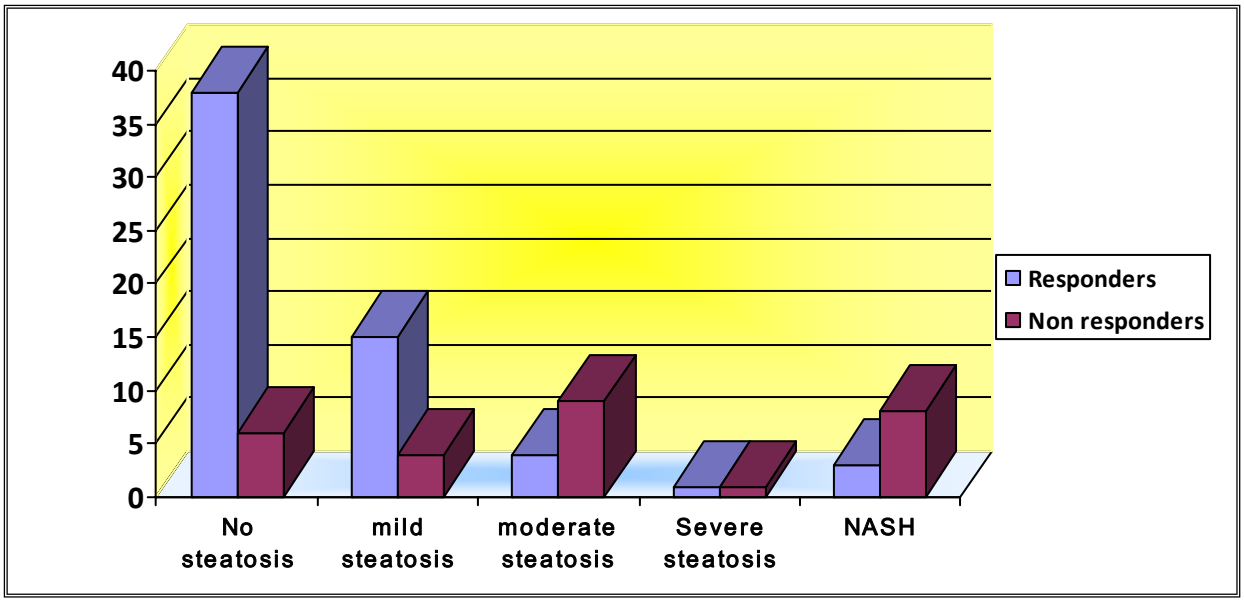

Figure 4: Response to therapy in relation to presence of NAFLD