Original Article

Liver Biopsy in Patients Infected with Chronic Hepatitis C and Persistently Normal Serum ALT Levels

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ABSTRACT

INTRODUCTION: Approximately 30 to 40% of patients with Chronic Hepatitis "C" (CHC) have persistently normal serum alanine aminotransferase (ALT) levels. Historically, these patients have been classified as healthy or asymptomatic and have not received any treatment for CHC infection. However, definition and clinical significance of persistently normal ALT in CHC have been recently revised as new information on liver disease which is now getting available.

AIMS: To evaluate the histological feature of liver of patients suffering from CHC with persistently normal ALT levels.

METHODS: In this prospective observational study we recruited consecutive patients infected with CHC with persistently normal ALT since last six months, visited our hepatology clinic from September 2004 to April 2005.The METAVIR scoring system was used for liver histology grading (degree of inflammation) and staging (degree of fibrosis).

RESULTS: A total of 55 patients were recruited from outpatient clinic with normal ALT during a follow up of six months. Mean age of these patients was 36.7± 9.78 years; out of these 39 (70.9%) were male. All these patients were diagnosed to have hepatitis C by HCV RNA PCR method. There were 24 (43.6%) patients with stage (fibrosis) equal or greater than 2 and 33 (60%) had biopsy grade equal or greater than 2. Eighteen (32.7%) patients had steatosis on liver biopsy. Twelve patients with stage > 2 had steatosis while 6 patients with stage < 2 had steatosis (p< 0.01).

CONCLUSION: There was no correlation found between the transaminase level and biopsy scores. Approximately 44 % of the patients have fibrosis equal to or greater than stage 2. The extent of steatosis is directly related to the biopsy score of the patients.

INTRODUCTION

Approximately 30-40% of the patients with chronic hepatitis "C" have persistently normal alanine aminotransferase (ALT) levels[1]. Antiviral treatment for patients with CHC infection has generally been limited to those with significantly abnormal serum ALT activity (2). Previously patients with normal ALT which consists mostly of female population and have lesser degree of inflammation and fibrosis used to be excluded from clinical trials (3, 4, 5, and 6). In past normal ALT levels even have been used as a surrogate marker for treatment response (7). It is true that if ALT levels are persistently normal, the possibility of significant and progressive liver disease tends to be low, but a significant proportion of patients with persistently normal ALT levels show some histological signs of fibrosis, the degree of which is usually mild but sometimes more marked and in rare cases, cirrhosis may be present (2). In fact in some series up to 14 to 20% of patients with normal ALT have advanced cirrhosis and advanced fibrosis on liver biopsy. It is a recognized fact that all of them do not have mild liver disease and slower disease progression[1, 3, 8]. Around 90% of HCV carriers with persistently normal ALT (PNALT) have normal to mild inflammation and fibrosis on liver histology. Long term follow-up studies suggest that 30% of such carriers became candidates for antiviral therapy within 5 years[9]. Persistently normal ALT in most studies is defined as "at least three normal ALT levels over a six month period". The degree of liver injury in patients with PNALT may not differ from the matched controls with elevated ALT[10]. The efficacy and safety of pegylated interferon alfa-2a and ribavirin combination therapy in patients with chronic hepatitis C and PNALT are similar to that in patients with elevated ALT. The indication for treatment of hepatitis C can be evaluated independently from baseline ALT activity[11]. In many studies it is shown that normal ALT levels do not preclude the abnormal liver histology so we decided to perform this study to evaluate correlation between PNALT levels in patients with CHC and histological features of liver at our tertiary care center of Hyderabad, Pakistan.

METHODS

In this prospective observational study, we recruited consecutive patients infected with chronic hepatitis "C" having persistently normal ALT (men < 55 IU/L and women < 33 IU/L) during last six months with three consecutive readings[12, 13]. Duration of recruitment was from September 2004 to April 2005. The liver biopsy was performed percutaneously through suction technique using 16 gauge spinal needle (NESCO Japan). All the patients were undergone ultrasound scan of abdomen prior to biopsy. The METAVIR scoring system was used for histological assessment of inflammation (grading) and fibrosis (staging) of liver. Fat accumulation (steatosis) in hepatocytes was also noted. For the sake of simplicity inflammatory grade and fibrotic stage from 0 to 1 labeled as insignificant and from 2 to 4 as significant inflammation and fibrosis respectively. This further labeling of grade and stage also quoted in the American Gastroenterological Association technical review on the management of Hepatitis "C" (Gastroenterology 2006, 130: 231-264). Similarly steatosis was labeled absent if no fat accumulation and present if fat accumulation seen whatever was the severity. All the patients who had PNALT but sonological evidence of cirrhosis, platelets count less than $150x10^9$/L and prothrombin time more than 3 seconds higher than the control were excluded. Aspartate aminotransferase (AST) was also checked in all patients. Statistical analyses. Database management, all statistical analyses were performed with SPSS version 11.0. The mean $\pm$ standard deviation was calculated for quantitative variables. The X^2 (chi-square) test and independent T-test, as required by the sample size were used to assess the significance of difference. All available information on each variable was used. P (probability) vale of 0.05 or less was considered to indicate statistical significance.

TABLE I:
CHARACTERISTICS OF PATIENTS WITH REGARDS TO INFLAMMATION OF LIVER

	Insignificant Inflammation (Grade 0 – 1) % (n)	Significant Inflammation (Grade 2 – 4) % (n)	p- Value
Age (years):	34.95/$\pm$ 8.39	37.97 $\pm$ 10.5	0.267
Sex			0.53
Men	27.3% (15)	45.5% (25)	
Women	12.7% (7)	14.5% (8)	
Weight (Kg)	60.77 $\pm$ 15.8	66 $\pm$ 12.2	0.12
ALT (IU/L)	34.55 $\pm$ 12.0	37.36 $\pm$ 9.5	0.337
AST (IU/L)	29.0 $\pm$ 8.14	36.42 $\pm$ 13.7	0.03
AAR (AST/ALT Ratio)			0.634
Less than 1	29% (16)	40% (22)	
More than 1	11% (6)	20% (11)	
Genotype			1.0
Genotype 3	36.4 (20)	54.5% (30)	
Other than3	3.6%(2)	5.5% (3)	
Severity of Fibrosis			0.002
Insignificant	32.7% (18)	23.6% (13)	
Significant	7.3% (4)	36.4% (20)	
Presence of Steatosis			0.19
Present	9.1% (5)	23.6% (13)	
Absent	30.9% (17)	36.4% (20)	

TABLE II:
CHARACTERISTICS OF PATIENTS RELATED TO LIVER FIBROSIS

	Insignificant Fibrosis (Stage 0 – 1) % (n)	Significant Fibrosis (Stage 2 – 4) % (n)	P-value
Age (years)	35.26 ± 8.8	38.7 ± 10.76	0.197
Weight (Kg)	62.84 ± 13.8	66.25 ± 14.1	0.37
Sex			0.78
Man	41.8% (23)	30.9% (17)	
Women	14.5% (8)	12.7% (7)	
ALT (IU/L)	34.32 ± 10.0	38.7 ± 10.8	0.128
AST (IU/L)	30.61 ± 9.1	37.2 ± 14.8	0.04
Severity of inflammation			0.002
Insignificant (Gr.0-1)	32.7% (18)	7.3% (4)	
Significant (Gr.2- 4)	23.6%(13)	36.4%(20)	
Presence of steatosis			0.016
Yes	11%(6)	21.8%(12)	
No	45.5%(25)	21.8%(12)	
AAR (AST/ALT Ratio):			0.732
Less than 1	40%(22)	29%(16)	
More than 1	16.4%(9)	14.5%(8)	
Genotype			0.439
Genotype 3	527%(29)	38.2%(21)	
Other than 3	3.6%(2)	5.5%(3)	

RESULTS

There were 40 (72.7%) men and 15 (27.3%) women. Mean age 36.76 ± 9.7 years and mean body weight was 64.33 ± 13.94 kilogram (kg). Only 2 patients were diabetic in this cohort. Genotype 3 was seen in 91% and genotype other than 3 was in 9% of patients. However, there was no patient with genotype 2, noted in this cohort. Other results were as follows:

Hepatic Inflammation:
Approximately 60% of patients had inflammation equal to or greater than grade 2. There was no significant relationship seen between the severity of inflammation, age, sex, weight, AST/ALT ratio, level of ALT and genotype, but a significant correlation was seen with mean AST level and severity of fibrosis as shown in table1.

Hepatic Fibrosis:
At least 43.6% of patients showed fibrosis equal to or greater than stage 2 (METAVIR F ≥ 2). Cirrhosis (F4, fibrosis) was seen in 7.3% of patients. A significant correlation was seen between severity of fibrosis and mean AST level, severity of inflammation and presence of steatosis as shown in Table 2.

Hepatic Steatosis (Fat accumulation):
Steatosis was seen in 18 (32%) patients. There was no significant correlation between the presence of steatosis and sex, mean ALT level, mean AST level, AST/ALT ratio, severity of inflammation and genotype of the patients. But a significant correlation was seen with mean age, mean weight and significant fibrosis (F ≥ 2) as shown in table 3.

DISCUSSION

Natural course of HCV infection in patients with persistently normal ALT remains poorly defined. This study reports demographic and liver histological finding in 55 HCV infected patients with PNALT, which is the first series of such patients yet reported from Pakistan in prospective manner. Overall, 60% of patients

TABLE III:
CHARACTERISTICS OF PATIENTS RELATED TO STEATOSIS IN LIVER

	Steatosis Absent % (n)	Steatosis Present % (n)	P- value
Age:	34.73 ± 8.9	40.9 ± 10.3	0.02
Sex:			0.48
Men	70% (28)	30%(12)	
Women	60%(09)	40% (06)	
Weight (Kg):	61.97 ± 13.5	69.17 ± 13.7	0.07
ALT:	35.4 ± 11.54	37.89 ± 8.2	0.42
AST:	32.38 ± 10.9	35.78 ± 14.7	0.34
AAR (AST/ALT ratio):			0.78
Less than 1	47.3% (26)	21.8% (12)	
More than 1	20% (11)	10.9 (6)	
Inflammation			
Insignificant	30.1% (17)	9.1% (05)	0.197
Significant	36.4% (20)	23.6% (13)	
Fibrosis:			0.016
Insignificant	45.5% (25)	11% (6)	
Significant	21.8% (12)	21.8% (12)	
Genotype:			0.716
Genotype 3	61.8% (34)	29.1% (16)	
Other than 3	5.4% (3)	3.6% (2)	

in our series had significant (≥A2) grade of inflammation. This is much higher than reported by Kyrlagkitsis (16%) and European collaborative study[4, 5]. As reported by Kyrlagkitsis et al, we also could not identify genotypic and demographic factors (age, sex, weight) to predict significant inflammation.[(4)] With regard to fibrosis, available histological data convincingly demonstrate that around 20-25% of these cases have significant liver disease with periportal fibrosis (>F2 METAVIR), a type of finding that is unanimously considered at risk of further progression and indication for antiviral therapy. Indeed, in HCV patients with normal ALT having significant fibrosis (>F2) in the initial biopsy, progression to advanced fibrosis (F3) or cirrhosis (F4) occur in 5-10 years even in presence of persistently normal ALT levels.[(5)] In our series there was no significant relationship seen between the severity of fibrosis and age, sex, weight, AST/ALT ratio, level of ALT and genotype. But a significant correlation was seen with mean AST level and severity of inflammation as shown in table 2. We found that almost every second patient (43.6%) has significant (F ≥ 2) fibrosis in our series, which is very high comparative to report by others. Although majority of data seem to show that HCV carriers with PNALT have mild and stable disease with a favorable prognosis. Most published studies agree on the conclusion that around two thirds of HCV patients with PNALT have no or minimal activity and / or fibrosis in their liver biopsy [5, 6,14,19]. On the other hand significant fibrosis (≥ F2) has been reported in 5 – 30% of the cases, with large variation among studies, when inclusion criteria and length of base line follow up was different. The cumulative percentage of HCV patients with PNALT with significant (≥ F2) fibrosis in liver biopsy among 1154 such cases included in 23 published studies, was 22% [20]. The prevalence of cirrhosis in HCV patients with PNALT ranged from 1 - 6% in various studies[20] and one study

FIGURE I: GRADE OF INFLAMMATION

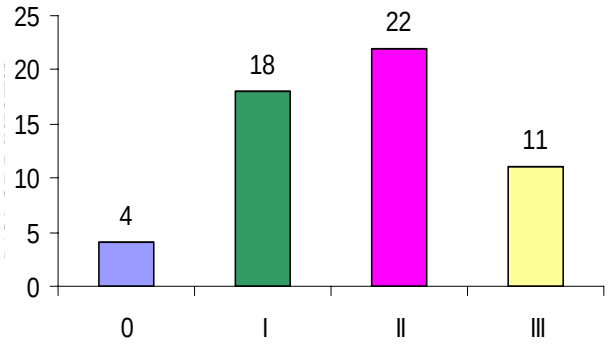
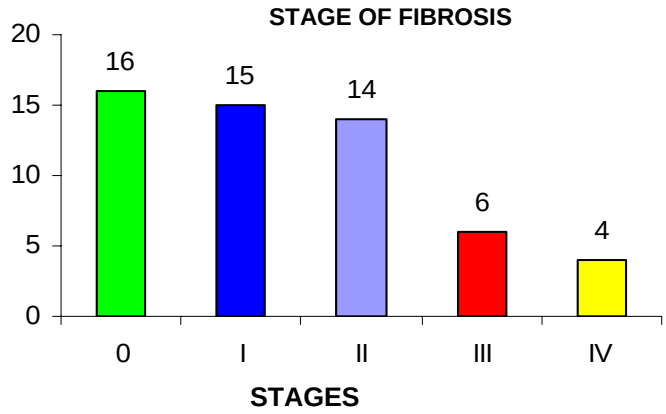


FIGURE II:
NECROINFLAMATION AND FIBROSIS OF PATIENTS



reported that up to 29% of these individuals have stage 3 or 4 fibrosis [20]. In our series we have reported 7.3% of cirrhosis in PNALT patients and 18% with stage 3 or 4 fibrosis. This is consistent with above reported literature. In our series, severity of fibrosis has significant correlation with severity of inflammation, level of AST and presence of steatosis. Above factors have already been validated in literature [4, 5, 11, 18]. We also found that age, weight, sex, AST/ALT ratio and genotype has no impact on fibrosis, where increasing age (> 35 years) and AST / ALT ratio > 1 has correlation with severity of fibrosis (21). Similarly, genotype also has no impact in reported study[4]. Significant fibrosis in almost 44% of patients in our series, despite PNALT could be explained by demonstration of significant necroinflammation (≥ A2) in 60% of patients at the time of liver biopsy. The significant hepatic inflammation (≥ A2) on liver biopsy is the most significant independent factor associated with severe liver damage and also fulfill histological criteria for antiviral treatment. These risk factors can only be identified by examining liver histology. Similarly steatosis and higher mean AST level may explain fibrosis in every second patient, because both of these are well established risk factors for advanced fibrosis. We have 2 limitations in our series; the number of patients is only 55 as compared with other studies and no comparative arm with elevated ALT.

CONCLUSIONS

We conclude that there was no correlation found between the transaminase level and liver biopsy scores (combination of grade and stage). Approximately 44 % of the patients have moderate fibrosis (≥F2) greater than stage 2. The extent of steatosis is directly related to the liver biopsy score of the patients. All potentially treatable CHC patients with PNALT should undergo liver biopsy and that antiviral treatment is considered on the basis of histological findings.

REFERENCES

1. Conry-Cantilena C, VanRaden M, Gibble J, Melpolder J, Shakil AO, Viladomiu L, et al. Routes of interferon, viremia, and liver disease in blood donors found to have hepatitis C virus infection. N Engl J Med 1996; 334:1691–6.
2. Mamori S, Suzuki F, Hosaka T, Akuta N, Someya T, Kobayashi M, et al. Interferon monotherapy for patients with chronic hepatitis C and normal serum aminotransferase levels at commencement of treatment. J Gastroenterol. 2004 Aug; 39(8):776-82.
3. Alberti A, Noventa F, Benvegnu L, Boccato S, Gatta A. Prevalence of liver disease in a population of asymptomatic persons with hepatitis C virus infection. Ann Intern Med. 2002 Dec 17; 137 (12):961-4. Summary for patients in: Ann Intern Med. 2002 Dec 17; 137(12):I36.
4. Kyrlagkitsis I, Portman B, Smith H, O'Grady J, Cramp ME. Liver histology and progression of fibrosis in individuals with chronic hepatitis C and persistently normal ALT. Am J Gastroenterol 2003, 98:1588-93
5. Pradat P, Alberti A, Poynard T, Esteban JI, Weiland O, Marcellin P, et al. Predictive value of ALT levels for histologic findings in chronic hepatitis C: a European collaborative study. Hepatology. 2002; 36(4 Pt 1):973-7.
6. Nutt AK, Hassan AK, Lindsay J. Liver biopsy in the evaluation of patients with CHC who have repeatedly normal or near normal serum ALT levels. Am J Med 2000; 109:62-4.
7. Jacobson IM, Russo MW, Lebovics E, Esposito S, Tobias H, Kilon F, et al. Interferon alfa-2b and ribavirin for patients with chronic hepatitis C and normal ALT: final results (abstract). Gastroenterology 2002; 122:627A.

8. Stanely SJ, Hayden GH, Pris J. Prevalence of liver disease in a population of asymptomatic persons with hepatitis C virus infection. Ann Intern Med 2002; 137:961-4.
9. Okanoue T, Makiyama A, Nakayama M, Sumida Y, Mitsuyoshi H, Nakajima T, et al. A follow-up study to determine the value of liver biopsy and need for antiviral therapy for hepatitis C virus carriers with persistently normal serum aminotransferase. J Hepatol. 2005 Oct; 43(4):599-605.
10. Puoti C, Magrini A, Stati T, Rigato P, Montagnese F, Rossi P, et al. Clinical, histological, and virological features of hepatitis C virus carriers with persistently normal or abnormal alanine transaminase levels. Hepatology. 1997 Dec; 26(6):1393-8.
11. Zeuzem S, Diago M, Gane E, Reddy KR, Pockros P, Prati D, et al. PEGASYS Study NR16071 Investigator Group. Peginterferon alfa-2a (40 kilo Daltons) and ribavirin in patients with chronic hepatitis C and normal aminotransferase levels. Gastroenterology. 2004; 127(6):1724-32.
12. Marcellin P, Levy S. Therapy of hepatitis C: patients with normal aminotransferase levels. Hepatology1997; 26:133S-1136.
13. EASL International Consensus Conference on Hepatitis C. Paris, 26 – 28, February 1999. Consensus Statement, European Association for the Stud of Liver. J Hepatol 1999. 30:956-61.
14. Hui CK, Monto A, Belaye T, Lau E, Wright TL. Outcomes of interferon alpha and ribavirin treatment for chronic hepatitis C in patients with normal serum aminotransaminase. Gut 2003; 52:1644-1648.
15. Shiffman ML, Stewart CA. Hofmann CM, Contos MJ, Luketic VA. Sterling RK, et al. Chronic infection with hepatitis C virus in patients with elevated or persistently normal serum Alanine aminotransferase levels: comparison of hepatic histologic and response to interferon therapy. J Infect Dis 2000; 182:1595-601.
16. Erardt A, Behlen-Wilm U, Adams O, Donner A, Heintges T, Haussinger D. Combination treatment of IFN alpha2b and ribavirin in patients with chronic hepatitis C and persistently normal ALTs. Dig: Dis: Sci: 2003; 48:921-25.
17. Herve S, Savoye G, Riachi G, Hellot MF, Goria O, Lerebours E, et al. Chronic hepatitis C with normal or abnormal aminotransferase levels: is it the same entity? Eur: J: Gastroenterol: Hepatol: 2001; 13:405-500.
18. Renou C, Halfon P, Pol S, Cacoub P, Jouve E, Bronowicki JP, et al. Histological features and HLA class II alleles in hepatitis C virus chronically infected patients with persistently normal alanine aminotransferase levels. Gut. 2002 Oct; 51 (4):585-90.
19. Jamal MM, Sony A, Quinn PG. Clinical features of hepatitis C infected patients with PNALT levels in the Southwestern United States. Hepatology 1999; 30:1307-11.
20. Albert A, Benvegnu L, Boccato S, Ferrari A, Sebastiani G. Natural history of initially mild chronic hepatitis C. Dig Liver Dis 2004;36:646-54.
21. Delladetsima JK, Rassidakis G, Tassopoulos N. Histopathology of chronic hepatitis C in relation to epidemiological factors. J Hepatol 1996;24:27-32.

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