ASSOCIATION OF RESPONSE TO COMBINED INTERFERON ALPHA-2b AND RIBAVIRIN THERAPY IN PATIENTS OF CHRONIC HEPATITIS C WITH SERUM ALANINE AMINOTRANSFERASE LEVELS AND SEVERITY OF THE DISEASE ON LIVER BIOPSY

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Background: Raised serum alanine aminotransferase (serum ALT) levels indicate active liver disease while liver biopsy has been considered the 'gold standard' for assessing the severity of disease in patients of chronic Hepatitis C. The response of these patients to standard treatment regimen of interferon (INF)-alpha- 2b and ribavirin for 24 weeks have been studied. **Objective:** The objective of this study was to evaluate the association of response to combined INF alpha-2b and ribavirin therapy in patients of chronic hepatitis C with serum ALT levels and severity of the disease on liver biopsy. **Methods:** This quasi experimental study was conducted in Department of Physiology at Army Medical College and Military Hospital, Rawalpindi from January 2006 to February 2007. One hundred and seven diagnosed non cirrhotic chronic hepatitis C patients were studied. Prior to the commencement of treatment, qualitative assay of Hepatitis C Virus (HCV) Ribonucleic Acid (RNA) was done by Polymerase chain reaction (PCR). Knodell Histopathological Index (HPI) was determined on liver biopsy. The standard treatment of INF-alpha-2b, 3 million units 3 times a week subcutaneous, and Ribavirin 800–1200 mg per day was given for 24 weeks. Serum ALT levels were determined before the start of treatment and later at weeks 12 and 24. Qualitative assay of HCV RNA was done by PCR at the end of treatment to determine the response to treatment. Statistical analysis was done on SPSS 15. **Results:** Out of 107 patients of chronic hepatitis C, 92 (69 males, 23 females) patients (84%) responded to INF-alpha-2b and ribavirin therapy and revealed negative qualitative assay of HCV RNA by PCR at the end of 24 weeks of treatment while serum ALT levels were normal in 88% of patients at 12 weeks and in 97% at the end of 24 weeks of treatment. Knodell HPI revealed mild, moderate and severe disease in 47.7%, 39.9% and 13.1% of patients respectively. No association was established between response to treatment and severity of the disease on liver biopsy (p<0.11) and serum ALT levels (p=0.09). **Conclusion:** Response to Interferon alpha-2b and ribavirin therapy in patients of chronic hepatitis C is not associated with the levels of serum ALT and the severity of the illness graded on liver biopsy.

Keywords: Chronic hepatitis C, interferon, liver biopsy, serum alanine aminotransferase

INTRODUCTION

Chronic hepatitis C is one of the commonest infectious diseases of the liver. According to World Health Organization (WHO) report, approximately 170 million individuals in the world population are suffering from this disease.[1] In Pakistan, the number of diagnosed patients of chronic hepatitis C is on the rise. The prevalence rate of anti HCV antibodies in Pakistani population, reported mostly in hospital based studies in patients, blood donors and in general population is 0.5 to 25.7 %.[2–4] Due to increasing awareness to health problems, there is increase in the number of people who are being screened, diagnosed and treated for the disease in Pakistan.

Non specific laboratory diagnostic test for chronic hepatitis C is persistently raised serum ALT levels; however it is not a reliable indicator of the disease.[5] Specific laboratory diagnostic tests for chronic hepatitis C are serological tests which detect anti-HCV in serum while molecular tests detect HCV RNA genome and assess qualitative and quantitative viral load.[6] Liver biopsy is not necessary for the diagnosis of chronic hepatitis C however it has been considered as the 'gold standard' for assessing the severity of the disease.

The indication for antiviral therapy is the presence of significant inflammation, fibrosis and necrosis on liver biopsy.[7] National Institute of Health (NIH) has recommended INF alpha-2b and Ribavirin for 24 weeks as the standard regimen for chronic hepatitis C.[8] The pattern of response varies in different individuals. Chronic hepatitis C treatment has shown response up to 70–80 % in international[9–11] and local studies.[4,12,13] The factors which determine the response to therapy are compliance to treatment, viral load, genotype of hepatitis C virus, age and sex of the patient.[14–16] Adherence to therapy, decreased viral load, genotype 3, female sex, young age and lower body weight are associated with better

virological response to therapy.[15,16] Severity of disease and serum levels of ALT are not associated with the outcome of the illness.[12–15] Serum ALT levels are not a reliable indicator of either the presence or the severity of the disease. Elevated serum ALT levels indicate active disease of liver but 25% of chronic hepatitis C patients may have normal or widely fluctuating levels of serum ALT.[14] Patients who respond to INF therapy and those who do not may have raised baseline ALT levels. The response to therapy is not associated with either the baseline serum ALT levels or its levels during treatment.[14–16]

Necroinflammatory damage to liver parenchyma assessed on liver biopsy indicates severity of the disease. Liver biopsies are scored by Knodell Histopathological Index (HPI) based on inflammatory, necrotic and fibrotic changes.[7] INF therapy is given to non cirrhotic patients and their response to treatment has not been associated with the extent of damage to the liver.[14–16]

This study was designed to determine the relationship of response to combined interferon alpha and ribavirin therapy in patients of chronic hepatitis C with serum levels of ALT and severity of the disease on liver biopsy.

MATERIAL AND METHODS

This study was conducted in department of Physiology at Army Medical College and Military Hospital, Rawalpindi from January 2006 to February 2007. One hundred and seven (80 males and 27 females) non cirrhotic chronic hepatitis C patients, age between 18–48 years; 35±7.12 years (Mean±SD) were included in this study by non probability convenience sampling after written and informed consent. The diagnostic criteria for non cirrhotic chronic hepatitis C was persistently raised serum ALT (>42 IU/L), positive anti–HCV antibodies by 4th generation Enzyme Linked Immunosorbent Assay (ELISA), positive HCV RNA by Polymerase Chain Reaction (PCR) and liver biopsy compatible with the diagnosis of chronic hepatitis C on the basis of Knodell HPI. These patients were planned to undergo treatment regimen comprising of combination of INF-alpha-2b, 3 million units 3 times a week subcutaneous, and oral Ribavirin 800–1200 mg daily in divided doses for 24 weeks. Patients treated previously with IFN and/or Ribavirin or suffering from severe cardiac or pulmonary disease and pregnant patients were excluded from the study on the basis of history, physical examination and laboratory tests including ECG, chest X-ray and urine for pregnancy test. Prior to the commencement of treatment, qualitative HCV RNA by PCR was done. Liver biopsy was performed and Knodell HPI was determined in these patients. Knodell HPI and qualitative HCV RNA by PCR were used to determine the severity of the disease and response to treatment respectively. Chi-Square tests were used to determine the association of response to combined interferon alpha-2b and ribavirin therapy with serum ALT levels and severity of the disease on liver biopsy. Independent student t test was applied to S. ALT values of baseline, at weeks 12 and 24. Statistical analysis was done using SPSS 15. Statistical significance was determined at $p<0.05$.

RESULTS

One hundred and twenty-five patients were screened for the study, out of which 11 patients were excluded due to the presence of decompensated liver disease based on the history of upper gastrointestinal bleed, ultrasonographic evidence of cirrhosis of liver or presence of ascites. Three patients declined to undergo treatment and 4 patients did not respond for the follow up. One hundred and seven patients met the inclusion criteria and were followed up till the end of the treatment.

The demographic data and baseline serum ALT levels (Mean±SD) and histology of liver biopsy are presented in Table-1. The age ranged from 18–48 (35±7.12) Years. Among 107 patients of chronic hepatitis C, 80 were males and 27 were females. Nineteen percent of the patients had the positive family history of chronic hepatitis C, out of which 2/3rd were spouses of the patients with history of chronic hepatitis C and 1/3rd were either siblings or relatives of persons with HCV infection living in the same house. Sixty two percent of patients did not have a history of exposure to any of the known risk factors of HCV transmission. Thirty-eight percent of patients had a history of exposure to one of the known risk factors for HCV transmission, e.g., use of un-sterilised instruments, injections and unscreened transfusions (Table-1). The histopathological changes in liver were sub grouped into mild, moderate and severe disease on Knodell Histopathological Index scoring system (HPI). Out of 107 patients, a total of 51 (47.7%) had mild disease on the basis of Knodell HPI while 42 patients (39.3%) had moderate and 14 (13.1%) had severe disease on liver biopsy (Table-1). The baseline ALT level was 93±63 IU/L with a range of 13–383 IU/L. The ALT level during treatment at 12 weeks of therapy was 38.53±30.86 IU/L and ranged between 14–201 IU/L. At the end of treatment, the mean ALT level was 33.85±24.02 IU/L with a range of 11–170 IU/L. At 12 weeks of therapy, 88% of all the patients and at the end of 24 weeks treatment, 97% of all the patients showed normalization of serum ALT levels. Among 107 patients of chronic hepatitis C, 92 patients (86%) showed complete response at the end of 24 weeks

treatment depicted by negative qualitative assay of HCV RNA by PCR at the end of treatment. Fifteen patients (14%) did not respond to therapy as depicted by positive qualitative assay of HCV RNA by PCR at the end of therapy (Table-2). There was no association between response to treatment and serum ALT (p<0.81). The patients who responded to treatment, majority of them (48.9%) suffered from mild liver disease while 35.9% had moderate and 15.2% had severe liver disease on liver biopsy on the basis of Knodell HPI (Table-3). Among the patients who did not respond to treatment, 40% suffered from mild liver disease while 60% had moderate and none had severe liver disease on liver biopsy on the basis if Knodell HPI. There was no association between severity of the disease and response to treatment on Chi-Square test (p=0.11). Response rates were almost similar in both genders. Among non responders, 13.75% of males and 14.81% of females did not respond to the treatment. Statistically response to therapy did not have any significant relationship with sex of the patient (p=0.89). All the patients completed anti viral therapy.

Table-1: Baseline Profile of patients of Chronic Hepatitis C (n=107)

Characteristics	Values
Age Mean±SD	35±7.12 years
Sex Male/Female ratio	80/27
Mean (range) S. ALT	93 (13–383) (IU/L)
Positive Family History	19%
Source of Infection	
Unknown	62%
Unsterilised instruments (surgical/dental)	21%
Injections	10%
Transfusion	7%
Knodell HPI on liver biopsy (%)	
Mild	47.7%
Moderate	39.3%
Severe	13.1%

Table-2: Response of Hepatitis C Patients to the Interferon and Ribavirin Therapy

HCV RNA by PCR	Patients (n)	Male:Female	Patients (%)
NEGATIVE	90	67:23	92 (86%)
POSITIVE	17	13:04	15 (14%)
Total:	107	80:27	100%

Table-3: Response of Hepatitis C patients to Interferon and Ribavirin therapy and severity of their disease on liver biopsy

Severity of disease on Knodell HPI	HCV RNA by PCR		Total
	Negative n (%)	Positive n (%)	
Mild	45 (48.9%)	6 (40%)	51 (47.7%)
Moderate	33 (35.9%)	9 (60%)	42 (39.3%)
Severe	14 (15.2%)	0	14 (13.1%)
Total:	92 (100%)	15 (100%)	107 (100%)

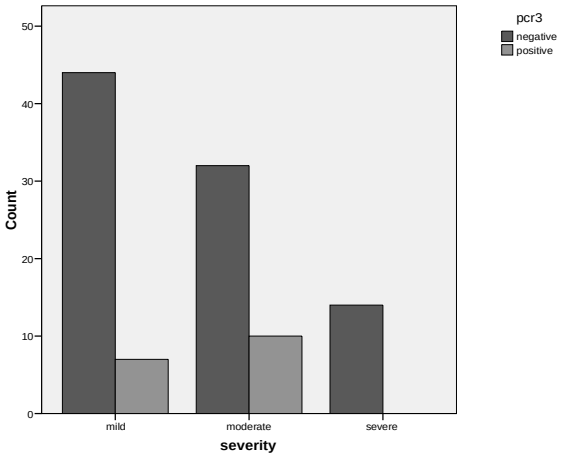

Figure-1: Bar Diagram Depicting Severity of Chronic Hepatitis C and Response to Treatment

DISCUSSION

The current regimen recommended by National Institute of Health for the patients of chronic hepatitis C is INF-Alpha-2b, 3 million units 3 times a week subcutaneous and oral Ribavirin 800–1200 mg daily in divided doses for 24 weeks.[12] Substantial research work has been carried out internationally and nationally to determine the response to INF-alpha-2b and ribavirin therapy Treatment response in our study was 84% similar to that of other local[8,16,17] and international studies.[13–15] After 12 weeks of therapy, 88% of patients while at the end of 24 weeks treatment 97% showed normalization of serum ALT levels. Most of the patients had baseline serum ALT levels between 2–4 times normal although 5% of the patients had normal baseline serum ALT levels as reported in literature.[9,18] The improvement in baseline serum ALT levels at 12 and 24 weeks was statistically significant (p=0.001) depicting the efficacy of treatment in normalizing the serum ALT levels. The improvement in serum ALT was because of INF therapy on liver parenchymal injury induced by hepatitis C virus.[9] Those patients who did not respond to treatment also had persistently raised serum ALT levels 3 to 4 times normal during 06 months of the study but the association between S. ALT at weeks 0, 12 and 24 of responders and non responders was not statistically significant (p=0.81). The findings are in accordance to those found in Batool *et al* study; however in their study the response was determined at the end of 24 weeks therapy at and again at 48 weeks to determine the frequency of relapsers. Giannini *et al* in their study revealed that most of the patients with normal ALT levels have mild disease, slow progression and better response to antiviral treatment. In our study only 5% of the patients had normal baseline serum ALT levels. Their response to therapy was not statistically different to those with 2–4 times higher serum ALT levels as documented by Batool *et al* study.[17] One probable

explanation is that the genotypes 1 and 2 of HCV are found in the western population whereas in this part of the world genotype 3 is more common. It is well established fact that genotype governs not only the pathogenesis but also the outcome of the illness. However studies with large sample size of chronic hepatitis C patients of local population with normal baseline serum ALT are required to be investigated to determine the response to treatment.

The indication to commence antiviral therapy is the presence of significant inflammation, fibrosis and necrosis on liver biopsy.[11] Knodell histopathological index (HPI) shows degree of fibrosis and grade shows inflammation.[11] Dienstag *et al* reported scores greater than 4 representing severe disease and piecemeal necrosis on any scoring system predicts non response to antiviral therapy. However, in present study, 14 (15.2%) patients had severe liver disease on Knodell HPI and all of them responded to the treatment. In the study of Batool *et al*, 9.55% patients had severe disease and those who responded had mild to moderate disease on liver biopsy. Most of the non responders had moderate to severe degree of disease but the difference was not statistically significant. In our study, the response to therapy was not associated with severity of the disease statistically (p=0.11). It could probably be due to the fact that different immune mechanisms are involved in the pathogenesis of disease than those processes that regulate the therapeutic response to HCV.[22]

Chronic hepatitis C is a global disease. More and more number of patients are being diagnosed and treated due to increasing awareness of the disease. Response to therapy can be improved by modifying factors by determining the viral load and genotype of the virus, early diagnosis and commencement of treatment and lowering the body weight, which influence the outcome of treatment.

CONCLUSION

It is concluded that response to Interferon alpha-2b and ribavirin therapy in patients of chronic hepatitis C is not associated with the levels of serum ALT and severity of illness graded on liver biopsy.

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