

## RELATIVE CHANGES IN HEPATITIS-C VIRUS RNA TITRE AND SERUM ALT LEVELS BEFORE, DURING AND AFTER TREATMENT WITH INTERFERON AND RIBAVIRIN

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### ABSTRACT

Hepatitis C is commonly treated with Interferon and Ribavirin combination therapy. The aim of this study was to find the relative change in the Hepatitis C virus (HCV) RNA titre and serum alanine aminotransferase (ALT) levels before, during and after completion of treatment with Interferon (IFN) and Ribavirin therapy in HCV positive patients.

*Patients and Methods:* This cross – sectional descriptive study was conducted from May 2010 to August 2010. Seventy hepatitis C patients reporting to the Laboratory of Army Medical College, Rawalpindi, were included in the study. Blood samples were first subjected to centrifugation and stored at –20°C till tested. HCV RNA titre was determined by Real Time PCR while ALT estimation on serum samples was done on Selectra-E by kinetic method. The age, gender and treatment status of the patients was also recorded.

*Result:* In a total of 70 patients, 30% were females while 70% were males. The mean age of patients was 38 ± 11 yrs. On applying, analysis of variance (ANOVA), we found that the change in serum ALT level was significant ( $F = 6.044$  and  $p = 0.004$ ) while it was not significant in case of HCV RNA ( $F = 1.93$  and  $p = 0.153$ ) treatment with Inj. IFN and Ribavirin.

*Conclusion:* There is a definite decreasing trend in the serum ALT levels while no definite relationship of the HCV RNA level to the treatment with combination therapy with IFN and Ribavirin.

*Key Words:* Hepatitis C, Interferon, Polymerase Chain Reaction.

### INTRODUCTION

HCV is a single stranded, linear RNA virus, belonging to the family Flaviviridae. HCV or non-A, non-B hepatitis was first recognized in 1975.<sup>1</sup> It is the major cause of post transfusion hepatitis i.e in 80 – 95% cases.<sup>1</sup> There are at least six different genotypes, with a varying prevalence in different parts of the

world [http://www.saudijgastro.com/article.asp?isn=1319-](http://www.saudijgastro.com/article.asp?isn=1319-3767;year=2002;volume=8;issue=1;page=9;epage=13;aulast=Akbar-ref3)

[3767;year=2002;volume=8;issue=1;page=9;epage=13;aulast=Akbar - ref3](http://www.saudijgastro.com/article.asp?isn=1319-3767;year=2002;volume=8;issue=1;page=9;epage=13;aulast=Akbar-ref3).<sup>1</sup> Approximately 150 to 200 million individuals are currently infected with the hepatitis C virus (HCV) worldwide and 3 to 4 million new cases of infection develop each year.<sup>2</sup>

The response to treatment depends not only on the genotype but also on the viral load.<sup>3,4</sup> Those with lower load respond are better than those with higher load.<sup>3</sup> In HCV genotype – 2 and 3, often requiring short course with combination therapy, the sustained response rate is 65% while in genotype – 1 which often requires a longer treatment regimen, it is 30%.<sup>2,4</sup> The results of interferon monotherapy are not satisfactory and those with combination therapy

are about 25 – 60% and only about 41 – 48% remain HCV negative in the subsequent 6 months period after treatment.<sup>5-8</sup> The option of high doses of Pegylated (PEG) – IFN should be especially considered in situations in which short – course treatment regimens are used or in cases of infection with genotype 1.<sup>1</sup>

Normal serum ALT levels are observed in approximately 25 – 30% of people with chronic HCV infections, called healthy carriers.<sup>9</sup> The aim of this study was to find out the relative change in the HCV RNA titre and serum ALT levels before, during and after completion of treatment with interferon therapy in HCV positive patients.

### PATIENTS AND METHODS

This cross sectional and descriptive study was conducted in the Department of Pathology, Army Medical College Rawalpindi, National University of Sciences and Technology, Islamabad, from May 2010 to August 2010. Seventy, non-duplicate patients were included in the study.

Routine blood samples were first subjected to centrifugation and then stored at –20°C till tested. The RNA extraction was done using the Ribo virus

(HCV Real – TM Qual) kit of Sacace Biotechnologies, as per guidelines. This was followed by annealing, amplification and finally, Hepatitis – C RNA detection was done by Real Time PCR, running for forty cycles. ALT estimation of the HCV RNA positive serum samples was done on automated analyzer Selectra – E by kinetic method while maintaining a strict internal and external quality control. The age, gender and treatment status of the patients was also recorded.

The results were interpreted using Statistical Package for the Social Sciences (SPSS) version 17. The p-values equivalent 0.05 or < 0.05 were considered statistically significant.

**RESULTS**

Among a total of 94 patients, 27(30%) were females while 63 (70%) were males as shown in the Fig. 1. The average age of patients was 38 ± 11 years. The mean values of serum ALT and HCV RNA titres of the patients who had not yet started their treatment those taking combination therapy with Inj. interferon, 3MU three times a week, subcutaneously and capsule Ribavirin 600 mg orally, and those who had completed their course of treatment are summarised in table 1. On applying, analysis of variance (ANOVA), we found that the change in serum ALT level was significant (F = 6.044 and p = 0.004) while it was not significant in case of HCV RNA (F=1.93 and p = 0.153), during treatment with IFN and Ribavirin.

On the whole, there was no correlation of the age of the patients with the HCV RNA titre (r =

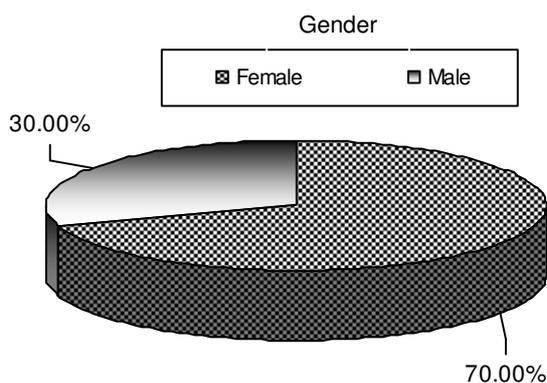


Fig. 1: Pie-chart showing the gender distribution of the patients.

Table 1: Mean values of the HCV RNA and serum ALT levels in the groups of before during and after treatment with IFN and Ribavirin.

Treatment status	HCV RNA titre (eq/ml)	Serum ALT (U/L)
Before	3×10 <sup>7</sup> ± 6×10 <sup>7</sup>	73 ± 4
During	3.8×10 <sup>6</sup> ± 1×10 <sup>7</sup>	49 ± 3
After	7.3×10 <sup>6</sup> ± 2.3×10 <sup>7</sup>	46 ± 23

0.135) or the serum ALT level (r = 0.037). Moreover, no correlation was found between the HCV RNA titre and serum ALT levels (r = 0.008) in the patients who had not started their treatment while a statistically significant correlation was found in the patients who had completed their treatment with IFN (correlation coefficient = 0.596, p = 0.004).

**DISCUSSION**

In the present study, we found that during treatment with Inj. Interferon and ribavirin, the serum ALT levels tend to decline significantly from before treatment to after completion of treatment, while we found no significant decreasing trend in the HCV RNA titre.

A study conducted by Anand et al, no correlation was found between HCV RNA levels and age of the patients (r = 0.181). Moreover, no correlation was observed between serum HCV RNA levels and the severity of liver disease, as judged by the value of ALT (r = 0.06) level.<sup>3</sup> Similarly in another study reported in 2005, Garson et al found a variable response of the patients to the interferon therapy, in terms of HCV RNA titre and ALT levels. In their study, HCV viraemia became undetectable within 1 month of commencing interferon in three of the five patients whose serum ALT levels decreased to normal on therapy.<sup>10</sup> In the remaining two patients, viraemia levels declined more slowly, becoming undetectable after a period of several months.<sup>10</sup> Recurrence of viraemia during therapy was observed in two cases. The one patient whose serum ALT levels remained elevated throughout therapy, showed no decline in viraemia.<sup>10</sup>

In another study conducted by Kakumu et al, out of a total of forty three hepatitis C patients, sixteen subjects showed sustained loss of viraemia with normalised ALT values. The other 27 subjects were non-responders (NR), who did not show a loss of viraemia.<sup>11</sup> Lee et al, in their study, conducted in 2001, found that serum ALT levels were not positively correlated with HCV RNA titers.<sup>9</sup> Similarly, in a study conducted by Ghany et al, a significant correlation was found between serum HCV RNA and ALT levels in the patients who received IFN therapy, but no correlation was observed in the untreated patients.<sup>12</sup>

From the above discussion, it is concluded that serum ALT level has a linear response to the combi-

ned treatment with IFN and ribavirin but HCV RNA has no linear relationship with this combination treatment. We recommend a large scale study, with complete follow up of the patients along with their genotyping to see the response of HCV RNA and ALT levels to the treatment regimen with IFN and ribavirin.

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#### REFERENCES

1. Akber HO. Significance of normal liver enzymes in patients with hepatitis C virus. *The Saudi journal of gastroenterology* 2002; 8 (1): 9-13.
2. Angerami RM, Junior FLG. Consensus of the Brazilian society of infectious diseases on the management and treatment of hepatitis – C – Therapeutic approach to acute hepatitis C. *Braz J Infect* 2007; 11 (6).
3. Anand BS, Velez M. Assessment of correlation between serum titers of hepatitis c virus and severity of liver disease. *World J Gastroenterol* 2004; 10 (16): 2409-2411.
4. Idrees M, Riazuddin S. Frequency distribution of hepatitis C virus genotypes in different geographical regions of Pakistan and their possible routes of transmission. *BMC Infectious diseases* 2008.
5. Puoti C. HCV carriers with persistently normal ALT levels : Not too much healthy, not true patients. *Romanian journal of gastroenterology* 2004; 13 (4): 329-334.
6. Russo MW, Brown Jr RS. Should patients with chronic hepatitis c who have normal alt levels be treated? *Current Gastroenterology Reports* 2001; 3 (1): 49-53.
7. Januszkiewicz – Lewandowska D et al. Presence of Hepatitis C (HCV) – RNA in peripheral blood mononuclear cells in HCV serum negative patients during interferon and ribavirin therapy. *Jpn. J. Infect. Dis.* 2007; 60: 29-32.
8. Radkowski M et al. Evidence of viral persistence in patients who test positive for anti-hepatitis C virus antibody and have normal alanine aminotransferase levels. *Infect. Dis.* 2005; 191: 1730-1733.
9. Lee YS et al. The Relationship of Histologic Activity to Serum ALT, HCV genotype and HCV RNA titers in Chronic Hepatitis C. *J Korean Med Sci* 2001; 16: 585-91.
10. Garson JA, Brillanti S, Ring C, Perini P, Miglioli M, Barbara L. Hepatitis C viraemia rebound after “successful” interferon therapy in patients with chronic non-A, non-B hepatitis. *Journal of medical virology* 2005; 37 (3): 210-214.
11. Kakumu S, Aiyama T, Okumura A, Iwata K, Ishikawa T, Yoshioka K. Earlier loss of hepatitis C virus RNA in interferon therapy can predict a long-term response in chronic hepatitis C. *Journal of Gastroenterology and Hepatology* 2008; 12 (6): 468-472.
12. Ghany MG, Chan TM, Sanchez – Pescador R, Urdea M, Lok ASF. Correlation between serum HCV RNA and aminotransferase levels in patients with chronic HCV infection. *Digestive diseases*.