Research Article **CODEN: IJPRNK** **ISSN: 2277-8713**
Gupta R, IJPRBS, 2013; Volume 2(5):414-423 **IJPRBS**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

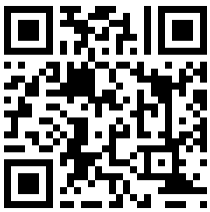
CORRELATION BETWEEN SERUM AMINOTRANFERASE LEVELS AND HCV RNA LOAD IN HEPATITIS C VIRUS RELATED CHRONIC LIVER DISEASE.

SINGH J[1], CHHINA DEEPINDER[1], GUPTA R[1], CHHINA RS[2], CHHINA RS[3] AND SURI POOJA[1]
[1]Department of Microbiology, [2]Department of Gastroenterology and [3]Department of Social & Preventive Medicine, Dayanand Medical College and Hospital, Ludhiana.

Accepted Date: 18/10/2013; Published Date: 27/10/2013

Abstract: **Background:** Hepatitis C virus (HCV) is a common infection that can lead to end stage liver disease in India. Viral load in chronic hepatitis is considered a strong predictor for the disease progression, it is unclear whether there is any link between the increasing viral loads with the other circulating biomarkers. **Aim & Objectives**: The aim of this study was to investigate the relationship between serum HCV RNA titre and serum aminotranferase levels in patients with HCV related chronic liver disease. **Methods:** A total of 33 patients with HCV related chronic liver disease were included in the study. Anti HCV antibodies were determined either by rapid card test (TRIDOT) or by IIIrd generation ELISA. Serum alanine aminotranferase (ALT)/ aspartate aminotranferase (AST) levels were measured by an automatic biochemical analyzer. The COBAS®TaqMan®HCV test, v 2.0, an *in-vitro* nucleic acid amplification test was used for the quantitation of HCV RNA in the patient's serum. The correlation between ALT/AST levels and HCV RNA titre were analyzed by calculating Spearman rank correlation coefficient and $p<0.05$ was considered significant. **Results:** An average value of ALT was found to be 64.15 IU/ml with a standard deviation of 65.03 (Range 16-370 IU/ml) and that of AST was 69.0 IU/ml with a standard deviation of 60.8 (Range 17-315 IU/ml). Average HCV RNA titre was evaluated as $10.5x10^6$ IU/ml (Range $8.83x10^2$ - $9.8x10^7$ IU/ml). A statistically significant positive correlation was observed between serum ALT levels and HCV RNA titre, however the positive correlation observed between HCV RNA titre and serum AST levels was not statistically significant. **Conclusion:** A positive correlation was observed between HCV RNA titre and the serum aminotranferase levels in patients with chronic liver disease. In addition to viral load, HCV genotype and histological damage has to be determined to assess the degree of liver damage, though decreasing viral load in response to therapy has a prognostic value.

Keywords: Alanine aminotransferase, Hepatitis C, Chronic, HCV RNA load



PAPER-QR CODE

Corresponding Author: Dr RAMA GUPTA

Access Online On:

www.ijprbs.com

How to Cite This Article:

Gupta R, IJPRBS, 2013; Volume 2(5):414-423

INTRODUCTION

The patients infected with hepatitis C virus (HCV) have different clinical outcomes, ranging from acute resolving hepatitis to chronic liver disease including liver cirrhosis or hepatocellular carcinoma.[1] Chronic HCV infection is often silent, and clinical symptoms are absent or minimal unless the disease is severe or cirrhosis is diagnosed. Many attempts to identify the natural history, progression and treatment of HCV infection have been made, but several aspects remain to be elucidated.[2] In chronically infected individuals, viral load, genotype, and elevated serum alanine aminotransferase (ALT) levels may have clinical relevance.[3-5] Approximately 25-30% of individuals with chronic HCV infections have persistently normal ALT level[2,6] and these individuals are usually referred to as healthy carrier of HCV.[7] However, several studies have demonstrated that the histological features of most healthy carriers showed chronic liver damage of a variable degree, ranging from mild hepatitis to liver cirrhosis.[8-12] In general, chronic hepatitis C patients with elevated ALT levels and high HCV RNA titers in the sera are considered to have active HCV replication in the liver and to be at risk for continued liver injury on a clinical basis. Also, the serum ALT level is recognized as a marker reflecting the degree of the histological damage and has served as a parameter for starting therapy or judging response to antiviral treatment in chronic hepatitis.[1] However, a number of recent studies showed ambivalent results in the relationships among the degree of histological damage, serum ALT level, HCV RNA titers and HCV genotype in chronic hepatitis C.[13-16] The aim of the present study was to determine the relationship between the HCV RNA load and serum aminotransferase levels in patients with HCV related chronic liver disease.

MATERIALS AND METHODS

Patients

This is a retrospective analysis of 33 consecutive patients with HCV related chronic liver disease. They consisted of 27 men and 6 women with ages ranging from 17-72 yrs (mean=47.9 yrs). The diagnosis of chronic hepatitis C was made on the basis of elevated serum ALT/ aspartate aminotranferase (AST) levels, positivity for anti-HCV antibody, and confirmation of HCV RNA by reverse transcription-polymerase chain reaction (RT-PCR).

Biochemical and Serological Tests

Anti HCV antibodies were determined either by rapid card test (TRIDOT) or by IIIrd generation ELISA. Serum ALT and AST levels were measured by an automatic biochemical analyzer. Other biochemical parameters evaluated include total and direct bilirubin, prothrombin index (PTI) and international normalized ratio (INR).

Quantification of serum HCV RNA

The COBAS®TaqMan®HCV test, v 2.0, an *in-vitro* nucleic acid amplification test was

used for the quantitation of HCV RNA genotype 1-6, in the patient's serum. The test was carried out as per kit protocol. The test procedure consisted of three major processes:

- Manual specimen preparation using high pure system viral nucleic acid kit.
- Automated reverse transcription of the target RNA to generate complementary DNA (cDNA).
- PCR based amplification of target cDNA using HCV specific complementary primers, and simultaneous detection of cleaved dual fluorescent dye labeled oligonucleotide probe that permit quantification of HCV target amplified product (amplicon).

COBAS Taqman 48 analyzer was used for automated amplification and detection of HCV RNA. The titer of circulating HCV RNA was defined by log IU/ml of HCV RNA. Linear range of detection of the test system is 25 IU/ml to 3.9 x 10^8 IU/ml and one IU is equivalent to 4 copies of HCV RNA.

Statistical Analysis: The data was analyzed using standard statistical methods. Variables were presented as mean ± standard deviation (SD). Correlation between HCV RNA titre and ALT/AST levels were assessed by calculating Spearmen Rank order correlation coefficeint and $p<0.05$ was considered significant.

RESULTS

Demographic and virological features of the patients are shown in Table-1. All the 33 patients were positive for anti HCV antibodies. An average value of ALT was found to be 64.15 IU/ml with a standard deviation of 65.03 (Range 16-370 IU/ml) and that of AST was 69.0 IU/ml with a standard deviation of 60.8 (Range 17-315 IU/ml). Average HCV RNA load was evaluated as $10.5x10^6$ IU/ml (Range $8.83x10^2$ - $9.8x10^7$ IU/ml).

Figure I shows the relationship between serum ALT levels and HCV RNA titre in patients with chronic hepatitis C. A direct correlation was observed between the two parameters, which was found to be statistically significant (r= 0.364, *p*=0.037). At the same time serum AST levels also showed a direct correlation with the viral load, (Figure II) but this correlation was not statistically significant (r=0.257; *p*=0.149)

Relationship of HCV viral load with other biomarkers of liver tissue damage were also evaluated. Direct bilirubin levels showed a statistically significant positive correlation with viral load (r=0.454; p=0.008). However, INR and PTI showed a negative correlation with HCV RNA load, though it is not statistically significant. (Table II)

DISCUSSION

The World Health Organization estimated that approximately 3% of the population worldwide has been infected with HCV thus far. There are about 170 million patients

with HCV in the world and three to four million individuals are diagnosed as new cases every year.[17-19] The clinical outcome of HCV infections is believed to depend mostly on the balance between the rate of replication of the infecting virus and the capacity of the immune system to mount rapid, multi-specific and efficient virus-specific responses to inhibit infection before the virus can devise strategies to evade immune surveillance.[20] Approximately 80% of patients with hepatitis C virus develop chronic infection and progression to cirrhosis occurs in 20% of these subjects.[21] Since most cases of HCV related liver cirrhosis remain undetected for many years and are often diagnosed at the stage of hepatocellular carcinoma occurring in compensated cirrhosis, suitable and reliable clinical examinations are needed to predict the severity of liver injury caused by HCV infection. Evaluation of HCV infection involves biochemical, virological and histological examination. The studies regarding the role of serum HCV RNA titre and ALT levels in assessing the degree of liver damage in patients with chronic HCV remains unclear and conflicting.[22-25] The aim of this study was to address whether there was a correlation between the serum ALT/AST levels and in chronic hepatitis C. The results of this study revealed a positive significant correlation between serum ALT levels and HCV RNA titers, however the direct correlation between AST and HCV RNA titre was found to be statistically insignificant. In addition bilirubin, a biochemical marker of liver tissue damage, significantly correlated with HCV RNA viral load. The previous reports in this regard are variable and conflictiong. Lau *et al* and Zeuzem *et al* have observed that there is no correlation between HCV viral load and serum ALT levels and at the same time extent of histological damage.[15,26] On the other hand Kato *et al*[27] observed significantly higher HCV RNA titre in patients with chronic active hepatitis and cirrhosis compared to those with milder histological abnormalities such as persistent chronic hepatitis. In another study it has been documented that there is no significant correlation between HCV RNA viral load and AST levels, while the two amino-tranferases (ALT/AST) levels were in positive correlation.

Bozdayia *et al*[28] has reported that relationship between ALT levels and HCV RNA load is dependent on the serotype of HCV. They suggested that hepatitis C patients infected with genotype 1b may show a relatively weaker immune response, resulting in lower ALT levels and higher viremia levels, while patients with higher ALT levels and lower viral load have more active immune response to chronic viral infection.

In conclusion, our study shows that viral load or ALT/AST levels does not accurately predict the degree of liver injury in chronic HCV carriers as progression of the disease is determined by many factors, including immune response to virus, however

decreasing viral load and amino-tranferases levels in response to therapy has a prognostic value. Thus, the histological evaluation would be the gold standard to accurately assess the degree of liver damage and to decide therapeutic plan in patients chronically infected with HCV.

Table I. Mean and Standard Deviation of Serum Biomarkers and Viral load

Serum Biomarkers	Mean ± S.D	Range
ALT (U/L) (range)	64.15±65.03	16 – 370
AST (U/L) (range)	69.0±60.8	17 – 315
Total bilirubin (mg/dL)	0.99±0.98	0.21 - 4.59
Direct bilirubin (mg/dL)	0.59±1.00	0.04 – 5.46
Prothrombin Index (sec)	15.7±2.9	11.90 – 21.20
INR	1.3±0.27	0.98 – 1.98
HCV RNA (IU/ml)	$1.05x10^7 \pm 2.19x10^7$	$8.83x10^2 – 9.84x10^7$

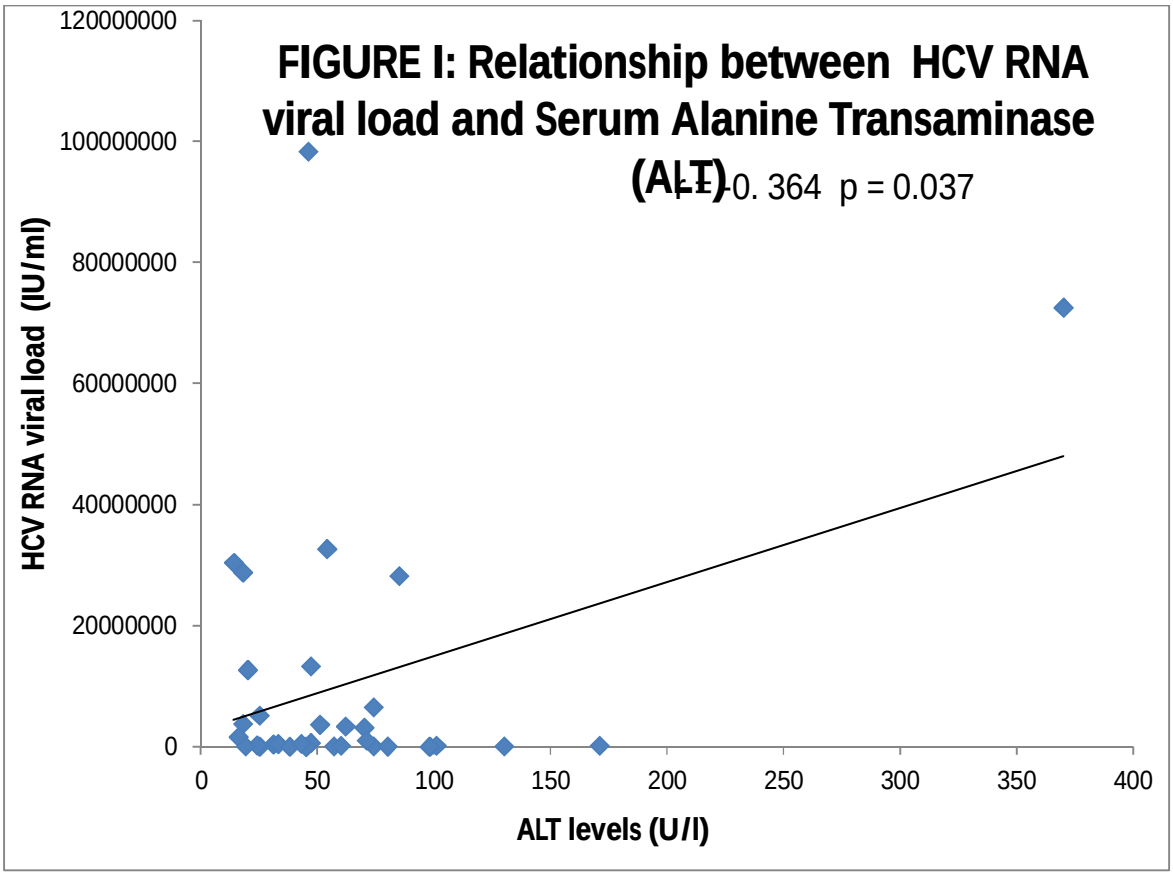


FIGURE I: Relationship between HCV RNA viral load and Serum Alanine Transaminase (ALT)
r = 0. 364 p = 0.037
HCV RNA viral load (IU/ml)
120000000
100000000
80000000
60000000
40000000
20000000
0
0
50
100
150
200
250
300
350
400
ALT levels (U/l)

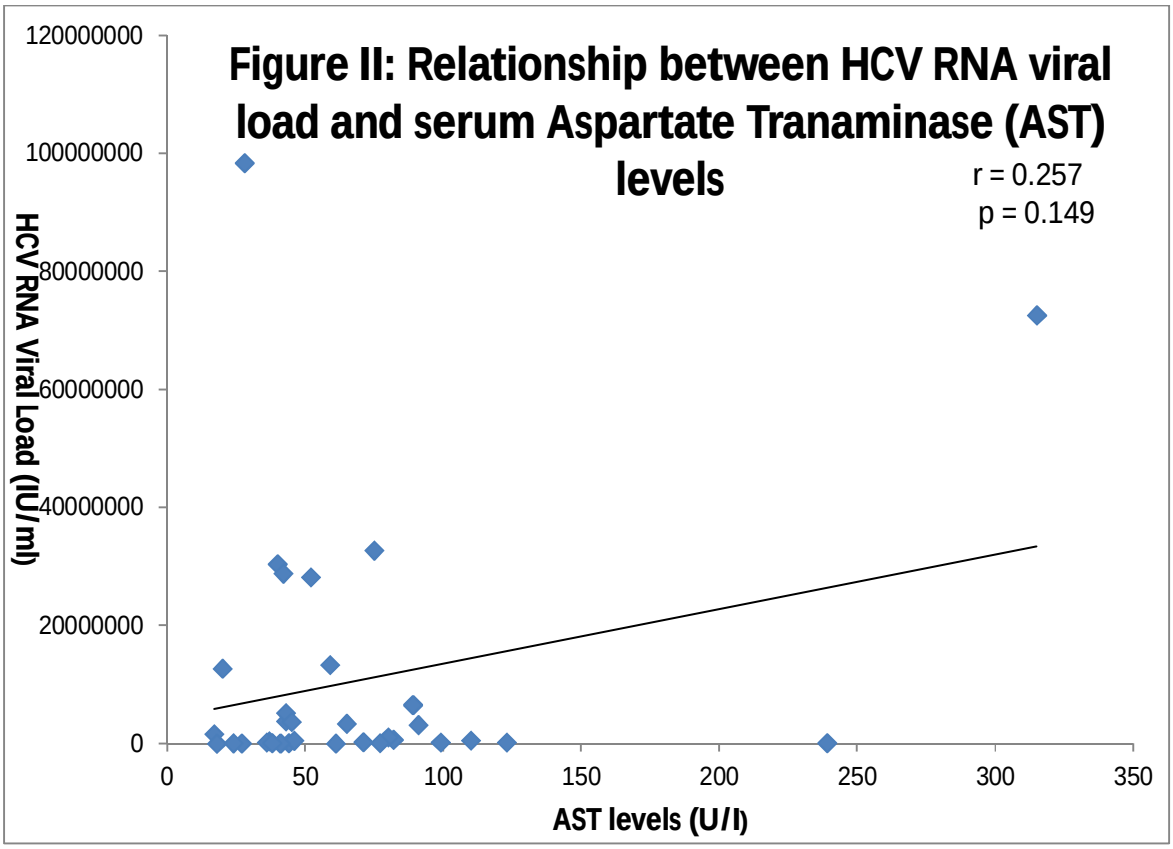


Table II: Correlation of HCV viral load with various biomarkers.

Serum Biomarker	r – value	p - value
AST (U/L)	0.257	0.149
ALT (U/L)	0.364*	0.037
Total Bilirubin (mg/dl)	0.295	0.096
Direct Bilirubin (mg/dl)	0.454**	0.008
PTI (sec)	0.271	0.127
INR	-0.242	0.175

* Correlation is significant at < 0.05 level. ** Correlation is significant at 0.01 level

REFERENCES

1. Young SL, Seung KY, Eun SC, Si Hyun B, Jong YC, Joon YH, *et al.* The relationship of histologic activity to serum ALT, HCV genotype and HCV RNA titers in chronic Hepatitis C. Korean Med Sci 2001;16:585-91

2. Hoofnagle J H, Di Bisceglie A M. The treatment of chronic viral hepatitis. N Engl J Med. 1997;336:347–56.

3. Lau J Y, Davis G L, Kniffen J, Qian K P, Urdea M S, Chan C S, *et al*. Significance of serum hepatitis C virus RNA levels in chronic hepatitis C. Lancet. 1993;341:1501–1504.

4. Magrin S, Craxi A, Fabiano C, Marino L, Fioretino G, Lo Iacono O, *et al*. HCV viraemia is more important than genotype as a predictor of response to interferon in Sicily (Southern Italy). J Hepatol. 1996;25:583–90.

5. Mizokami M, Orito E, Gibo Y, Suzuki K, Ohba K, Ohno T, *et al*. Genotype, serum level of hepatitis C virus RNA and liver histology as predictors of response to interferon-alpha 2a therapy in Japanese patients with chronic hepatitis C. Liver. 1996;16:23–7.

6. Marcellin P, Levy S, Erlinger S. Therapy of hepatitis C: patientsn with normal aminotransferase levels. Hepatology 1997; 26: 133S-6S.

7. Brillanti S, Foli M, Gaiani S, Masci C, Miglioli M, Barbara L. Persistent hepatitis C viraemia without liver disease. Lancet 1993;341:464-5.

8. 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; 26: 1393-8.

9. Puoti C, Stati T, Magrini A, Rigato P, Romagnoli G, Rossi P, *et al*. Liver histology in anti-HCV positive subjects with normal ALT levels. Ital J Gastroenterol Hepatol 1997; 29:383-4.

10. Alberti A, Morsica G, Chemello L, Cavalletto D, Noventa F, Pontisso P, *et al*. Hepatitis C viraemia and liver disease in symptomfree individuals with anti-HCV. Lancet 1992; 340(8821): 697-8.

11. Prieto M, Olaso V, Verdu C, Cordoba J, Gisbert C, Rayon M, *et al*. Does the healthy hepatitis C virus carrier state really exist? An analysis using polymerase chain reaction. Hepatology 1995; 22: 413-7.

12. Healey CJ, Chapman RW, Fleming KA. Liver histology in hepatitis C infection: a comparison between patients with persistently normal or abnormal transaminases. Gut 1995; 37: 274-8

13. Magrin S, Craxi A, Fabiano C, Simonetti RG, Fiorentino G, Marino L, *et al*. Hepatitis C viremia in chronic liver disease: relationship to interferon-alpha orcorticosteroid treatment. Hepatology 1994; 19: 273-9.

14. Gretch D, Corey L, Wilson J, dela Rosa C, Willson R, Carithers RJr, *et al*. Assessment

of hepatitis C virus RNA levels by quantitative competitive RNA polymerase chain reaction: high-titer viremia correlates with advanced stage of disease. J Infect Dis 1994; 169: 1219-25.

15. Lau JY, Davis GL, Kniffen J, Qian KP, Urdea MS, Chan CS, *et al*. Significance of serum hepatitis C virus RNA levels in chronic hepatitis C. Lancet 1993; 341(8859): 1501-4.

16. Kumar U, Thomas HC, Monjardino J. Serum HCV RNA levels in chronic HCV hepatitis measured by quantitative PCR assays: correlation with serum AST. J Virol Methods 1994; 47: 95-102.

17. Lo Re V, Kostman JR. Management of Chronic hepatitis C. Postgrad Med J 2005;81: 376-82.

18. Ray KW. Global epidemiology and burden of hepatitis C. Microbes Infect 2004;4:1219-25

19. Bhatty SA, Shaikh NA, Akhter SS. Liver histology in hepatitis C virus positive patients with normal and elevated amino transferase levels. J Pak Med Assoc. 2009; http://www.jpma.org.pk/full_article_text.php?artcle_id=1874.

20. Doherty PC, Ahmed R. Immune response to viral infection. In: Nathanson N, ed. Viral pathogenesis. Philadelphia: Lippincott-Raven: 1997: 143-61.

21. Alter MJ. The epidemiology of hepatitis C virus in the west. Semin Liv Dis 1995; 15: 5 -14

22. Adinolfi LE, Utili R, Andreana A, Tripodi MF, Marracino M, Gambardella M, *et al*. Serum HCV RNA levels correlate with histological liver damage and concur with steatosis in progression of chronic hepatitis C. Dig Dis Sci.2001;46:1677–83.

23. Zechini B, Pasquazzi C, Aceti A. Correlation of serum aminotransferases with HCV RNA levels and histological findings in patients with chronic hepatitis C: The role of serum aspartate transaminase in the evaluation of disease progression. Eur J Gastroenterol Hepatol. 2004;16:891–6.

24. Anand BS, Velez M. Assessment of correlation between serum titers of hepatitis C virus and severity of liver disease. World J Gastroenterol. 2004;10:2409–11.

25. Bacon BR. Treatment of patients with hepatitis C and normal serum aminotransferase
levels. Hepatology. 2002;36:S179–S184.

26. Zeuzem S, Franke A, Lee JH, Herrmann G, Ruster B, Roth WK. Phylogenetic analysis of hepatitis C virus isolates and their correlation to viremia, liver function tests, and histology. Hepatology 1996; 24: 1003-9.

27. Kato NK, Hosoda K, Ito Y, Ohto M, Omata M. Quantification of hepatitis C virus by competitive reverse transcription' polymerase chain reaction: increase of the virus in advanced liver disease. Hepatology 1993; 18:16-20.

28. Bozdayia MA, Uzunalimoglua O, Aslana N, Bozkayab H, Türkyilmaza AR, Kayhanc B, *et al*. Influence of viral load and alanine aminotransferase on viral genetic heterogeneity in patients with chronic hepatitis C virus infection. Intervirology 2000; 43: 61-6.