ORIGINAL ARTICLE

Assessment of Serum Alpha-Glutathione S-Transferase and Alanine Aminotransferase Levels in Patients with Interferon Treated Hepatitis C

FAISAL KHAN[1], MARIA SARFRAZ[2], BUSHRA GOHAR SHAH[3], *MUHAMMAD KAMRAN AMIR[4], FAIZA MEHBOOB[5], MUHAMMAD NAZIR AWAN[6], ASMA SAEED[7]*
[1]Associate Prof. Biochemistry, Multan Medical & Dental College, Multan
[2]Assistant Prof. Biochemistry, Rawal Institute of Health Sciences, Islamabad
[3]Associate Prof. Physiology, Sahara Medical College, Narowal
[4]Associate Prof. Anatomy, Multan Medical & Dental College, Multan
[5]Associate Prof. Anatomy, Multan Medical & Dental College, Multan
[6]Assistant Prof. Anesthesia, Islamic International Medical College, Rawalpindi
[7]Prof. Pharmacology, Multan Medical & Dental College, Multan
Correspondence to: Faisal Khan, Email: faisalkhan1900@gmail.com, Cell: 03215667305

ABSTRACT

Background: Hepatitis C virus infection is worldwide health issue.
Aims & Objectives: To compare and correlate the mean levels of biochemical parameters i.e. total bilirubin, ALT, AST, α-GST and PCR quantitative at day zero and one month after the start of the treatment with Interferon alpha-2b and ribavirin.
Materials & methods: The study consists of 40 patients with normal liver and clinical evidence of compensatory liver disease (early stage cirrhosis) on ultrasound examination. Before the start of IFN alpha-2b and ribavirin treatment, at zero day, all of the 40 patients were HCV- RNA positive. One month after the start of IFN alpha-2b and ribavirin treatment (Ribavirin 500mg B.D and IFN alpha-2b 4.5 mega units,(3times per week for 6 months) these 40 patients were again tested. On the basis of the presence of HCV-RNA virus, they were classified into two groups (A&B).
Results: After treatment, viral load was significantly reduced in group A (n=31), and significantly reduced in patients of group B (n=9). ALT and AST levels were reduced significantly in patients of Group A (n=31) and insignificantly reduced in patients of group B after treatment. After treatment, Alpha-GST level was significantly elevated among patients of Group A (n=31) who remained HCV-RNA positive and significantly reduced in HCV-RNA negative patients in group B (n=9)
Conclusion: In hepatic insults, α-GST levels show an early rise in serum. So, α-GST level is a sensitive method for evaluation of hepatic insults without ALT elevation.
Key words: alpha-glutathione s-transferase (α-GST); alanine aminotransferase(ALT); interferon; hepatitis c

INTRODUCTION

Health burden due to Hepatitis C virus infection is on a rise at an alarming rate. Although the incidence has fallen, but its healthcare burden is still expected to rise in the upcoming four decades.[1] Early therapeutic intervention may slow the progression towards various complications.[2]

Late occurance of complications mostly occuring after about 20 years of infection, provides a time window for earlier detection & managing hepatic insult.[3] Making a clinical diagnosis of liver fibrosis by noninvasive means has always been challenging. History, clinical evaluation, routine LFTs and determination of serum bilirubin have not served as sensitive markers of progression of liver disease[4]. Liver biopsy is the best method for liver fibrosis assessment, but has shortcomings such as poor sampling of liver tissues, risk of bleeding, pain and sampling errors and dependency on observer[5].

Noninvasive histological methods & tests are needed due to the marked adverse effects of liver biopsy[6]. For finding the progressive fibrosis, number of markers have revealed assurance, though their sensitivities for identification of mild fibrosis are low. None of the serum marker has yet proven its efficacy in appropriately diagnosing and assessing the degree of hepatic fibrosis. Therefore, there is a need for an appropriate, noninvasive indicator of liver fibrosis .

Although alanine transaminase is used as guide for hepatocellular damage, its rise is found in only 40.2 % of HCV patients[7]. Furthermore, HCV-RNA levels are not well reflected by aminotransferase levels. Several non-invasive tests & approaches to anticipate liver disease severity have been suggested. The non-invasive tests include platelet count, serum albumin, INR & elastography to check for the liver stiffness[8,9].

Alanine Aminotransferase and Aspartate Aminotransferase are part of LFTs but their concentrations around the portal regions is found higher while α-GST has a uniform distribution in both the centrilobular and periportal regions of the hepatocytes[10].

One of the enzyme responsible for cellular detoxifying processes is Alpha-Glutathione S-Transferase (a-GST). Factors which make monitoring for hepatocellular damage by Alpha-Glutathione S-Transferase (a-GST) useful than routine liver function tests are its: uniform distribution in hepatic tissues, high concentration in the cytosol, and short plasma half-life . Is has been reported to better reflect liver damage as compared to aminotransferases[11]

The present study aimed to estimate serum α-GST levels along with aminotransferases levels in patients with hepatitis C. It was further determined if alpha-GST is a

sensitive method for evaluation of hepatic insult without ALT elevation. Early rise of serum α-GST levels in various hepatic insults has been shown in a number of studies[12,13,14]. For assessing and managing HCV patients on interferon-alpha therapy, studies have validated its role as a more better marker[15].

MATERIALS & METHODS

It was cross sectional study was carried out at biochemistry department of PGMI, Lahore & department of gastroenterology, Lahore General Hospital, Gujranwala liver foundation and Chughtais Lahore laboratory from January 2013 – September 2013. Recently diagnosed untreated patients of HCV, aged 15-60 of both sexes, presenting in the outpatient gastroenterology/liver clinic, with normal liver and clinical evidence of compensatory liver disease (early stage cirrhosis) on ultrasound examination were included in the study.

Exclusion Criteria: The HCV patients with definite ultrasonic or clinical confirmation of decompensated liver disease (cirrhosis with complication) were excluded. Other patients excluded were those with any other type of hepatitis or known liver disease, who had interferon or ribavirin therapy, with any other known debilitating disease such as tuberculosis, malignancy or HIV, those with the history of upper gastro-intestinal bleeding who have not had upper gastro-intestinal endoscopy yet, those suffering from a condition other than hepatitis that can alter the trail tests e.g. coagulopathies including drugs like heparin and warfarin.

Study Population: HCV diagnosis was confirmed with PCR and decompensated liver disease (cirrhosis with complication) was excluded in recently diagnosed 40 patients. Anti HCV therapy was initiated with Interferon alpha-2b and ribavirin and the first day was taken as day zero. Lab. tests were performed at zero day and then repeated after one month of therapy. Patients were then grouped as: Group A (Non-responders): This group included 31 patients who persisted as HCV-RNA positive (RVR negative) thereafter one month of therapy. Group B (Responders): This group included 9 patients who eventually turne HCV-RNA negative (RVR positive) after one month of therapy.

The project was allowed by the Ethical Research Committee of Post Graduate Medical Institute (PGMI) Lahore. Informed written consent was taken from each study participant. Informed written consent of all the subjects included in the study was obtained, history taking & clinical information were entered in the proforma. Five milliliter's of blood samples were collected from all cases from the vein above elbow under aseptic condition. Serum for biochemical markers were separated and preserved at -20ºC for assay and haematological markers were assayed on the day of collection.

Biochemical Parameters: Biochemical parameters were Total bilirubin**,** ALT**,** AST**,** α-GST**,** (PCR) for estimation of HCV-RNA**,** Estimation of HCV antibodies (Initial screening)

The kit used for the estimation of serum Alanine Aminotransferase (ALT) was manufactured by Diasys Diagnostic Systems Gmbh, Holzheim, Germany (cat No 1.14820-001 Ecoline RS+). Reference Range:Female ≤ 31U/l; Male ≤ 41U/l[16]. The kit used for the estimation of AST was manufactured by Diasys Diagnostic Systems Gmbh, Holzheim, Germany (cat No 1.14829.0001 Ecoline RS+). Reference Range:Female ≤ 31U/L; Male ≤ 35U/l[17,18,19].

Test employed for Quantification of HCV-RNA was Abbott RealTime™ HCV. It provided lower limit of detection of 12 IU/mL, a more than 99.5% specificity & from 12 to 10,000,000 IU/mL linear amplification range independent of the HCV genotype[18,19].Hepatitis C diagnosis was done by an enzyme immunoassay (EIA) that detects antibodies to HCV, which were used as an initial screening test[20].

The Argutus Medical HEPKIT®-Alpha kit was used which provided quantitative determination of human serum alpha Glutathione S-Transferase(α-GST) and sodium-heparinized plasma. HEPKIT®-Alpha is a specific, precise immunoassay for α-GST[21]. Argutus Medical HEPKIT®-Alpha is a quantitative enzyme immunoassay. ELISA HEPKIT by Biotrin International, Dublin, Ireland, was used for the determination of α-GST as per the manufacturer's instructions where α-GST levels ≤ 8 ng/ml were considered normal[22].

Statistical Analysis: Entry & analysis of data was done using Statistical Package for Social Sciences, version 17 was used for data entry & its analysis.

Quantitative data were expressed as mean and standard deviation ± SD and paired sample t-test was performed. For comparison of mean levels of ALT, AST and α-GST one way ANOVA was applied. If differences existed among the means, then post hoc test and pair-wise multiple comparisons were applied to determine which means differ. A p-value of <0.05 was considered statistically significant.

The effects of ALT, AST and α-GST on virological response were displayed by roc curve to determine which test had a significant role in classification and discrimination of patients with virus before and after treatment. Pearson correlation coefficient 'r' was used for Correlation analysis

RESULTS AND OBSERVATIONS

The study consisted of 40 newly diagnosed HCV patients with normal liver and clinical evidence of compensatory liver disease (early stage cirrhosis) on ultrasound examination.

One month after the onset of treatment with IFN alpha-2b and ribavirin (Ribavirin 500mg B.D and IFN alpha-2b 4.5 mega units,(3times per week for 6 months) these 40 patients were again tested.

Means ± S.D of group A patients was 39.77+ 9.09 and of group B was 37.22+ 8.303. Out of 21 males, 16 were in group A & 5 in group B were 5. Out of 19 females, 15 were in group A and 4 in group B).

To detect presence of HCV-antibodies in the blood, HCV antibody test was performed in all 40 patients initially. At zero day, 31 patients in group A and 9 patients in group B were reactive for HCV antibodies. Normal level: Cutoff index < 1.0.

Comparison of mean levels of ALT by paired t-test at zero day and after one month of therapy, indicated a significant decrease in group A (n=31) patients who

retained HCV-RNA positive status. While insignificant reduction was shown by group B patients (n=9) who were HCV-RNA negative after treatment. Normal level: Male ≤ 41U/l; Female ≤ 31U/l. ALT (Table 1, 2)

Comparison of mean levels of AST by paired t-test at zero day and after a month of treatment, indicated a significant decrease in group A (n=31) patients who retained HCV-RNA positive status. While insignificant reduction was shown by group B patients (n=9) who were HCV-RNA negative after treatment. Normal level: Normal level: Female ≤ 31U/L; Male ≤ 35U/l. AST

Comparison of mean levels of α-GST by paired t-test at zero day and after one month of treatment, indicated a significant elevation in group A (n=31) patients who retained HCV-RNA positive status. While significant reduction was shown by group B patients (n=9) who were HCV-RNA negative after treatment. Normal level: Normal level: ≤ 8 ng/ml. Alpha-GST (Table 1, 2)

Table 1- ALT, AST, Alpha-GST and PCR Quantitative in Groups A & B at Zero Day and One Month

Laboratory Investigations		Zero Day HCV- RNA		One Month HCV-RNA	
		Group A	Group B	Group A	Group B
AST(IU/L)	≤ 40	6(19.4%)	7(77.8%)	10(32.3%)	6(66.7%)
	>40	25(80.6%)	2(22.2%)	21(67.7%)	3(33.3%)
ALT(IU/L)	≤ 40	7(22.6%)	4(44.4%)	7(22.6%)	6(66.7%)
	>40	24(77.4%)	5(55.6%)	24(77.4%)	3(33.3%)
Alpha-GST (ng/ml)	≤ 8.0	5(16.1%)	2(22.2%)	3(9.7%)	6(66.7%)
	>8.0	26(83.9%)	7(77.8%)	28(90.3%)	3(33.3%)
PCR Quantitative (IU/ml)	>12.0	31(100%)	9(100%)	31(100%)	0(0%)
	< 12.0	0(0%)	0(0%)	0(0%)	9 (100%)

ALT=Alanine Transaminase, AST=Aspartate Transaminase, α-GST= Alpha-Glutathione S-Transferase, PCR=Polymerase Chain Reaction.

Table-2: ALT, AST, Alpha-GST and PCR Quantitative in Groups A & B at Zero Day and One Month

Laboratory Investigations	Group A			Group B		
	Time of Investigation		Paired t-test(Before treatment Vs After Treatment) p-Value	Time of Investigation		Paired t-test(Before treatment Vs After Treatment) p-Value
	Zero Day	One Month		Zero Day	One Month	
Serum ALT(IU/L)	88.90±55.49	57.8±26.97	0.003*	47.0±20.56	39.6±20.53	0.398
Serum AST(IU/L)	70.5±45.50	50.5±19.69	0.015*	36.8±10.28	37.44±15.12	0.907
Serum α-GST (ng/ml)	14.7±4.68	17.3±6.60	0.028*	13.9±4.92	8.6±3.19	0.035*
PCR Quantitative (IU/ml)	42.61± 21.27	23.81± 7.06	0.000*	20.44± 8.41	0.000± 0.0000	0.000*

P value ≤ 0.05 is considered significant and highly significance at <0.001.
ALT=Alanine Transaminase, AST=Aspartate Transaminase, α-GST= Alpha-Glutathione S-Transferase, PCR=Polymerase Chain Reaction,*Significant.

DISCUSSION

People belonging to low socioeconomic group cannot afford the chronic hepatitis C treatment because of its cost & furthermore assistance from government & non-government resources is quite limited[23] Standard interferon therapy introduction was a big hope for chronic hepatitis C patients[24].

The liver fibrosis assessment through liver biopsy is risk associated and hence concordance is low[25]. Liver biopsy is an expensive procedure and requires expert help for its performance and interpretation[26]. Finding other methods of evaluation which are simpler, cost-effective & noninvasive is therefore important[27].

The result of ALT, AST, α-GST and viral load levels in one geographical location may not mean the same as in other geographical location trials. As chronic hepatitis C shows race dependent behaviour[28,29].

In our study indicates that ALT and AST levels were reduced significantly in group A and insignificantly reduced in group B after treatment at zero day & one month later

They concluded the decline of ALT after 2 & 4weeks of combination therapy with IFN and RBV from baseline to be a good indicator of an SVR[30]. The result of our study showed similar results. Use of ALT as a surrogate marker for treatment effect in patients with elevated ALT was suggested by Ribeiroet al[31].

Sharawy et al. showed that comparison of ALT & AST before and after treatment, among non responders to combination therapy (interferon & ribavirin) showed significant variation as P value < 0.05[32,33]. Thus serum α-GST proved to be a good test to detect the presence of HCV virus, at both zero day and one month.

Nelson et al. data demonstrated similar diagnostic ability of alpha GST as serum aminotransferases & speculated that it can be used for monitoring patients on interferon treatment[15].

Study conducted by Abdel-Moneima and Sliem found significantly higher mean values as compared to the controls of ALT, AST and Alpha-GST in HCV patients with 82% sensitivity. Results of our study showed similar results[34].

CONCLUSION

Alpha-GST shows early elevation of serum α-GST levels in hepatic insults & is a sensitive method for evaluating the liver injury without ALT elevation.

REFERENCES

1. Davis GL, Albright JE, Cook S, Rosenberg D. Projecting the future healthcare burden from hepatitis C in the United States. InHepatology 1998 Oct 1 (Vol. 28, No. 4, pp. 390A-390A). independence square west curtis center, ste 300, philadelphia, pa 19106-3399 usa: wbsaunders co.
2. Attia MA. Prevalence of hepatitis B and C in Egypt and Africa. AntivirTher. 1998;3(Suppl 3):1–9.
3. Strickland GT. Liver disease in Egypt: Hepatitis C superseded schistosomiasis as a result of iatrogenic and biological factors. J. hepatology; 2006; 43: 915–922.

4. Fortunato G, Castaldo G, Oriani G, Cerini R, Intrieri M, Molinaro E, et al. Multivariate discriminant function based on six biochemical markers in blood can predict the cirrhotic evolution of chronic hepatitis. Clin Chem. 2001, 47(9): 1696-700.
5. Cadranel JF, Rufat P, Degos F. Practices of Liver Biopsy in France: results of a Prospective Nationwide Survey. Hepatology. 2000 Sep; 32(3):477-81.
6. Afdhal NH. Diagnosing fibrosis in hepatitis C: is the pendulum swinging from biopsy to blood tests? Hepatology. 2003 May; 37(5):972-4.
7. Mohamed MK, Bakr I, El-Hoseiny M, Arafa N, Hassan A, Ismail S, et al. HCV-related morbidity in a rural community of Egypt. J Med Virol. 2006 Sep 1; 78(9):1185-9.
8. Castera L. Non-invasive assessment of liver fibrosis in chronic hepatitis C. Hepatol Int. 2011 Jun; 5(2): 625–34.
9. Bain VG, Bonacini M, Govindarajan S, Ma M, Sherman M, Gibas A, et al. A multicentre study of the usefulness of liver biopsy in hepatitis C. J viral hepat. 2004 Jul; 11(4):375-82.
10. Beckett GJ, Hayes JD. Glutathione S-transferases: biomedical applications. InAdvances in clinical chemistry 1993 Jan 1 (Vol. 30, pp. 281-380). Elsevier.
11. Trull AK, Facey SP, Rees GW, Wight DG, Noble-Jamieson G, Joughin C, Friend PJ, Alexander GJ. Serum alpha-glutathione S-transferase--a sensitive marker of hepatocellular damage associated with acute liver allograft rejection. Transplantation. 1994 Dec; 58(12):1345-51.
12. Sherman M, Bass NM, Campbell JA, Kirsch RE. Radioimmunoassay of human ligandin. Hepatology. 1983; 3(2):162-9.
13. Beckett GJ, Hayes PC, Hussey AJ, Bouchier IA, Hayes JD. Plasma glutathione S-transferase measurements in patients with alcoholic cirrhosis. Clinicachimicaacta. 1987 Oct 30; 169(1):85-9.
14. Hayes PC, Hussey AF, Keating J, Bouchier IA., and Williams, R. Glutathione S-transferase levels in autoimmune chronic active hepatitis: a more sensitive index of hepatocellular damage than aspartate aminotransferase. Clin. Chim. Acta. 1988; 172(2)**:** 211-16.
15. Nelson DR, Lim HL, Oliver D, Qian KP, Davis GL, and Lau JY. Alpha-glutathione S-transferase as a marker of hepatocellular damage in chronic hepatitis C virus infection. Am. J. Clin. Pathol. 1995, 104(2):193-198.
16. Schumann, G., Bonora, R., Ceriotti, F., Ferard, G., Ferrero, C.A., and Franck., P.F.H., 2002. IFCC primary reference procedure for the measurement of catalytic activity concentration of enzymes at 37ºC. Part 5: Reference procedure for the measurement of catalytic concentration of aspartate aminotransferase. Clin. Chem.Lab. Med., 40(7): 725-733.
17. Schumann G, Bonora R, Ceriotti F, Férard G, Ferrero CA, Franck PF, Gella FJ, Hoelzel W, Jørgensen PJ, Kanno T, Kessner A. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 C. Part 4. Reference procedure for the measurement of catalytic concentration of alanine aminotransferase. Clin Chem. Lab. Med, 2002 Jul 24; 40(7):718-24.
18. Michelin BD, Muller Z, Stelzl E, Marth E, Kessler HH. Evaluation of the Abbott Real Time HCV assay for quantificative detection of hepatitis C virus RNA. J. Clin.Virol. 2007 Feb 1; 38(2):96-100.
19. Sábato MF, Shiffman ML, Langley MR, Wilkinson DS, Ferreira-Gonzalez A. Comparison of performance characteristics of three real time reverse transcription-PCR test systems for detection and quantification of hepatitis C virus. J. Clin. Microbiol.,2007 Aug 1;45(8):2529-36.
20. Thomas L. Alanine aminotransferase (ALT), Aspartate aminotransferase (AST). Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft. 1998; 2:55-65.
21. Rees GW, Trull AK, Doyle S. Evaluation of an enzyme-immunometric assay for serum alpha-glutathione S-transferase. Ann. Clin.Biochem.1995 Nov; 32(6):575-83.
22. Loguercio C, Tuccillo C, Caporaso N, Del Vecchio Blanco G, Morisco F, and Guerriero, C. Determination of plasma alpha-glutathione S-transferasesin patients with HCV-related chronic infection: its significance and possible clinical relevance. Liver1998:18(3), pp. 166-172.
23. Heathcote J. and Main J. Treatment of hepatitis. J. Viral. Hepat. 2005., **3**, 223- 235.
24. McHutchison JG, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK.1998. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. N. Engl J Med 1998. 339(21): 1485–92.
25. Hui AY[1], Chan HL, Wong VW, Liew CT, Chim AM, and Chan FK, Sung JJ. Identification of chronic hepatitis B patients without significant liver fibrosis by a simple noninvasive predictive model. Am J Gastroenterol. 2005 Mar; 100(3):616-23.
26. Dufour DR[1], Lott JA, Nolte FS, Gretch DR, Koff RS, S eeff LB.Diagnosis and monitoring of hepatic injury.I. P erformance characteristics of laboratory tests.Clin Chem. 2000 Dec; 46(12):2027-49.
27. Poynard T, Imbert-Bismut F, Munteanu M, Messous D, Myers RP, Thabut D. Overview of the diagnostic value of biochemical markers of liver fibrosis (Fibro Test, HCV FibroSure) and necrosis (ActiTest) in patients with chronic hepatitis C. Comp. Hepatol., 2004; 3:8.
28. Mukherjee KL,and Swarajit G Medical Laboratory Technology. Teta McGraw-Hill Publishing Company Limited, 1998. 1, pp. 303-307, 372-375.
29. Richer SS. Laboratory assays for diagnosis and management of hepatitis C Virus infection. J Clin Microbiol. 2002 Dec; 40(12):4407-12.
30. Turbide C[1], Soulellis C, Deschênes M, and Hilzenrat N. Does a rapid decline in the hematological and biochemical parameters induced by interferon and ribavirin combination therapy for the hepatitis C virus predict a sustained viral response? Can J Gastroenterol. 2008 Feb; 22(2):149-52.
31. Ribeiro RM[1], Layden-Almer J, Powers KA, Layden TJ and Perelson AS. Dynamics of alanine

aminotransferase during hepatitis C virus treatment. Hepatology. 2003 Aug; 38(2):509-17.
32. Sharawy, M.A., Okasha, A.M., Shata, M.A.A., and El Latif, F.F.A., 2010. [Online]. Biochemical studies on oxidative stress factors on chronic hepatitis c virus patients treated by peglated interferon and ribavirin combination therapy. Available at: mu.minia.edu.eg/Medecine_Ar/images/stories/Jun%202010/1122. doc[cited on 2013 Jul. 15].
33. Witthoft, T.H., Moller, B., and Wiedmann, K.H., 2007. Safety and tolerability of peginterferon alfa-2a (Ro25-8310) monotherapy and combination therapy with ribavirin (Ro20-9963) in patients with chronic hepatitis C. J.Viral Hepatitis.
34. Abdel-Moneima SM and Sliem H. Significance of serum alpha-glutathione S-transferase assessment in hepatitis C patients with different alanine aminotransferase patterns.Gastroenterology Res. 2011 Feb; 4(1): 13–19.