

Significance of Hepatic Profile and Malondialdehyde as Marker of Lipid Peroxidation in HCV Patients: A Perspective Study from Local Population of Punjab

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ABSTRACT

Objective: All over the world Hepatitis C virus (HCV) remains to be a main etiological mediator of liver disease. Approximately, 10 million people in Pakistan are diseased with HCV. Pegylated interferon plus ribavirin signifies the gold standard therapy but various side effects may occur.

Study Design: Comparative study.

Place and Duration of Study: This study was conducted at Jinnah Hospital Lahore from August 2013 to March, 2014.

Materials and Methods: Thirty five patients of hepatitis C virus and Twenty three age and sex-matched clinically apparently healthy individuals were eligible for inclusion in the study at Jinnah Hospital Lahore during the year 2013-2014. 1.0 ml blood sample were taken and subjected to centrifuge at 3000-4000 rpm for 10-15 minutes for the separation of serum. All the analytical work was performed at the Institute of molecular biology and biotechnology (IMBB), and Centre for research in molecular medicine (CRiMM), The University of Lahore-Pakistan.

Results: The estimation of AST, ALT, ALP, TP and T.Bilirubin were estimated. The AST level in HCV patients was increases (47.88 ± 40.49) as compared to the control persons (31.43 ± 7.31) and statistically significant ($0.02 < 0.05$). Total Protein level in HCV patients was (4.20 ± 0.61) and in healthy individuals (6.23 ± 0.51) and statistically significant ($0.000 < 0.05$). MDA level in HCV patients was increases remarkably (8.58 ± 1.19) and in control persons (1.47 ± 0.54) and it was statistically significant ($0.000 < 0.05$).

Conclusion: There is a relationship between oxidative stress and ALP, ALT, AST and Albumin. The results of the present study confirmed a perfect sketch regarding the circulating biochemical markers and lipid peroxidation (MDA) profile between the studied groups i.e., control and HCV patients with interferon induced Hepatitis C virus infection.

Key Words: MDA, ALT, AST, ALP, HCV, Interferon.

INTRODUCTION

Hepatitis C virus (HCV) is the main mediator of liver diseases in all over the world. HCV virus is mainly transferred during surgical operations i.e. replacement of organs, blood transfusions or by using contaminated syringes. This virus belongs to the gene Hepacivirus and it is RNA virus (Ogata et al., 1991; Simmonds et al., 1994)^{1,2}. There are six main types of HCV genotypes (Kuiken and Simmonds, 2009)³. Out of six, genotype 2, 3, 5 and 6 were reported frequently (Al-Faleh et al., 1995; Osaba et al., 2000; Karkar, 2007; Alzahrani et al., 2009)^{4,5,6,7}. Those people which receive organs, blood or blood products from those people which were already affected by the HCV-virus have a greater risk of this infection (Alter et al., 1989; Esteban et al., 1990; Vander et al., 1990)^{8,9,10}. Liver cirrhosis and hepatocellular carcinoma (HCC) are caused by the chronic HCV (Seeff et al., 1992; Kaneko et al.,

1994)^{11,12}. A person with strong immune system has the ability to boost the HCV allowance (Cooper et al., 1999; Rehermann and Nascimbeni, 2005)^{13,14}. It is estimated that approximately 10 million people are affected by HCV in pakistan.3a followed by 3b and 1a is the most dominant genotype in Pakistan (Ahmad et al., 2010)¹⁵.

As genotype 3a largely exist in Pakistan due to which HCV genotyping is not suggested for patients infected by HCV by Gastroenterology Society of Pakistan (Hamid et al., 2003).¹⁶ Secondly due to inadequate facilities and the expense on genotyping test, most patients deprived of genotyping test. But on the contrary, genotyping gives information about strain variation, potential association with severity of the disease and treatment response (Derbula et al., 2006; Kabir et al., 2006)^{17,18}. Pegylated interferon and ribavirin proves a valuable therapy for Hepatitis C virus (HCV) but also has some side effects (Dusheiko, 1997;

Negro, 2010)^{19,20}. Some adverse effects are also reported which may lead to life-threatening results. In the patients, liver fibrosis, cirrhosis and hepatocellular carcinoma (HCC) are the major causes of HCV (Giannini and Brechot, 2003)²¹. From last few years, scientists strived to compare viral and biochemical factors including ALT, AST, bilirubin, genotype with each other and liver damage but no inference was formulated (El-Serag, 2002; Skehel, 1992; Kato and Eggers, 1969; Pratt and Kaplan, 2000)^{22,23,24,25}.

MATERIALS AND METHODS

Source of Data:

I. Thirty five patients of hepatitis C virus were eligible for inclusion in the study at Jinnah Hospital Lahore during the year 2013-2014. Detailed history, clinical complications, habits in particular smoking and tobacco chewing were collected from subjects of the study by giving them a questionnaire. Clinical diagnosis of the patient was also taken into consideration.

II. Twenty three age and sex-matched clinically apparently healthy individuals were included as controls.

III. All the analytical work was performed at the Institute of molecular biology and biotechnology (IMBB), and Centre for research in molecular medicine (CRiMM), The University of Lahore-Pakistan.

Method of Collection of Data: Blood samples were collected with aseptic precaution. Informed consent from subjects was obtained before collection of blood samples.

Sample and Sampling Technique: Blood samples of Patients and controls were collected and processed. 5ml blood was collected in EDTA-Vaccutainers and centrifuged.

Chemicals: All chemical reagents of analytical grades were purchased from Sigma Chemical Co. (St. Louis, Mo, USA).

Following Parameters were Estimated:

Biochemical Assays for Liver Function Tests: Blood sample were taken and subjected to centrifuge at 3000-4000 rpm for 10-15 minutes for the separation of serum. The estimation of AST, ALT, ALP, TP and T.Bilirubin were estimated by using commercially available Bio Merux and Randox kits. Malondialdehyde (MDA) was estimated by Ohkawa et al., (1979)²⁶ method.

RESULTS

The data presented in table: 1 is the clear cut picture of the different parameters estimated in HCV patients. It was observed that the level of ALT in HCV patients was (40.51) as compared to the healthy individuals (32.78) but observed that it was statistically non-significant. The AST level in HCV patients was

elevated remarkably (47.88) as compared to the control persons (31.43) and it was statistically significant ($0.02 < 0.05$). When the ALP level was estimated, it was observed that ALP level was increased in HCV patients (202) while in healthy individuals it was (84). Total Protein level in HCV patients was (4.20) as compared to controls (6.23). It shows that total protein level was decreased in HCV patients and was statistically significant ($0.000 < 0.05$). Total bilirubin level in HCV patients was observed (0.95) while in healthy individuals it was (0.88). When the Albumin was observed in patients it was found that albumin level was decreased in HCV patients (3.10) as compared to the control (4.12) and also found that it was statistically significant ($0.000 < 0.05$). MDA level was increased remarkably (8.58) as compared to the healthy individuals (1.47) and it was statistically significant ($0.000 < 0.05$).

Table No.1: Comparison of different parameters in HCV patients and control

Variables	Control (n=23)	HCV Patients (n=35)	(P < 0.05)
Age	33.04 ± 6.63	40.94 ± 11.53	0.002
ALT	32.78 ± 20.46	40.51 ± 41.69	0.413
AST	31.43 ± 7.31	47.88 ± 40.49	0.024
ALP	84 ± 7.10	202 ± 76.26	0.375
Total Protein (TP)	6.23 ± 0.51	4.20 ± 0.61	0.000
Total Bilirubin (T.Bili)	0.88 ± 0.27	0.95 ± 0.46	0.492
Albumin (ALB)	4.12 ± 0.48	3.10 ± 0.21	0.000
Malondialdehyde (MDA)	1.47 ± 0.54	8.58 ± 1.19	0.000

Table No.2: Comparison of different parameters between genders of HCV patients

Variables	Males (n=28)	Females (n=30)	(P < 0.05)
Age	35.71 ± 10.36	39.76 ± 10.53	0.146
ALT	38.75 ± 40.25	36.23 ± 29.54	0.786
AST	40.82 ± 30.14	41.86 ± 35.30	0.094
ALP	192 ± 71.16	198 ± 77.85	0.756
Total Protein (TP)	5.26 ± 1.17	4.78 ± 1.09	0.114
Total Bilirubin (T.Bili)	0.94 ± 0.32	0.91 ± 0.46	0.782
Albumin (ALB)	3.54 ± 0.57	3.47 ± 0.64	0.627
Malondialdehyde (MDA)	5.05 ± 3.86	6.43 ± 3.36	0.154

ALT: IU/L; AST: IU/L; ALP: IU/L; TP: mg/dL; ALB: mg/dL; MDA: nmol/mL

Table No.3: Spearman's correlation coefficients of different variable

Age Vs TOTAL BILIRUBIN (r= -0.474**)
ALT Vs TOTAL BILIRUBIN (r= 0.362*)
AST Vs TOTAL PROTEIN (r= -0.359**)

The data presented in table 2 shows the comparison between males and females suffering from HCV. 28 HCV infected males and 30 female patients were taken. The ALT level in males was observed (38.75) while in females it was (36.25). Data showed that the AST level in males was (40.82) and in females was (41.86). When ALP level was measured in Males and it was found to be (192) as compared to females (198). Total protein level in males was (5.26) and in females (4.78). When MDA level was observed in males it was found to be (5.05) while in females patients it was (6.43). Data also showed that all the parameters are statistically non-significant ($P>0.05$) and also observed that in the progression of disease, gender does not matter.

Table 3 shows the correlation exists between different parameters. Data showed that inverse correlation exists between AGE and total bilirubin ($r = -0.474^{**}$). It means that with the increase of AGE the amount of total Bilirubin decreases. Positive correlation was found between ALT and Total Bilirubin ($r = 0.362^{*}$). Negative correlation was found between AST and Total Protein level ($r = -0.359^{*}$).

DISCUSSION

Hepatitis C virus (HCV) is a RNA virus which has been known to cause acute and chronic necroinflammatory disease of the liver. It infects more than 170 million people worldwide. In Western countries, HCV is the leading cause of end-stage liver disease and hepatocellular carcinoma, as well as the main indication for liver transplantation (Kuiken and Simmonds, 2009; Khattab et al., 2011)^{3, 27}. In more than 70% of the infected people, the disease becomes chronic and leads to chronic hepatitis, 5-20% develops cirrhosis, and 1-5% died from cirrhosis or liver cancer. Furthermore, use of contaminated syringes, drug abuse, and use of barber razor, dental procedures, tattooing, ear piercing, acupuncture and high-risk sexual behavior are other modes of transmission (Grobusch et al., 1999)²⁸. The first discovery of Interferon (IFN)- α , a cytokine produced after stimulation of leukocytes or fibroblasts with virus infection or nucleotide treatment, growing numbers of subtypes of IFN have been identified. Of these, IFN- α and IFN- β species have been used in the treatment of hepatitis. IFN therapy is an effective method of clearing the hepatitis C virus (HCV) from serum, normalizing biochemical liver function and improving liver histology in chronic hepatitis C patients. Nevertheless, only about 40 of patients respond to this therapy and up to 60 of responders showed reactivation of the disease after IFN withdrawal (Fried and Hoofnagle, 1995)²⁹.

The study was assessed to evaluate the different parameter levels in Hepatitis C virus patients ALP, Albumin, ALT, Total protein, AST and oxidative biomarker MDA was measured in acute hepatitis and chronic necroinflammatory disease of the liver patients.

Parameters were calculated in these studies on 35 patients and 23 controls. Oxidative stress conditions are categorized with a raise in the concentration of reactive oxygen specie that can cause destruction at different cellular level association (Feron et al., 1991)³⁰. Negative correlation exist between age and total bilirubin ($r = -0.474^{**}$) and this is significant correlation. While there is positive correlation exist between ALT and Total bilirubin ($r = 0.362^{*}$) and this is significant correlation. Whereas Between AST and Total protein negative correlation ($r = -0.359$) occur and it is also significant correlation.

CONCLUSION

In this current study it is established that there is a relationship between oxidative stress, ALP, ALT, AST and albumin of Hepatitis C virus patients. Biochemical study of the Hepatitis C virus patients receiving interferon showed that oxidative stress and ALP, AST, ALT and Albumin play important key role in the progression of HCV. It was also determined that the Hepatitis C virus patients have remarkably high lipid peroxidation due to which the level of MDA was increased. And here also levels of AST and TB were increased in this estimation. The results of the present study confirmed a perfect sketch regarding the circulating biochemical markers and lipid peroxidation (MDA) profile between the studied groups i.e., control and HCV patients with Hepatitis C virus infection receiving interferon.

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