

## Biochemical Parameters and Protein Oxidation Relationship for Hepatitis C Patients and Healthy Ones

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**Abstract.** Globally hepatitis C virus is recognized as one of the basic health issues of liver and result in chronic liver diseases whose diagnosis is difficult and possible only with symptoms until it may leads to liver cancer. DNA damage along with lipids and protein oxidation is caused as result of oxidative stress in hepatitis patients. This work was carried out to search out the oxidation of protein and lipids per-oxidation with relative to DNA damage for hepatitis C patients. Sampling was done in 50 suspected patients among them 20 were positive for HCV, while 30 were negative. With the help of 2-4, dinitrophenyl hydrazine assay quantification of protein carbonyls was done and results showed much increased values than normal. Biochemical parameters of HCV such as complete blood count, triglycerides and cholesterol level were recorded. The results were significant in positive cases than the control group. The study elaborated a new window in the research of chronic HCV patients for severe biochemical parameters alterations, over oxidation of proteins and liver dysfunction. It was therefore concluded that oxidative stress was responsible for increased level of protein oxidation and altered biochemical parameters that leads to damage of liver.

**Keywords:** biochemical parameters, cirrhosis, hepatitis C virus, lipid and protein oxidation, oxidative stress

### Introduction

HCV a global health issue causes chronic disease of the liver which leads to death of the patient (Cooke *et al.*, 2013). Chronic HCV infection causes Liver cirrhosis and also severely damage liver. Death is ultimate result of Hepatocellular carcinoma (Lauer and Walker, 2001). Global HCV burden estimates are closely related to chronic liver diseases. With lack of resources it is not easy to reduce and control HCV burdon by public health teams especially in undeveloped countries. The preventive resources against the HCV such as primary, secondary and tertiary of a region are the pre-requisite to establish preventive targets for it (Zanetti, 1999). The estimation of disease burden should be based on regional, national and global level. Its global prevalence is 2.2% which shows 13 million positive patients worldwide (WHO, 2004). HCV infection prevalence perception is not similar in both geographic and temporal (Thomson and Liang, 2000).

When proteins reaction occurs under the same conditions, it results reaction products *i.e.* oxygen dioxide and hydrogen peroxide or a mixture of these were synthesize. Reaction starts mainly with OH, while protein modification occurs during reactions and further results were concluded, while oxygen availability is responsible for other mechanism of oxidation. Overall these reactive oxygen species can result in fragmentation of protein, while oxidation of residual side chains of amino acids resulted in the protein-protein cross-linkages formation (Levine *et al.*, 1994; Garrison, 1987). Under the control of nitration activity of key enzyme and Signal transduction networks provide a cellular mechanism (Hunter, 1995). In model substrates with the help of protein tyrosine kinases, the tyrosine residual nitration takes place in the phosphorylation (Gow *et al.*, 1996; Kong *et al.*, 1996). With the help of adenylating process meanwhile during reaction of nitration with residual of tyrosine, *E. coli* glutamine synthetase motivates to produce single tyrosine debris for each enzyme sub unit (Berlett *et al.*, 1996). Free radical products are formed from the iron based oxidative stress

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of amino acid produced through gamma irradiation, oxidative stress of hexoses, pentoses and carbohydrates is responsible for free radical production too (Bird and Draper, 1984). HCV results in cirrhosis and may hepatocellular carcinoma too. It is also responsible for liver cirrhosis and reactive oxygen species over production in serum (De Maria *et al.*, 1996; Farinati *et al.*, 1995). In aerobic life processes ROS is the important part and for variety of imperative biochemical reactions these are the normal by-products. At higher level it is deadly to cells while at normal level it is physiological necessary while over production is deleterious to living organisms and known as oxidative stress (Valko *et al.*, 2006). Macro molecules are cellularly bruised by ROS and result in alterations of biochemical parameters and protein (Valko *et al.*, 2006). The goal of this study is to investigate the changes in the protein oxidation and biochemical profile of HCV patients. Oxidation of protein and biochemical profile is affected by the oxidative stress of ROS. This study elaborated a unique window in the research of chronic HCV infection without comes of abnormal biochemical profile, over proteins oxidation and severed liver dysfunction. It is helpful for the calculation of biochemical parameters in the HCV patients to observe the oxidative stress.

### Materials and Methods

**The study was carried out in two steps.** 1st phase contained sampling of HCV serum samples from various diagnostic labs and hospitals while in 2nd phase calculation of biochemical parameters and protein oxidation from samples was done. For calculation of protein carbonyl performed 1,4 dinitrophenyl hydrazine (DNPH) assay and biochemical parameters included glucose, CBC, cholesterol and triglycerides level of HCV patients. Following methodology was adopted which is discussed here in detail accordingly.

**HCV serum samples.** All samples were taken from Dera Ismail Khan district headquarter hospital. First of all, confirmed HCV samples by using surface antigens device. Insta answer one step diagnostic test devices was used. Then storage and transportation of positive samples was carried out at 4 °C.

Protein carbonyl quantification was done with the help of 2,4 di-nitrophenyl hydrazine (DNPH) assay. To check out the quantification of protein carbonyl, 2,4-dinitrophenyl hydrazine (DNPH) assay was performed. All 20 hepatitis C serum samples and 4 normal samples as control was used to compare result with each other.

Reagents required for DNPH assay (500 µL of 10 mM DNPH) included 500 µL of 2.5M HCl, 150 µL trichloroacetic acid, 1% streptomycin sulfate, ethanol/ethyl acetate, 2 mL of 6M guanidine-HCl, 1 mg/mL Bovine serum albumin.

Quantification of total protein was done with the help of bovine serum albumin standard curve (1 mg/mL) in 6 M guanidine-HCl. Protein carbonyl was calculated with the help of DNPH coefficient at (22,000/mole/cm) 370 nm, then absorbance was recorded. Result of concentration was noted as nM carbonyl/mg protein. Data was presented as mean ± SD. All experimental data of protein oxidation by DNPH assay quantification were statistically analyzed. The unpaired Student's t-test was used to calculate the statistical significance of protein oxidation by DNPH assay. The level of significance was  $P < 0.05$ . In DNPH assay, calculated Hydrozones were observed at 370 nm using UV visible double beam spectrophotometer.

**Biochemical parameters.** All HCV positive samples were performed by biochemical tests. These tests were performed in repetitive lab and hospitals. Aim of this biochemical parameter was to compare chronic HCV patients with healthy control. Tests include, blood CBC, triglycerides and cholesterol level.

**Complete blood count.** CBC was carried out in Microbiology laboratory, FVAS, Gomal University, Dera Ismail Khan. Blood counts were measured using Sysmex XE 5000 with Autostainer. In the current study only red blood cells (RBC) and white blood cells (WBC) were quantified.

**Cholesterol and triglycerides level.** The triglycerides reagents (FS Co. KG, Holzheim, Germany) were used to quantify the triglycerides. With the help of biochemistry system (S.A., Barcelona, Spain) a Spectrum CCX II device is used to quantify the level of cholesterol.

### Results and Discussion

Different parameter like protein carbonyls and biochemical parameters such as, cholesterol, triglycerides, glucose and CBCs are quantified in patient samples and compared with healthy control group.

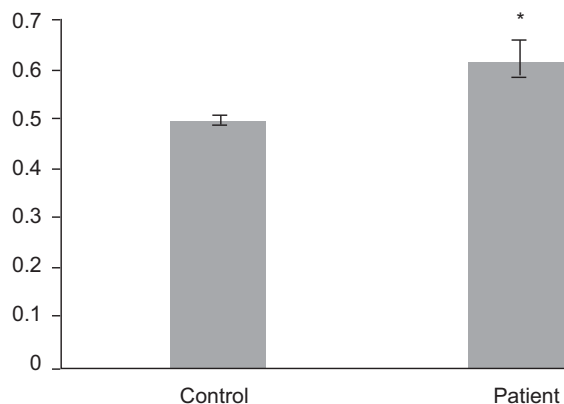
**Carbonylated serum proteins quantification.** The protein carbonyl level of chronic HCV patients was significantly higher compared to those of healthy control ( $P < 0.05$ ).

In protein structure, the carbonation of protein is non-reversible mechanism. It leads to risk of liver cirrhosis, malignancy and disturbs the normal mechanism of homeostasis. As a result of oxidative stress protein damage is occurred which directly involved due to amino acids oxidation and indirectly makes conjugates with the lipid peroxidation end products. One of the major bio markers for identification and detection of damage of protein is protein carbonyl (Dalle-Donne *et al.*, 2003). Results of our study are significantly higher for proteins carbonyl in HCV samples than the healthy ones which are according to findings of (Alou-El-Makarem *et al.*, 2014) in Fig. 1.

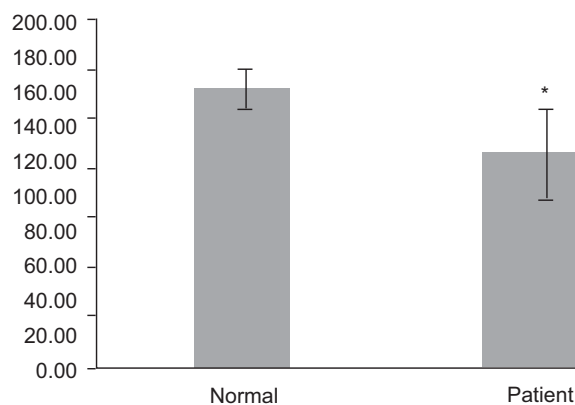
**Biochemical parameters.** Different biochemical parameters like cholesterol and triglycerides were quantified using the chronic HCV patients' serum samples. The range of cholesterol normal values is from 150-200 mg/dl. The quantification of cholesterol level was done in all 50 samples. In HCV patients' serum samples there was a significantly decline in the cholesterol level than the normal ones (Fig. 2). The statistical analysis of the data showed a significantly variation (\* $P < 0.05$ ). The result of present study are in accordance with previous studies in which cholesterol level was lower in acute HCV patients and lower LDL level too in case of chronic HCV patients (Corey *et al.*, 2009; Marzouk *et al.*, 2007). In case of chronic HCV patients triglycerides level was also lower along with cholesterol than the normal healthy samples as represented in (Fig. 3). The standard triglycerides range is 150-200 mg/dl. In HCV samples triglycerides level was significant low than the control group (\* $P < 0.05$ ).

The values for triglycerides was also found lower, demonstrated by (Perlemuter *et al.*, 2002) and thus similar finds are in favour of our hypothesis that hypo-lipidemia is developed from chronic HCV infection along with low level of triglycerides. The normal range of white blood cell count is 6.8-8.0 and its unit is  $10^3/\text{mL}$  in our current lab settings. The finding about the white blood cells in patients and normal group samples same values (Fig. 4). So a non-significant difference was noticed as a result of statistical analysis of the data. It is concluded from our results that in hepatitis C patients, level of white blood cells level remained same. It is also concluded that in HCV samples no affect was noticed for CBC by the oxidative stress.

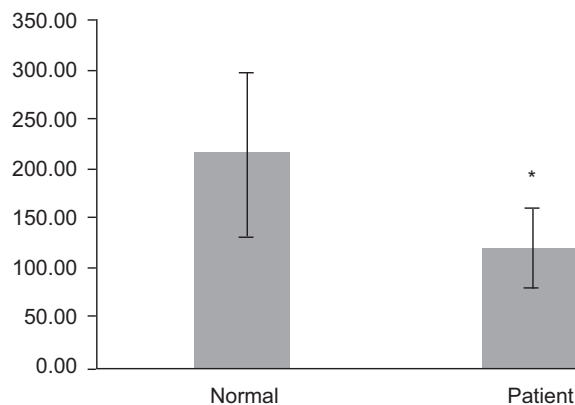
Along with this it is also demonstrated that HCV RNA also did not change for patient's WBCs samples in mixed with catrimox. When it is stored at room



**Fig. 1.** Quantification of serum carbonylated protein level in chronic hepatitis C patients vs normal control (\* $P < 0.05$ ).

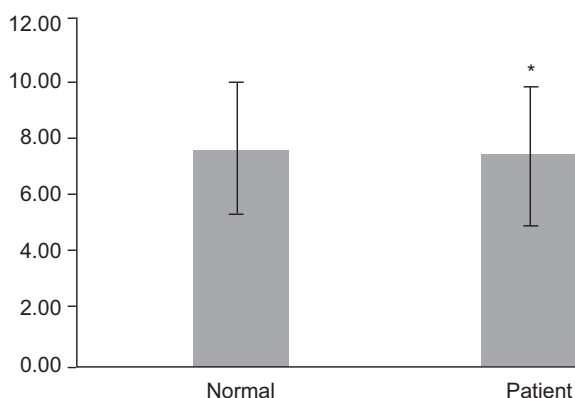


**Fig. 2.** Quantification of cholesterol level in chronic hepatitis C patient vs normal control (\* $P < 0.05$ ).

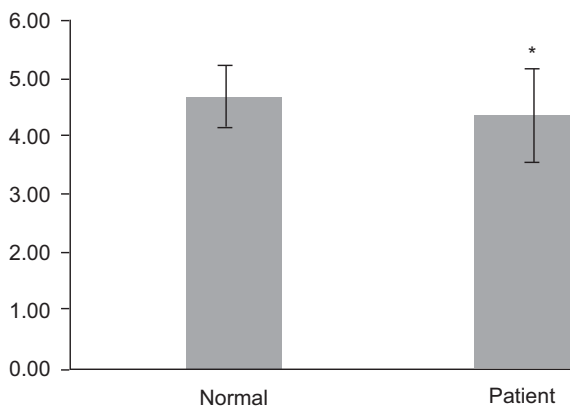


**Fig. 3.** Quantification of triglycerides level in chronic hepatitis C patient vs normal control (\* $P < 0.05$ ).

temperature does not decrease up to 7th day (Schmidt *et al.*, 1992). The normal range of red blood cells count is 4.5-5.5, having unit of ( $10^3/\text{mL}$ ) in our current lab settings. The results were non-significant ( $*P < 0.05$ ). The results of study showed that in chronic HCV patients, lowest HCV-RNA level was found in red blood cells and granulocytes (Schmidt *et al.*, 1998; Schmidt *et al.*, 1997). In granulocytes case, HCV-RNA may be engulfed through phagocytosis and by RBCs (Bronowicki *et al.*, 1998), shows in Fig. 5.



**Fig. 4.** Quantification of white blood cells level in chronic hepatitis C patient vs normal control.



**Fig. 5.** Quantification of red blood cell level in chronic hepatitis C patient vs normal control.

## Conclusion

It is concluded from the above study and discussion that level of protein oxidation and damage of DNA in Hepatitis C patients had significant level of increase

than the control group. Protein dysfunction is also clear from the over production of protein carbonyl and result in instability of cytoskeleton. In comparison to normal samples lipid peroxidation and their conjugated diens including MDA, lipid hydroperoxides and biochemical parameters are also abnormal and effected from oxidative stress. Further study and research would be fruitful to find out the relationship among liver cancer, lipid peroxidation level and these biomarkers. For the prevention and treatment of HCV infection assessment of the antioxidant defence systems, oxidative stress and augmentation may be result oriented and beneficial.

**Conflict of Interest.** The authors declare no conflict of interest.

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