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Research Article

Relationship among protein oxidation and biochemical parameters of healthy and hepatitis C patients

Umar Khitab[1], Ikram Ullah[1], Ali Zaman[2], Haseena Baloch[3], Shakeebullah[2], Muhammad Shoaib Khan[2], Ambrina Tariq[4], Khalid Muhammad[2], Naimat ullah[2], Muhammad Inamullah Malik[2*], Saqib Ali Rustam[2] and Muhammad Noman[5]

1. Centre for Interdisciplinary Research in Basic Science, Faculty of Basic and Applied Sciences, International Islamic University, Islamabad-Pakistan
2. Faculty of Veterinary and Animal Sciences, Gomal University, D.I.Khan-Pakistan
3. Department of Veterinary Microbiology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam-Pakistan
4. Department of Livestock and Dairy Development, KPK-Pakistan
5. Department of Epidemiology, University of Veterinary and Animal Sciences, Lahore-Pakistan

**Corresponding author's email: malikinamgu@gmail.com*

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Abstract

HCV is internationally recognized health issue as it causes chronic liver diseases which are difficult to diagnose with symptoms until it causes sever liver damage which may leads to liver cancer. Oxidative stress caused by hepatitis leads to the damage of the DNA and oxidation of both lipid and protein. In the present study research was conducted to find and correlate hepatitis C with protein oxidation, DNA damage and lipid peroxidation. Samples were collected from 50 suspected individuals including 20 with hepatitis C virus (HCV) and 30 healthy ones. The protein carbonyls quantification was performed by using 2, 4 dinitrophenyle hydrazine assay (2,4 DNPH assay). The results of DNPH assay clearly showed that protein Carbonyl level was much higher than normal controls. Finally, HCV biochemical parameters i.e. cholesterol, triglycerides and complete blood count were compared. The results indicated that biochemical parameters were also significantly changed in patients when compared with control. This project elaborates a new era particularly the chronic HCV patients having severe liver dysfunction, abnormal proteins oxidation and biochemical parameters. On the basis of the current results, we concluded that oxidative stress was involved in abnormal biochemical parameters, protein oxidation and lead to liver damage.

Keywords: Biochemical parameters; HCV; Liver Cancer; Oxidative stress; Protein oxidation

Introduction

HCV is internationally recognized health issue as it causes chronic diseases and mortality **[1]**. Liver cirrhosis occurs due to chronic HCV infection, which also damages liver. Hepatocellular carcinogenesis leads to death **[2]**. HCV causes severe damage to liver cells and mortality in both highly developed and industrialized countries. Global HCV

burden calculations which are still unpublished should be essentially related with the chronic liver diseases. It is very difficult task for public health teams to control and reduce HCV burden in under developed countries with low resources. Before setting preventive targets of HCV in specific regions, their preventive resources like primary, secondary and tertiary should be properly estimated [3]. The burden of disease should be nationally, regionally and globally estimated by national health policies. Hepatitis C (HCV) infection is 2.2%, global prevalence, due to this estimation 13 billion person worldwide having HCV-positive [4]. Temporal and geographic both have differences in the perception of HCV infection [5].

When protein reaction occurred under the same conditions, resulted into byproducts i.e O_2 and OH or their mixture. Reaction mainly started with OH and protein is modified during reaction. The availability of O_2 starts other mechanisms of oxidation process or its protonated form (HO_2). Collectively, these ROS can lead to protein fragmentation when oxidation of amino acid residues side chains, which further leads to the formation of protein-protein cross-linkages [6, 7]. Signal transduction networks and activity of key enzyme provides a cellular mechanism according to the nitration [8]. The nitration of tyrosine residues in model substrates undergoes the phosphorylation process with the help of protein tyrosine kinases [9, 10] and E.coli glutamine synthetase novitiates during manifestation with nitration of tyrosine residues with help of adenylation process agnate to single tyrosine debris in each subunit of the enzyme [11].

Iron based oxidative damage of amino acid form free- radical products originated by gamma irradiation. Carbohydrates, pentoses and hexoses also undergo oxidative stress to form free radical [12]. Hepatitis C virus leads to HCC (hepatocellular carcinoma) and may also cause cirrhosis. HCV causes over production of reactive oxygen species, manifested by increased serum and cirrhosis of liver [13, 14]. Reactive oxygen species (ROS) are the normal product of a variety of imperative biochemical reactions and the generation of ROS is an obligatory outgrowth of aerobic life. Normal level of ROS has physiological functions, whereas it is deadly to the cells at high level. Overproduction of reactive oxygen species either endogenous or exogenous to living organisms and is termed oxidative stress. Oxidative stress can bruise cellular macromolecules, leading to protein and biochemical alteration [15].

The objective of study was to investigate the alterations in biochemical profile and protein oxidation of hepatitis patients. The ROS causes oxidative stress which affects the biochemical profile and protein oxidation in chronic HCV patients. This study elaborated a new era for the chronic HCV patients particularly with severed liver dysfunction, abnormal biochemical and proteins oxidation. This study will help in quantification of hepatitis C patient's biochemical parameters to check out how much oxidative stress took place in biochemical parameters.

Materials and methods

The work was divided into two main phases. First of all sampling of HCV serum from different repetitive hospitals and diagnostic labs and second was quantification of protein oxidation and biochemical parameters from HCV serum samples. For quantification of protein carbonyle 1, 4 dinitrophenylehydrazine (DNPH) assays was performed and biochemical parameters of HCV patients, which includes triglycerides, cholesterol, CBC and glucose level were observed. The whole experiment was conducted in CIRBS (Center for Interdisciplinary Research in Basic Sciences) Laboratory.

HCV serum samples

All HCV serum samples were confirmed by using surface antigens device. The HCV ab rapid test cassette is a sandwich lateral flow chromatographic immunoassay for qualitative detection of antibodies. It is not

only helpful in diagnostic purpose but as a tool for screening test. After confirmation HCV positive samples were stored in CIRBS lab at 4°C.

Quantification of protein Carbonyl by 2, 4 dinitrophenyle hydrazine (DNPH) assay

Total protein quantification was carried by standard curve of bovine serum albumin (1 mg/mL) in 6 M guanidine–HCl. Carbonyl concentration was examined using the coefficient of DNPH at 370 nm (22,000 per mole per cm), and the values were estimated as nM carbonyl per mg protein. Use of graphs to compare the control with HCV patients for quantification of carbonyl groups by DNPH assay in protein provided a suitable technique for measuring oxidative modification of protein. 2, 4 dinitrophenylhyrdazine (DNPH) combine with protein carbonyls to generate hydrazones. With the help of (DNPH) assay, calculated Hydrazones should be observed by UV visible double beam spectrophotometer at an absorbance of 370 nm or by fluorescence.

Biochemical Parameters

Biochemical parameters included Complete Blood Count, Triglycerides and cholesterol level.

Complete Blood Count

It was measured by using Sysmex XE 5000 with Auto stainer. Complete blood count contains Hb, WBCs, Platelets, RBCs, Neutrophils, Lymphocytes and Monocytes etc. but in our research, we quantified white blood cells and red blood cells.

Triglycerides and Cholesterol Level

The quantification of the triglycerides level was performed by the reagents Triglycerides FS Co. KG, Holzheim, Germany, while Cholesterol quantification by Biochemistry system S.A., Barcelona, Spain according to the enumeration of the assemble in a Spectrum CCX II device. The tests were arranged with the CCX Multicalibrator System (Abbott), with curves of 3 points.

Statistical analysis

Data was statistically analyzed. The statistical significance was calculated by using unpaired Student'st-test. When $P < 0.05$, then results were considered significant.

Results and discussion

Carbonylated serum proteins quantification

The protein oxidation was lead to protein carbonyl. Carbonylated serum protein level was significantly higher in chronic HCV patients compared to healthy control (Figure 1). The data was statistically analyzed and found significantly different from control group (*p<0.05).

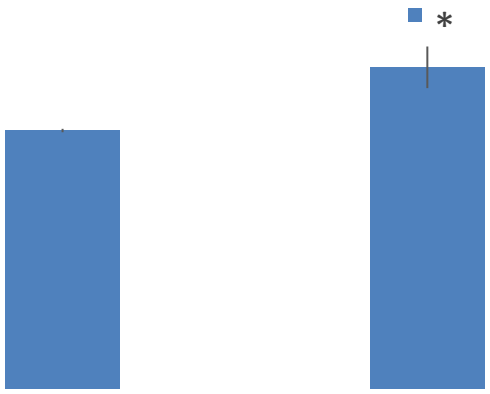

Figure 1. Qunatification of serum Carbonylated protein level in chronic heptitis C patients vs normal control (*p<0.05)

The protein carbonation was an irreversible modification in proteins structure. It interferes with the normal homeostasis and contributes to the risk of malignancy and liver cirrhosis. The protein carbonyl is one of the major bio markers for protein damage due to oxidative stress and involved directly with the oxidation of amino acids, in other words indirectly forms conjugates with the end product of lipid peroxidation [16]. Our results showed significant increase in hepatitis C patients' serum carbonylated proteins compared to control groups. The current results were in line with literature data [17].

Biochemical parameters

The normal values for cholesterol lie in the range from 150-200 mg/dl in our current lab settings. The cholesterol level was quantified in 20 HCV samples and 30 normal ones. The results showed a significant decreased level of cholesterol in HCV patient's samples versus normal healthy control (Figure 2). The data was statistically analyzed, and a significant difference was observed (*p<0.05).

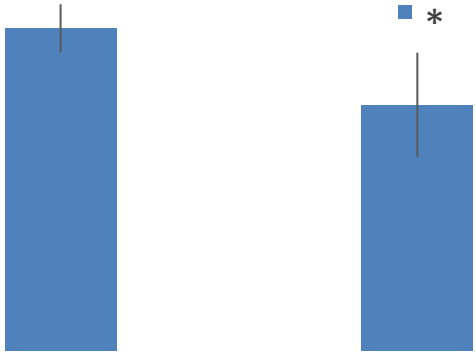

Figure 2. Qunatification of Cholestrol level in chronic heptitis C patient vs normal control (*p<0.05).

The current data was consistent with the findings where they demonstrated a low level of cholesterol in acute HCV patients and low level of LDL and cholesterol in chronic HCV patients [18, 19]. Along with cholesterol we also observed low level of triglycerides in case of chronic HCV patients as compared to healthy control as shown in (Figure 3). The normal range of cholesterol ranged from 150-200 mg/dl. The level of triglycerides was significantly lower in the HCV patients versus healthy control (*p<0.05). The low level of triglycerides was consisted with the literature data [20] and these results further strengthen our hypothesis that chronic HCV infection leads to hypolipidemia and as well low triglycerides.

The normal values for white blood cell count lie in the range from 6.8-8.0, which was calculated by unit of (10^3/ ul). The results were non-significant because normal controls and HCV patients has same white blood cell count (Figure 4). The data was statistically analyzed and a non-significant difference was observed.

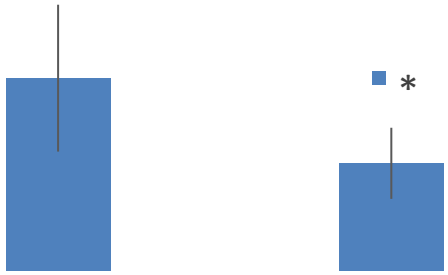

Figure 3. Qunatification of triglycerides level in chronic heptitis C patient vs normal control (*p<0.05).

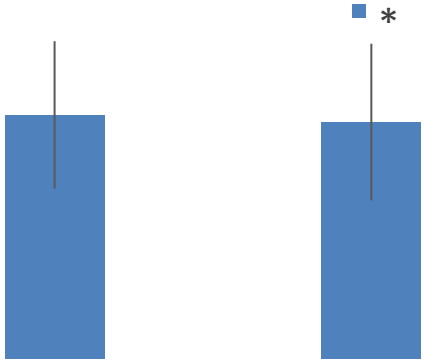

Figure 4. Qunatification of white blood cells level in chronic heptitis C patient vs normal control.

Our data suggested that white blood cell level remained constant in hepatitis C patients. Oxidative stress has no effect on complete blood count of hepatitis C patients, in addition it was demonstrated that hepatitis C viral RNA remained in patient white blood cell samples mixed with catrimox does not decrease when maintained at room temperature for up to seven days **[21]**.

The normal values for red blood cells count lie in the range from 4.5-5.5, which was calculated by unit of (10^3/ ul). The results were non-significant because normal controls and HCV patients have same red blood cell count (figure-5). The data was statistically analyzed and a non-significant difference was observed (*p<0.05). Our data suggested that lowest level of HCV-RNA in chronic HCV patient was found in granulocytes and in red blood cells **[22, 23]**. While in the case of granulocytes, the HCV-RNA may simply be ingested by phagocytosis and in red blood cells **[24]**.

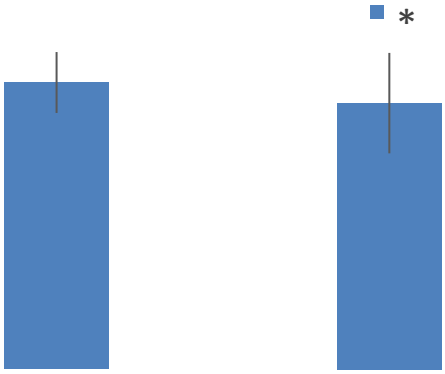

Figure 5. Qunatification of red blood cells' level in chronic heptitis C patients vs normal control

Conclusion

The results obtained here finalized that oxidative stress was involved in Hepatitis C. We found that the Hepatitis C patients had significantly increased the levels of protein oxidation and DNA damage versus control subjects, the overhead level of protein carbonyl was also a sign of protein dysfunction, which also preceded the cytoskeleton instability. Due to oxidative stress increase in the lipid peroxidation take place and their conjugated such as lipid hydroperoxides and MDA. The mechanism remained to be fully revealed. Furthermore, it has also concluded that due to stress HCV biochemical parameters were also affected when compare to normal controls. It would be valuable to further study the correlation between these biomarkers and levels of lipid peroxidation and liver cancer. Evaluation of augmentation and oxidative stress of the antioxidant defense systems may be beneficial for the treatment and prevention of Hepatitis C patients.

Authors' contributions

Conceived and designed the experiments: U Khitab, I Ullah & A Zaman, Performed the experiments: U Khitab, Shakeebullah, MS Khan, Analyzed the data: K Muhammad, SA Rustam & A Tariq, Contributed reagents/materials/analysis tools: N Ullah, H Baloch & M Noman, Wrote the paper: MI Malik.

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