ORIGINAL ARTICLE

Assessment of Oxidative Stress in Hepatitis C Patients Receiving Interferon Therapy

Arif Malik[1], Muhammad Saeed Qureshi[2], Abdul Manan[3], Sadia Saleem[4], Mamoona Munir[5], Atiya Fatima[6], Mahwish Arooj[7], Mahmood Husain Qazi[8]

ABSTRACT

Background: Oxidative stress and antioxidative status caused by hepatitis C therapy plays a significant role in aggravating the disease. A number of reactive oxygen species are responsible for the damaging of cell machinery and ultimately disturbing the homeostasis of the cell.

Objectives: To assess enzymatic, non-enzymatic antioxidants and circulating biomarkers in HCV patients receiving interferon therapy.

Methods: Study subjects were divided into two groups; patients and controls. The levels of the Thiobarbituric acid reactive substances (TBARS, as a marker of lipid peroxidation), superoxide dismutase (SOD), glutathione (GSH), catalase (CAT) and lipid peroxidation product (MDA) in the serum were estimated.

Results: There was statistically difference between patients and healthy controls in levels of CAT($p< 0.000^{**}$), SOD($p< 0.000^{**}$), GSH ($p< 0.000^{**}$)and MDA($p< 0.000^{**}$). Similarly, the levels of ALT ($p< 0.048^{*}$), AST ($p< 0.005^{*}$) and ALP ($p< 0.000^{**}$) were also statistically different between two groups.

[1] **Arif Malik**

Institute of Molecular Biology and Biotechnology, University of Lahore.

[2] **Muhammad Saeed Qureshi**

Department of Biochemistry, Allama Iqbal Medical College, Lahore.

[3] **Abdul Manan**

Institute of Molecular Biology and Biotechnology, University of Lahore.

[4] **Saadia Saleem**

Institute of Molecular Biology and Biotechnology, University of Lahore.

[5] **Mamoona Munir**

Institute of Molecular Biology and Biotechnology, University of Lahore.

[6] **Atiya Fatima**

Institute of Molecular Biology and Biotechnology, University of Lahore.

[7] **Mahwish Arooj**

Center for Research in Molecular Medicine, University of Lahore.

[8] **Muhammad Husain Qazi**

Center for Research in Molecular Medicine, University of Lahore.

Corresponding Author

Arif Malik

Conclusion: Imbalanced levels of superoxide dismutase, catalase, reduced glutathione, MDA and serum enzymes (e.g. ALT, AST, ALP) revealed that interferon itself play a crucial rule in disturbing oxidative vs. antioxidative status which ultimately results in tissue damaging. Increased levels of MDA have a significant correlation with disease development during the course of therapy.

KEY WORDS: *ALT, AST ALP, Antioxidants, MDA (Malonyldialdehyde) Superoxide dismutase, Catalase, Reduced Glutathione*.

INTRODUCTION

The symptoms observed during liver inflammation are jaundice, liver distension, abdominal, gastric malaise and unusual liver functions etc. In most of the cases, scarring of its tissues has been observed when severe hepatitis progresses including cirrhosis or may lead to liver cancer also known as hepato cellular carcinoma (HCC). The common cause of hepatitis is infection due to virus or excessive consumption of alcohol. Infections due to hepatitis C virus (HCV) have arrested the attention of many scientists. Alter[1] observed that deaths due to HCV would exceed in number than the mortality due to AIDS in following years only in USA.

Cirrhosis, liver damage or failure and hepato cellular carcinoma prevail due to HCV infection.[2] Moreover, other than the genetic factors in the subjects alcohol had played an infectious role particularly in males.[3] Alcohol had a greater impact regarding fibrosis of liver than virus itself.[4] It has been reported that alcoholics have HCV infection with thirty five percent incidence, other latent risk factors included are intravenous drug addiction and blood transfusions.[5]

Many studies have been conducted to evaluate the oxidative stress in the patients suffering from the hepatitis B and/or hepatitis C.[13,14,15,16,17] De-Maria[18] showed that MDA may be elevated in the liver and the blood. Paradis[19] also studied adducts of MDA-protein by immunohistochemically in infected liver tissue. The increased MDA concentrations and elevated SOD activity were also observed in patients of chronic hepatitis C when peripheral blood mononuclear cells were analyzed. Romero[20] observed the higher serum MDA concentration in chronic HCV patients as compared to healthy individuals before giving the interferon treatment to the patients. The combination therapy of interferon with ribavirin is a well renowned therapy for chronic hepatitis C patients. It has been observed that the response of this combination treatment is not 100 percent and showed some side effects too.[21]

METHODOLOGY

The inclusion criteria for participants was to select patients diagnosed with chronic hepatitis C (CHC) and taking interferon therapy. Those patients having any other disease like diabetes, or any other viral infection were excluded from the study. 50 patients with chronic hepatitis C (CHC) and 10 healthy individuals were selected for the present study. Two antioxidant enzymes including superoxide dismutase (SOD) and catalase (CAT) and one non-enzymatic antioxidant reduced glutathione (GSH) were evaluated. Moreover, malondialdehyde (MDA) was also investigated in patients and control group by spectrophotometrically. Venous blood was taken from control and patient groups and sera was separated with the help of centrifugation and employed for biochemical analysis. Estimation of liver enzymes: Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and alkaline phosphate (ALP). Blood was centrifuged at 4000 rpm for 10 minutes and serum was separated. Serum liver enzymes (AST, ALT and ALP) were estimated by using Human Diagnostic enzymatic kits. The sample were processed and analyzed for the Superoxide dismutase (SOD) activity was determined by the method of Kakkar.[22] GSH was estimated according to the method of Moron.[23] Lipid peroxidation level in the serum samples were expressed by MDA. It was measured according to procedure of Ohkawa.[24] CAT activities were determined by measuring the decrease in hydrogen peroxide

concentration at 230 nm by the method of Beutler.[25] The results are presented as the mean and standard deviation Data presented in the form of Mean ± SD and observations were analyzed using the statistical package SPSS windows (ver. 16). Independent student t-test was applied to find out the statistical significant difference ($p<0.05$) between the groups. Moreover, Pearson's correlation was used to figure out the correlation among the circulating biomarkers.

RESULTS

In the present study the level of MDA were significant ($p<0.01$) and were increased than in healthy patients and this is agree with the work of Padmaja.[26] There is positive correlation between the concentration of MDA and SOD ($r = 0.532^{**}$). The high levels of MDA in the subjects showed the damaging of the cell membrane of the hepatocytes. Since the high levels of MDA is indicator of lipid peroxidation. Reduced glutathione (GSH) levels in the red cells were observed statistically significant and lower in patients with chronic liver disease (both viral and/or other etiology) as compared to healthy individuals. The decreased blood GSH levels may be due to less production in the body or less flow from the hepatocytes.[27,28] In the present study the level of GSH were statistically significant ($p<0.05$) and were decreased than in healthy patients and this is agree with the work of Bianchi.[28] There is negative correlation between the concentration of GSH and SOD ($r = 0.726^{**}$).

Table 1: Antioxidant and circulating biomarkers status of HCV patients receiving interferon therapy

Groups	SOD	CAT	GSH	MDA	ALT	AST	ALP
Control (n=10)	0.9202±1.79	4.29±0.83	9.82±1.32	3.0±1.36	23.4±5.69	26±5.5	29±4.73
Diseased (n=50)	119±46.31	0.71±0.29	0.63±0.34	34.65±4.1	28.4±7.4	32.6±8.0	110±27.1
P value	0.000**	0.000**	0.000**	0.000**	0.048*	0.005*	0.000**

Values are expressed as means ± SD
-GSH expressed as U/mg Protein
-SOD expressed as nmol/g tissue
-TBARS expressed as nmol/g tissue
-Catalase expressed as U/mg Protein
- ALT, AST and ALP expressed as (dl/ml)

Table 2: Correlation matrix of antioxidant and circulating biomarkers status of HCV patients receiving interferon therapy

	SOD	MDA	CAT	GSH	ALT	AST	ALP
SOD	1	.532** .000	-.708** .000	-.726** .000	.235 .071	.148 .259	.587** .000
MDA		1	-.901** .000	-.933** .000	.270* .037	.349** .006	.751** .000
CAT			1	.965** .000	-.213 .102	-.298* .021	-.713** .000
GSH				1	-.238 .067	-.315* .014	-.734** .000
ALT					1	.542** .000	.120 .361
AST						1	.073 .580
ALP							1

The increased activities of hepatic enzymes (ALT, AST and ALP) were observed in hepatitis C patients. Increased levels of hepatic enzymes give insight into the cellular damaging and loss of physiological integrity of plasma membrane in hepatocytes. These enzymes are found in cytoplasm, serum as well as in bile. In the present study the levels of the serum enzymes like ALT, AST and ALP showed variations in their values and were elevated as compared to control, this is agree with the work of Padmaja.[26] The increased lipid peroxidation in the RBC's is also is also due to the changing in the biological activity of enzymatic (SOD and CAT) and non-enzymatic (GSH) parameters of oxidative stress system. Moreover, glutathione peroxidase (GPx) system is built up by several chemical components, one of which is reduced glutathione.[29]

DISCUSSION

The oxidative stress (OS) can be defined as an imbalance of oxidants and antioxidants that can lead to cell damaging and production of free radicals which are highly reactive. In other words, oxidative stress is the presence of excessive levels of oxygen free radicals. Oxygen

radicals know how to steer cell damaging by attacking fundamental macromolecules in the cell, such as proteins which account for the cell's genetic material and fats the main physiological part of the cell membrane. For example, OS is responsible for inducing enhanced metabolism of fatty acids in the form of lipid peroxidation (LPO) that may generate biologically active molecules mainly MDA. Some of these molecules occasionally lead to the progression of fibrosis. However, the creation of oxygen radicals is a usual process and many metabolic processes are involved. On the other hand, cell shows different protective systems to detoxify radicals. These systems make use of molecules called antioxidants, which are present in our daily diet or could be generated by the cell itself. The glutathione (GSH), vitamin E and vitamin C are commonly found antioxidants in biological system. These compounds have a number of mechanisms of action e.g. GSH can neutralize oxygen radicals by relocating hydrogen to the reactive molecules, thus forming a more stable chemical structure.[6,7]

Hepatic oxidative stress had been investigated in animals as well as in human beings for many years and showed that lipid peroxidation (LPO) and the variations in the levels of hepatic antioxidants confer a fine initiative about the state of the liver. On the other hand, hepatic dysfunction may present without considerable changing in blood oxidative status hence the liver biopsy left the best criteria for determining the unbalancing or status of oxidants and antioxidants in the liver tissue. By evaluating antioxidants status in the liver provides a perfect assessment about the function of the liver that allows the diagnosis of hepatic dysfunction, and facilitates to wrap up the degree of decline in the hepatic cells.[8]

Like the other tissues in the body, the liver is also sound equipped with various enzymatic and non-enzymatic antioxidants. Active microsomal system is also present in hepatocytes that generate reactive oxygen species (ROS) in the cellular environment. Moreover, during the normal metabolic reactions mitochondria are responsible for generation of ROS.[9,10] Oxidative stress (OS) in the biological system can be observed when the balancing between ROS and antioxidants production is disturbed. The host cell suffering from destruction utilizes the enzymes which are antioxidants in nature such as superoxide dismutase (SOD) and catalase.[11]

Rosser[12] found that proteins and nucleic acids are damaged by ROS and set off lipid peroxidation (LPO) process and reported that hepatocytes are damaged by LPO and enhances the concentration of malondialdehyde (MDA), which is a biochemical marker of oxidative stress in the biological system.

Metabolism of various endogenous and exogenous compounds and viruses generate reactive oxygen species (ROS) which are involved in the pathogenesis of liver diseases [30,31,32]. ROS rapidly react with a variety of molecules and thereby interfere with cellular function. Cells are equipped with non-enzymatic and enzymatic protective systems that play an important role in the scavenging of free radicals. In present study, it was observed that MDA, the indicator of lipid peroxidation was significantly elevated in hepatitis C. There is association between increased levels of MDA and hepatitis C. Free radicals might be attack on unsaturated fatty acids in membrane and organelles to produce lipid peroxides. This free radical may cause loss of membrane and which may decrease in membrane permeability. Thus loss of membrane causes cellular damage and necrosis of liver in hepatitis C. Lipid peroxidation is an indicator of oxidative stress in cells and tissues. Lipid peroxides derived from polyunsaturated fatty acids are unstable and are decomposed to form a series of compounds, including malondialdehyde (MDA). The quantization of MDA is widely used as an indicator of lipid peroxidation.[33]

Superoxide dismutase (SOD) and catalase (CAT) are responsible for catalyzing or decomposing superoxide radical (O_2^{-2}) and hydrogen peroxide (H_2O_2) in biological system and act as defending component of antioxidant defense system. The SOD produces H_2O_2 during catalyzing and/or removal of superoxide radical. In the next phase, catalase in the peroxisomes protect the cellular environment from harmful effects of H_2O_2 by decomposition into oxygen (O_2) and water and no further free radicals are produced[34]. Demirdag[16] observed the lower activity of SOD and catalase significantly in patients suffering from hepatitis B and hepatitis C than healthy individual group. The available literature presents no unequivocal theory concerning the function of the antioxidative barrier in chronic HCV infection.[35] The observations are not isolated; it was confirmed also by other authors, who observed

decreased activity of SOD. There are also papers demonstrating SOD values increase in the HCV infected group in comparison with healthy subjects.[36,37,38,39]

The results concerning CAT are contradictory; some authors demonstrate decreased activity, whereas others suggest its increase in comparison with healthy subjects [15,36,40]. In the present study the levels of CAT was decreased in the patients of chronic hepatitis C and was significant ($p<0.05$). While the levels of SOD were increased in patients which is in agreement with the work of Ko and Par.[37,38]. Moreover, concentration of SOD was significant ($p<0.05$) and positive in correlation with concentration of MDA ($r = 0.532^{**}$) while it showed significant level and inverse relation with the concentration of CAT ($r = -0.708^{**}$) and GSH ($r = -0.726^{**}$).

CONCLUSION

Imbalanced levels of superoxide dismutase, catalase, reduced glutathione, MDA and serum enzymes (e.g. ALT, AST, ALP) revealed that interferon itself play a crucial rule in disturbing oxidative vs. antioxidative status which ultimately results in tissue damaging. Increased levels of MDA have a significant correlation with disease development during the course of therapy.

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