IJBPAS, February, 2019, 8(2): 430-449

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

CORRELATION OF PRIDICTIVE VARIABLES AND THEIR INTERPLAY TO DEVELOP OXIDATIVE STRESS IN HCV PATIENTS RECEVING INTERFERON THERAPY

MEHWISH IFTIKHAR[3], SAIMA RUBAB KHAN[2], MUHAMMAD WAQAS[4], RABIA RASOOL[1], RABAIL ALAM[1], ARIF MALIK[1]

1 Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore-Pakistan

2-University College of Medicine and Dentistry (UCMD), The University of Lahore-Pakistan

3-Azra Naheed Medical College, Superior University, Lahore-Pakistan

4-Johar Institute of Professional studies, Lahore-Pakistan

***Corresponding Author: Arif Malik (Ph.D); Cell: 0321-8448196; Tel: +92 42-7515460-7, Fax: +92-42-7515519; E Mail: arifuaf@yahoo.com**

Received 16th Sept. 2018; Revised 4th Oct. 2018; Accepted 10th Nov. 2018; Available online 1st Feb. 2019

https://doi.org/10.31032/IJBPAS/2019/8.2.46455

ABSTRACT

BACKGROUND: Hepatitis C has become a worldwide concern because of its association with hepatocellular carcinoma and liver cirrhosis. Interferon have antiviral properties that causes modulations in the cytokines cascade that increase the synthesis of reactive oxygen species and result in oxidative stress and is associated with many inflammatory conditions, which patients develop soon after the initiation of therapy. **AIMS:** To assess the oxidative stress in hepatitis C patients after receiving interferon therapy. **METHODOLOGY:** For the current study, blood samples of the Hepatitis C patients who received interferon therapy (n=100) and healthy individuals were selected (n=100). Plasma was tested for stress markers by their respective spectrophotometric protocols. ELISA kits were used for AOPPs and AGEs. **RESULTS:** The results of present study demonstrate significantly higher levels of MDA and AOPPs and AGEs along with reduced levels of GSH that support the presence of a pro-oxidant state in Hepatitis C patients after therapy. Level of MDA, AOPPs and AGEs in

subjects were significantly increased (3.88±1.06 nmol/ml, 1.32±0.31 µ mol/g and 3.77±0.27 µ mol/g) as compared to the healthy control group (1.43±0.36 nmol/ml, 0.849±0.04 µ mol/g and 2.56±0.10 µ mol/g). Whereas, levels of GSH, CAT, GPX and GRX decreased significantly (3.12±1.40 µg/ml, 1.51±1.15 nmol/mol Prot., 6.53±0.46 U/L, and 3.40 ±0.41U/L) as compared to healthy controls (9.79±1.22 µg/ml, 3.95±0.81 nmol/mol Prot., 8.22±0.36 U/L, 1.47±0.22U/L) **CONCLUSION:** Hepatitis C patients who received interferon therapy exhibit low levels of antioxidants along with increased protein and lipid peroxidation due to presence of inflammation. Interferon therapy in conjunction with antioxidants and micro nutrients like zinc, iron etc. can help achieve better outcomes by counteracting the free radicals.

Keywords: Hepatitis C, MDA, IL-1, TNFα, IFNγ, AOPPs, AGEs

INTRODUCTION

Hepatitis C is a disease which has become a global concern of both the developed and the developing countries. Hepatitis prevalence is around 3 % in the world and is highest in the African countries as well as the East Asia region. Each year approximately 4 million new cases of hepatitis C are reported (World Health Organization, 2000). Hepatitis C virus is not only responsible for chronic hepatitis but is also a cause of hepatocellular carcinoma and fibrosis leading to liver cirrhosis. Hepatitis C is a member of the Flaviviridae family. It comprises of a single positive strand RNA comprising of 9600 bases (Carvajal-Yepes *et al*., 2009; Bartenschlager *et al*., 2011). Hepatitis C is transmitted by the infected blood or its products and is mainly transmitted through the parenteral route (Donahue *et al*., 1992). Hepatitis C is more common in drug abusers than HIV and Hepatitis B infection (Wasley and Alter, 2000). Interferon (IFN) is a strong antiviral cytokine and is most commonly used in treatment of Hepatitis C (Hep. C).

The interferon α was believed to have antiviral properties only. The Hep. C virus is first of all counteracted by innate immunity response. Viral products are sensed by the innate immunity sensors and signaling cascade results in secretion of cytokines. The interferon antiviral signaling pathway or interferon-inducible 2'-5'-oligoadenylate synthase/ RNAse L pathway also abbreviated as OAS/RNAse L pathway involves expression of oligoadenylate synthase (OAS) molecules.

Interferon is produced by the immune cells upon identification of viral infection. This interferon binds to its receptors on cell infected by virus, upon entering the cell virus releases its genetic material which is detected by OAS and 2'-5'-oligoadenylate is produced. This then activates RNAse L which destroys any single stranded RNA found. RNAse thus halts the replication of the virus. RNAse is also capable of inducing apoptosis of the infected cell (Hovanessian and Wood, 1980 and Schroder *et al*., 1992). Melanoma differentiation association gene (MDA 5) is located in the cytoplasm. It identifies long ds RNAs and detects positive strand viruses it generates many inflammatory cytokines which result in overall increased inflammatory status of the patient. (Kato *et al*., 2008). There is sufficient evidence that only 10-40% patients after interferon therapy show rate of sustained virologic response, SVR (Pearlman and Traub, 2011).Recent research results have shown that interferon is not an exclusive antiviral agent but has a strong role in inflammation by interfering with the T-cell and cytokine cascade. IFN α promotes formation of T1 helper cells (Th1) instead of producing T2 helper cells (Th2). Th1 cells produce IFN-γ and interleukin-2, which show pro-inflammatory activities. This interference was brought to limelight due to the association of the interferon and its pro inflammatory effects. Interferon α treatment is shown to have associations with the rheumatoid arthritis, pancreatitis, insulin resistance, glomerulonephritis and many autoimmune diseases (Guttermann, 1995). Interferon developed inflammation of the pancreatic islets and type 1 diabetes was induced in mice in a study. IFN α antibody was introduced in this study which prevented the diabetes and inflammation (Stewart *et al*., 1993). IL-4 suppresses the IFN-γ synthesis. IFN α suppresses the inhibitory action of IL-4 on IFN –γ as well (Guttermann, 1995).

These inflammatory cytokines act to promote inflammation and lacks a specific antiviral response. This increased level of pro inflammatory cytokines is associated with specific symptoms labelled as sickness behavior. In humans, the interferon therapy in 70% of Hep C patients gives rise to depression due to increased inflammation and increased levels of cytokines in brain (Bonaccorso *et al*., 2002). Somatic symptoms like myalgias, tiredness and depression has been reported in Hep C patients few weeks after start of IFN- α therapy (Wichers *et al*.,

2005). This inflammation increases the production of reactive oxygen species especially in the cells that are infected by the virus. Hepatitis C virus replicates not only inside the cytoplasm of liver cells, but it also attacks and thrives inside the cells of the immune system. So the hepatocytes as well as the immune cells undergo stress. The deficient antioxidant response and the marked increase of the free radicals damages the lipids, proteins and DNA (Farinati *et al*., 1995).

MATERIALS AND METHODS

The inclusion criteria were to select patients infected with Hepatitis C and received interferon therapy. Patients with Hepatitis B, HIV or infected with any other virus were excluded. Any other co-existing disease like Diabetes mellitus or any other chronic inflammatory condition was ruled out. 100 patients who had received interferon therapy and 100 healthy controls were selected for the current study. Catalase, Superoxide dismutase, Glutathione, Glutathione peroxidase and Glutathione reductase were evaluated for the anti-oxidant status. Glutathione (GSH) was evaluated in the blood of patients according to the method of Moron *et al*., 1979. Catalase was determined according to the generalized procedure of Beutler, 1975. SOD activity was measured by Kakkar, 1972. Glutathione peroxidase was measured by the Aebi and Bergmeyer, 1983 method. Glutathione reductase was determined by the method of David and Richard (1983).Malondialdehyde (MDA) or Lipid peroxidation in the blood sample of the patients are evaluated calorimetrically by method of Ohkawa et al., 1979. AOPPs and AGEs were performed by commercially available ELISA kits.

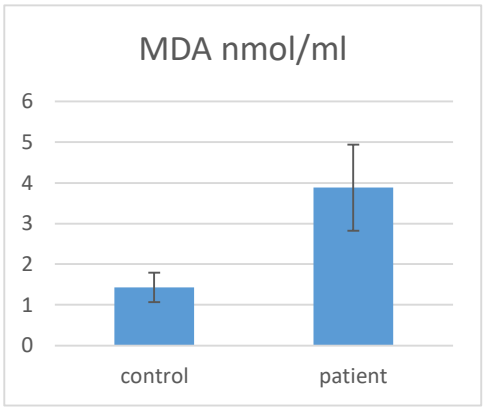
RESULTS

Results of the present study show that there is significant rise in oxidative stress who received interferon therapy as compared to controls. Table 01, fig 01 (a) shows significantly increased levels of MDA in the Hepatitis C patients who received interferon therapy (3.88±1.06 nmol/ml, p value= 0.00) as compared to healthy males (1.43±0.36 nmol/ml). In Table 01, fig 01 (b) shows significantly decreased levels of SOD in the patients with interferon therapy (0.09±0.40 µg/ml, p value= 0.00) as compared to healthy controls (0.497±0.07 µg/ml). The levels of GSH, CAT, GPX and GRX decreased significantly (3.12±1.40 µg/ml, p value= 0.00, 1.51±1.15 nmol/mol Prot., p value= 0.00, 6.53±0.46 U/L, p value= 0.00 and

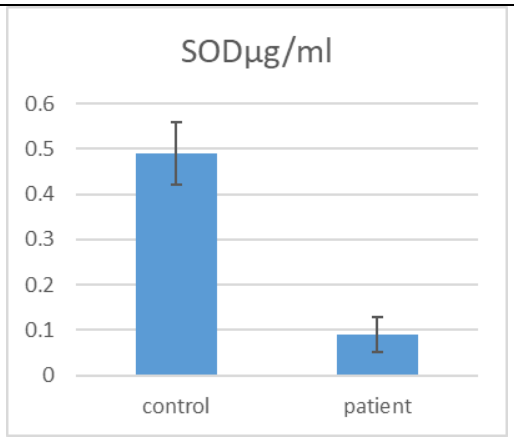
3.40 ±0.41 U/L, p value= 0.00) as compared to healthy controls (9.79±1.22 µg/ml, 3.95±0.81 nmol/mol Prot., 8.22±0.36 U/L and 1.47±0.22U/L) as shown in table 01, fig 01 (c,d,e and f). There was significant rise in levels of AOPPs in the Hep. C patients who had received interferon therapy (1.32±0.31 µ mol/g, p value = 0.00) compared to the healthy controls (0.849±0.04 µ mol/g) figure 01 (g). Similarly the AGEs were significantly high in the patients of Hep C with interferon therapy (3.77±0.27 µ mol/g, p value = 0.00) as compared to controls who have lower levels of AGEs (2.56±0.10 µ mol/g) figure 01 (h).

Table 1: Levels of different variables of medical importance in hep c patients who received interferon

VARIABLES	CONTROL (n=100)	SUBJECT (n=100)	P (≤0.05)
MDA (nmol/ml)	1.43±0.36	3.88±1.06	0.00
SOD (µg/ml)	0.49±0.07	0.09±0.04	0.00
GSH (µg/ml)	9.79±1.22	3.12±1.4	0.00
CAT (nmol/mol Prot.)	3.95±0.81	1.51±1.15	0.00
GP_X (U/L)	8.22±0.36	6.53±0.46	0.00
GR_X (U/L)	1.47±0.22	3.4±0.41	0.00
AOPPs (µ mol/g)	0.849±0.04	1.32±0.31	0.00
AGEs (µ mol/g)	2.56±0.1	3.77±0.27	0.00



a)



b)

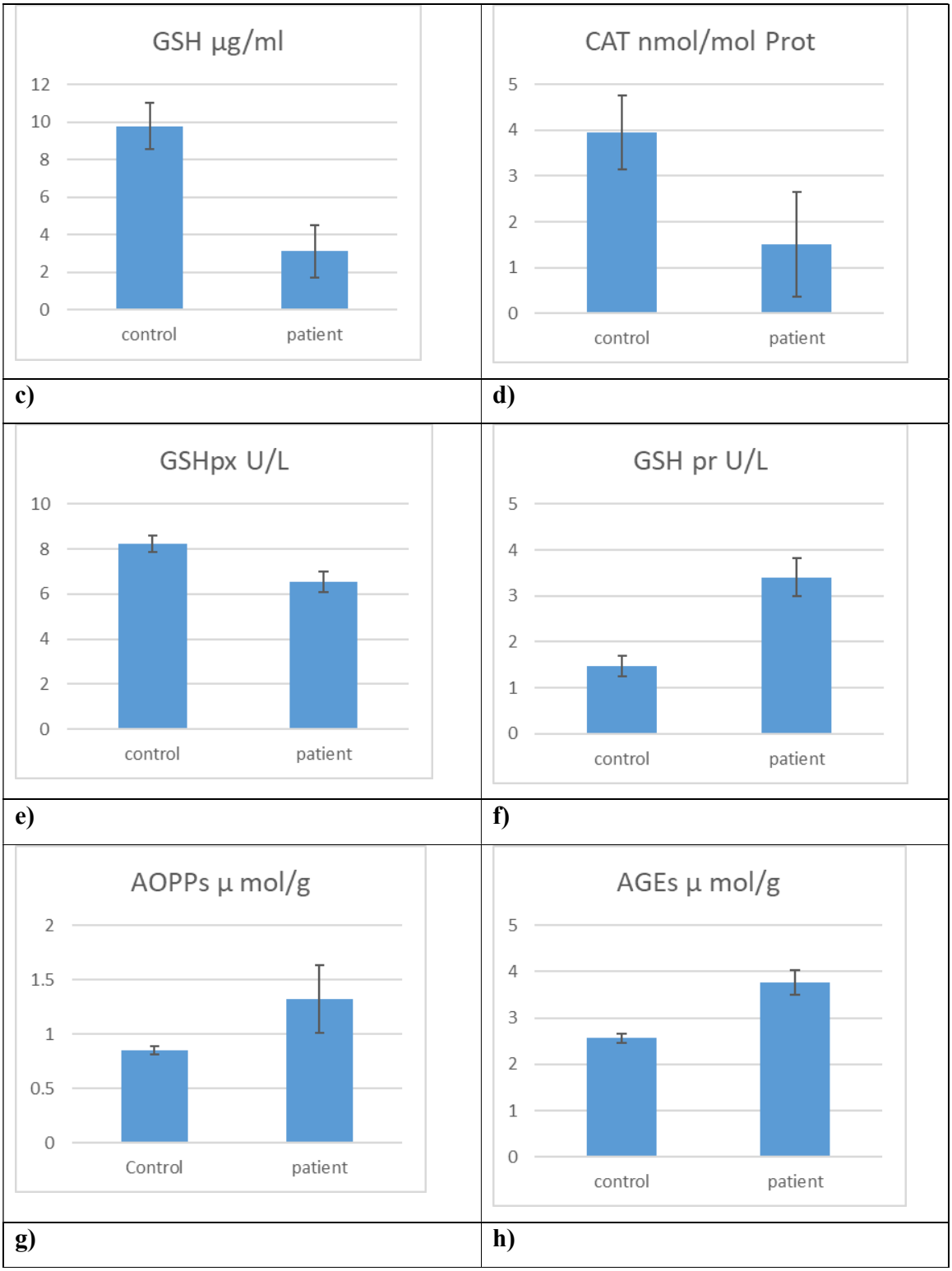


Figure 01: Levels Of Different Variables Of Medical Importance In Hepatitis C Patient After Interferon Therapy

	MDA	SOD	GSH	CAT	AOPPs	AGEs	GSHpx	GSHpr
MDA	1	-.726**	-.763**	-.604**	.582**	.340**	-.649**	.520**
		.000	.000	.000	.000	.000	.000	.000
SOD		1	.792**	.635**	-.620**	-.357**	.680**	-.584**
			.000	.000	.000	.000	.000	.000
GSH			1	.778**	-.667**	-.366**	.655**	-.580**
				.000	.000	.000	.000	.000
CAT				1	-.545**	-.309**	.662**	-.463**
					.000	.000	.000	.000
AOPPs					1	.386**	-.564**	.518**
						.000	.000	.000
AGEs						1	-.397**	.445**
							.000	.000
GSHpx							1	-.412**
								.000
GSHpr								1

Figure: 02 Correlation Matrix Of Antioxidant And Circulating Biomarkers Status Of Hcv Patients After Interferon Therapy

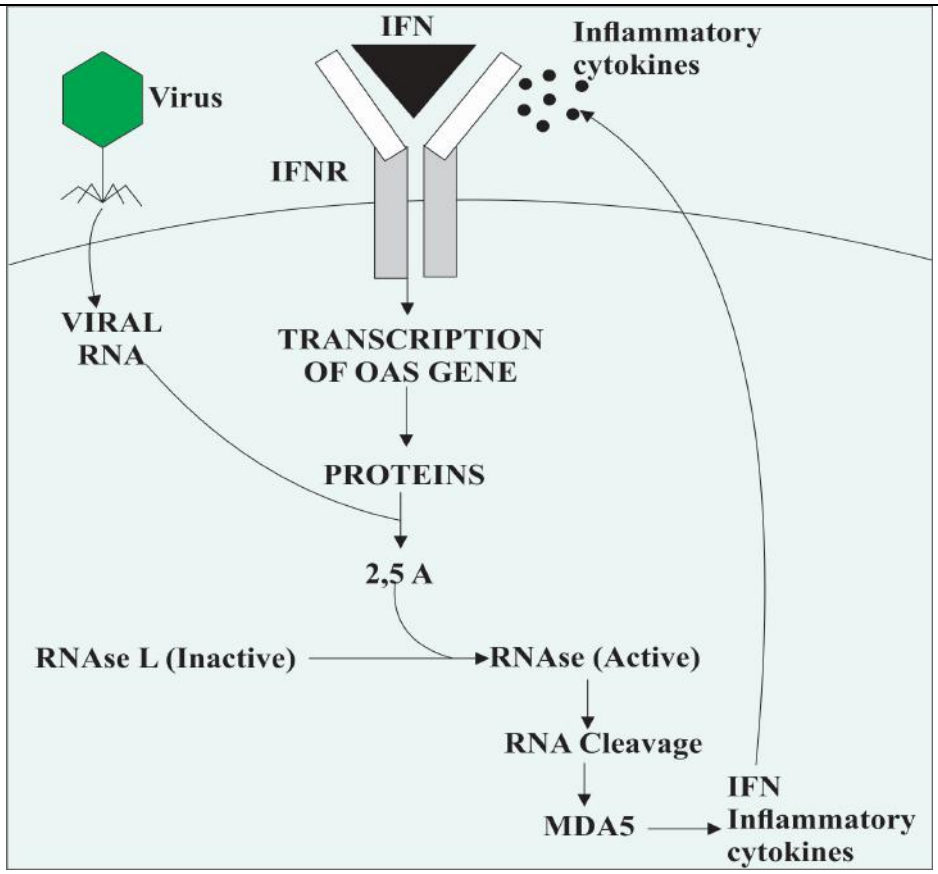


Figure 03: Shows a hepatocyte infected by hepatitis c virus. When interferon therapy is given, interferon binds to its receptor and it initiates transcription of 2'-5'- oligoadenylate synthase (OAS), which results in formation of 2'-5'- oligoadenylate proteins. These proteins convert the inactive RNAse to its active form. RNAse destroys the viral RNA strands found in the cell and halts the replication of virus. MDA 5, melanoma differentiation gene is also located in the cytoplasm. It not only detects positive long ds RNA strands but it also leads to synthesis and release of many inflammatory cytokines like, IL1, IFNγ, IFNα and TNFα. These cytokines in return increase the formation of reactive oxygen species and promote inflammation which in return will further increase oxidative damage

DISCUSSION

The present study results have shown that the patients with chronic hepatitis C who received interferon therapy exhibit oxidative stress due to the inflammatory response generated by the interferon. The response and outcome depends on the viral load, replication rate, immunity and the antioxidant status of the patient. Interferon binds to receptors of the Hep C infected cells and initiates a signaling which activates RNAse which destroys the viral strands. Upon activation of MDA5, various inflammatory cytokines are produced (Liu *et al*., 2007). Inflammatory cytokines like Interferon γ (IFN- γ) produced by lymphocytes, Interleukin 1 β (IL-1 β) and tumor necrosis factor α (TNF-α) produced by macrophages, are the main cytokines responsible for the inflammatory response. They exert actions on the host immune and defense as well as they are responsible for regulation of genes. TNF-α in hepatocytes have shown to increase ROS (Corda *et al*., 2001; Schulze-Osthoff *et al*., 1993). Interleukin-1 β also increases the ROS production and has been reported in many studies (Mendes *et al*., 2003; Brigelius-Flohe *et al*., 2004 Kaur *et al*., 2004). Similarly IFN- γ also increases ROS production in the lymphocytes (Pearl-Yafe *et al*., 2003; 2004). Watanabe *et al* (2003) also reported increase in ROS and ER stress (endoplasmic stress) in the hepatocytes undergoing apoptosis by the IFN- γ. Complex 1 and complex 111 are mainly responsible for the ROS produced by IFN α (Corda *et al*., 2001). TNF α is known to increase the ROS production by hampering with the electron transport chain (Schulze-Osthoff *et al*., 1992, 1993; Goosens *et al*., 1995; Corda *et al*., 2001). TNF α after binding to the receptor activates G protein which then leads to activation of phospholipase A2. It also activates protein kinase C by the triacylglycerol synthesis from arachidonic acid (Elbaz *et al*., 1991; Brach *et al*., 1993; Belka *et al*., 1995). These signaling cascades are also involved in the cell death. TNF regulates the cytotoxic and gene modulation via increased synthesis of ROS (Jones and Selby, 1989; Schulze-Ostholl *et al*., 1992).

Mitochondria are the main site of ROS production and ceramide has recently gained attention due to its role of a mediator in TNF induced ROS production by the mitochondria (Goosens *et al*., 1995; Adamson and Billings, 1992). Ceramide acts as a second messenger system and

increases the production of hydrogen peroxide. The study conducted by García-Ruiz et al., showed that there is significant increase in production of cermaide by the hepatocytes as an intermediate in the TNF induced ROS generation and signalling. Ceramide increases the hydrogen peroxide formation in the mitochondria by interfering with complex III of electron transport chain. Glutathione present in the mitochondrial depletes soon and as it is the only defense against the hydrogen peroxide generated from electron transport chain, hydrogen peroxide production rises further and leads to the lipid peroxidation of mitochondria. So the mitochondrial GSH levels are the first line of defense against ROS and its status will define the extent of damage by the ROS (García-Ruiz *et al*., 1997). Endoplasmic reticulum stress leads to release of calcium in the cytoplasm (Piccoli *et al.*, 2007; Tardif *et al*., 2005; Qadri *et al*., 2004 and Dionisio *et al*., 2009). There is increase in the uptake of calcium inside the mitochondria by increasing the uniporter activity. This is a beginning of a vicious cycle; the entry of calcium inside the mitochondria leads to increase in reactive oxygen species (ROS) production and these ROS further sensitize mitochondria to calcium by opening MPT pore (Li *et al*., 2007 and Wang *et al*., 2010). The lymphocytes undergo oxidative stress and damage to DNA is caused. It is known that PI3K pathway initiates in these lymphocytic cells. The interferon therapy targets the hepatocytes as well as the infected immune cells. The antiviral signaling pathway of interferon leads to the production of cytokines which contribute to inflammation and do not possess a specific antiviral action (Guttermann, 1995).

In the current study the altered ratio of oxidized to reduced glutathione also indicates loss of the antioxidant potential and it is in accordance with Malik *et al*., 2013. The excessive production of ROS and the poor antioxidant status of the cell leads to the damage of lipids, proteins and DNA. Lipid peroxidation results in the formation of malondialdehyde (MDA) a hallmark of lipid peroxidation and oxidative stress in cells (Rosser *et al*., 1995). The free radicals are catalyzed by enzymatic activity of the antioxidants; Superoxide dismutase (SOD) and Catalase. SOD catalyzes the superoxides and leads to formation of the hydrogen peroxide; another reactive species. This in turn is converted to oxygen and water by the activity of catalase. Low levels of activity of catalase and SOD are reported in

patients of Hepatitis C viruses (d trials in the treatment of viral hepatitis C in naive patients: 1999 update. J Demirdag *et al*., 2003). MDA levels were assessed to determine the amount of lipid peroxidation in the patients who have received interferon therapy. There is a lot of research data available on the increased levels of MDA in patients with chronic hepatitis (De Maria *et al*.1996; Paradis *et al*., 1997 and Boya *et al*., 1999). Values of MDA in all the patients were significantly high as compared to the controls. The explanation to this is the interference of the interferon with the cytokine cascade leads to increased free radical production and resulting in high MDA levels. The current study results are in accordance to Malik *et al*., 2013. Interferon regulates the OAS (2, 5-oligoadenylate synthetase). This OAS enhanced activity results in the inhibition of the MDA production from the cyclooxygenase pathway. The overall decrease in the production of free radicals and reduced lipid peroxidation is the major reason of the decreased MDA levels seen in some research studies (Hanigan and William, 1991 and Andreoni *et al*., 1993). Increased levels of MDA after interferon therapy are contrary with the results of Romero *et al*. (1998) who also observed a drop in MDA levels after interferon therapy. A strong negative co relation between MDA and GSH exists r = -0.763**. Liver metabolizes the xenobiotics and possesses high amounts of antioxidants. Protection against ROS is by antioxidant systems; enzymatic and non-enzymatic. Glutathione system is of particular importance in this regard. Glutathione peroxidase counteracts peroxides and uses GSH (reduced glutathione). Glutathione reductase regenerates the oxidized glutathione. In HCV infected patients, markedly reduced levels of this antioxidant is reported both in the hepatocytes as well as the serum of the patients. Nrf-2 pathway is reported to be suppressed by HCV which leads to decrease in the genes of glutathione system. (Carvajal-Yepes *et al*., 2011).

The anti-oxidant defense system comprises of enzymatic and non-enzymatic anti-oxidants. Catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase. Levels of glutathione reductase and glutathione peroxidase are also found to be decreased in Chronic Hepatitis C patients. Bhargava *et al*. (2011) showed decreased levels of glutathione reductase, glutathione peroxidase, superoxide dismutase and catalase in mono nuclear cells of the peripheral blood smear

of hepatitis C. Levent *et al*. (2006) studied the antioxidant defense system in hepatitis C patients before and after therapy with interferon. Antioxidant enzymes levels were found to be decreased in hepatitis patients but improved in patients who responded to interferon therapy. The current study results are in contrast to the Levent *et al*. (2006) and Osman *et al*., (2007). Decreased levels of SOD in hepatitis C patients has been identified by many researchers (Loginov *et al*., 1991; Yasuyama *et al*., 1988 and Irshad et al., 2002). Glutathione, Glutathione peroxidase, glutathione reductase, catalase and superoxide dismutase all were decreased in current study and similar were the findings of Malik *et al*., 2013. There was a strong correlation found between the GSH and SOD r =0.792** and also between GSH and Catalase r = 0.778**. Oxidative stress results in the synthesis of advanced glycation end products (AGEs) and advanced oxidation protein products (AOPPs). It is evident from research data that with the advancement of chronic diseases the serum levels of AGEs and AOPPs increase (Witko-Sarsat *et al*., 1996; 1998).AGEs and AOPPs both bind to a receptor called RAGE (receptor for advanced glycation end products). This receptor is expressed by the fibrogenic cells of liver; heaptic stellate cells and myofibroblasts. These cells are involved in the fibrosis of the chronic liver disease. AOPPs and AGEs after binding to RAGE are able to induce an inflammatory response. They initiate a signaling cascade by activation of NF-κB (Hyogo and Yamagishi, 2008). AGEs have shown to significantly increase inflammatory cytokines by increasing the transcription of the respective genes.

Thereby, increasing the production of interleukins, mainly inter leukin 6 (IL-6) and tumor necrosis factor α (Ekong *et al*., 2006). Zuwała-Jagiełło *et al*., (2012) studied the AOPPs, AGEs and various inflammatory cytokines and found a significant rise in the patients of chronic hepatitis C. AOPPs are known to be involved in many inflammatory diseases. They directly modulate pathogenesis of many diseases (Guo *et al*., 2008). There is sufficient evidence of increased concentration of AOPPs and AGEs in plama of chronic hepatitis patients (Zuwała-Jagiełło *et al*., 2011). Current study shows a significant rise in the levels of AGEs and AOPPs after interferon therapy due to the inflammation provoked by the cytokines.

ACKNOWLEDGEMENTS

The authors are highly thankful for the valuable contribution of Prof. Dr. M.H. Qazi, Director Center for Research in Molecular Medicine (CRiMM), The University of Lahore-Pakistan regarding financial support and critical review of the manuscript.

CONFLICT OF INTEREST

Authors declares no conflict of interest

REFERENCES

[1] Adamson GM and Billings RE. (1992). Arch. Biochem. Biophys. **294**, 223-229.

[2] Andreoni P, Cursaro C and Gasbarrini G (1993). Interferonα increases prostaglandin E2 production by cultured liver biopsy in patients with chronic viral hepatitis: Can non-steroidal anti-inflammatory drugs improve the therapeutic response to interferon? J Hepatol.**19**, 228-31.

[3] Barbaro G, Di Lorenzo G, Asti A, Ribersani M, Belloni G, Grisorio B, Filice G and Barbarini G. 1999. Hepatocellular mitochondrial alterations in patients with chronic hepatitis C: ultrastructural and biochemical findings. Am J Gastroenterol. **94 (8)**, 2198-2205.

[4] Bartenschlager R, Penin F, Lohmann V and Andre P. 2011. Assembly of infectious hepatitis C virus particles. Trends in Microbiology. **19(2),** 95-103.

[5] Belka C, Wiegmann K, Adam D, Holland R, Neuloh M, Herrmann F, Krönke M, Brach MA. 1995. Tumor necrosis factor (TNF)-alpha activates c-raf-1 kinase via the p55 TNF receptor engaging neutral sphingomyelinase. The EMBO journal. **14(6)**, 1156-65.

[6] Bhargava A, Raghuram GV, Pathak N, Varshney S, Jatawa SK, Jain D, Mishra PK. 2011. Occult hepatitis C virus elicits mitochondrial oxidative stress in lymphocytes and triggers PI3-kinase-mediated DNA damage response. Free Radical Biology and Medicine. **51(9)**, 1806-14.

[7] Bonaccorso S, Marino V, Biondi M, Grimaldi F, Ippoliti F and Maes M. 2002. Depression induced by treatment with interferon-alpha in patients affected by hepatitis C virus. Journal of Affective Disorders. **72**, 237-241.

[8] Boya P, Pena A, Beloqui O, Larrea E, Conchillo M and Castelruiz Y. 1999. Antioxidant status and glutathione metabolism in peripheral blood mononuclear cells from patients with chronic hepatitis. C. J Hepatol. **31**, 808-814.

[9] Brach MA, Gruss HJ, Sott C, Herrmann F.1993. The mitogenic response to tumor necrosis factor alpha requires c-Jun/AP-1. Molecular and cellular biology**. 13(7),** 4284-90.

[10] Brigelius-Flohe R, Banning A, Kny M and Bol GF. 2004. Redox events in interleukin-1 signaling. Arch Biochem Biophys. **423,** 66-73.

[11] Carvajal-Yepes M, Himmelsbach K and Schaedler S. 2011. Hepatitis C Virus Impairs the Induction of Cytoprotective Nrf2 Target Genes by Delocalization of Small Maf Proteins. The Journal of Biological Chemistry. **286(11)**, 8941-8951.

[12] Carvajal-Yepes M, Himmelsbach K, Schaedler S, Ploen D, Krause J, Ludwig L and Lavanchy D. 2009. The global burden of hepatitis C. Liver International. **29(1),** 74-81.

[13] Corda S, Laplace C, Vicaut E and Duranteau J. 2001. Rapid reactive oxygen species production by mitochondria in endothelial cells exposed to tumor necrosis factor-alpha is mediated by ceramide. Am J Respir Cell Mol Biol.**24,** 762-768.

[14] De Maria N, Colantoni A, Fagiuoli S, Liu GJ, Rogers BK and Farinati F. 1996. Association between reactive oxygen species and disease activity in chronic hepatitis C. Free Radic Biol Med. **21,** 291-295.

[15] Demirdag K, Yılmaz S and Ozdarendeli A. 2003. Levels of plasma malondialdehyde & erythrocyswte antioxidant enzyme activities in patients with chronic hepatitis B. Hepato-Gastroenterology. **50,** 766-770.

[16] Dionisio N, Garcia-Mediavilla MV and Sanchez-Campos S. 2009. Hepatitis C virus NS5A and core proteins induce oxidative stress-mediated calcium signalling alterations in hepatocytes. Journal of Hepatology. **50(5),** 872-882.

[17] Donahue JG, Munoz A and Ness PM. (1992). The declining risk of post-transfusion hepatitis C virus infection. N Engl J Med. **327**, 369-73.

[18] Ekong, U, Zeng S, Dun H, Feirt N, Guo J, Ippagunta N, Guarrera JV, Lu Y, Weinberg A, Qu W, Ramasamy R, Schmidt AM and Emond JC. 2006. Blockade of the receptor for advanced glycation end products attenuates acetaminophen-induced hepatotoxicity in mice. Journal of Gastroenterology and Hepatology, **21**, 682-688.

[19] Elbaz O, Budel LM, Hoogerbrugge H, Touw IP, Delwel R, Mahmoud LA, Löwenberg B. 1991. Tumor necrosis factor downregulates granulocyte-colony-stimulating factor receptor expression on human acute myeloid leukemia cells and granulocytes. The Journal of clinical investigation. **87(3)**, 838-41.

[20] Espinosa-Diez C, Miguel V, Mennerich D, Kietzmann T, Sánchez-Pérez P, Cadenas S, Lamas S. 2015. Antioxidant responses and cellular adjustments to oxidative stress. Redox biology. **6,** 183-97.

[21] Farinati F, Cardin R, De Maria N, Libera GD, Marafin C, Lecis E, Burra P, Floreani A, Cecchetto A, Naccarato R. 1995. Iron storage, lipid peroxidation and glutathione turnover in chronic anti-HCV positive hepatitis. J Hepatol. **22(4),** 449-456.

[22] Fujinaga H, Tsutsumi T, Yotsuyanagi H, Moriya K, Koike K. 2011. Hepatocarcinogenesis in hepatitis C: HCV shrewdly exacerbates oxidative stress by modulating both production and scavenging of reactive oxygen species. Oncology. **81 (1),** 11-17.

[23] García-Ruiz C1, Colell A, Marí M, Morales A, Fernández-Checa JC. 1997. Direct effect of ceramide on the mitochondrial electron transport chain leads to generation of reactive oxygen species. Role of mitochondrial glutathione. J Biol Chem.**1,** 11369-77**.**

[24] Goossens V, Grooten J, De Vos K, Fiers W. 1995. Direct evidence for tumor necrosis factor-induced mitochondrial reactive oxygen intermediates and their involvement in cytotoxicity. Proceedings of the National Academy of Sciences. **92(18)**, 8115-9.

[25] Guo ZJ, Niu HX, Hou FF, Zhang L, Fu N, Nagai R, Lu X, Chen BH, Shan YX, Tian JW, Nagaraj RH, Xie D, Zhang X. 2008. Advanced oxidation protein products activate vascular endothelial cells via a RAGE-mediated signaling pathway. Antioxid Redox Signal. **10,** 1699-1712.

[26] Guttermann JU (1995). Cytokine therapeutics: lessons from interferon a. Proc Natl Acad Sci USA. **91,** 1198-1205.

[27] Hanigan GE and BRG William BRG. 1991. Interferon signalling through arachidonic acid-dependent pathway: A clue to adjuvant therapy for chronic viral hepatitis. Hepatology.**14,** 392-4.

[28] Hino K, Hara Y, Nishina S. 2014. Mitochondrial reactive oxygen species as a mystery voice in hepatitis C. Hepatology Research. **44(2),** 123-132.

[29] Hoofnagle JH. 1999. Management of hepatitis C: current and future perspectives. J Hepatol. **1,** 264-268.

[30] Hovanessian AG and Wood JN. 1980. Anticellular and antiviral effects of pppA(2'p5'A)n. Virology. **101,** 81-90.

[31] Hyogo H and Yamagishi S. 2008. Advanced glycation end products (AGEs) target genes by delocalization of small Maf proteins. J. Biol. Chem. **286,** 8941-8951.

[32] Irshad MC and Joshi YK. 2002. Superoxide dismutase and total anti-oxidant levels in various forms of liver diseases. Hepatol Res. **23,** 178-184.

[33] Ivanov AV, Bartosch B, Smirnova OA, Isaguliants MG, and Kochetkov SN. 2013. HCV and oxidative stress in the liver. Viruses. **5,** 439-469.

[34] Jones AL, Selby P. 1989. Tumour necrosis factor: clinical relevance. Cancer surveys. **8(4),** 817-36.

[35] Kato H, Takeuchi O, Mikamo-Satoh E, Hirai R, Kawai T, Matsushita K, Hiiragi A, Terence, Dermody S, Fujita T, Akira S. 2008. Length-dependent recognition of double-stranded ribonucleic acids by retinoic acid–inducible gene-I and melanoma differentiation–associated gene 5. Journal of Experimental Medicine. **205(7),** 1601-1610.

[36] Kaur J, Dhaunsi GS, Turner RB. 2004. Interleukin-1 and nitric oxide increase NADPH oxidase activity in human coronary artery smooth muscle cells. Med Princ Pract. **13,** 26-29.

[37] Levent G, Ali A, Ahmet A, Polat EC, Aytaç C, Ayşe E, Ahmet S. 2006. Oxidative stress and antioxidant defense in patients with chronic hepatitis C patients before and after pegylated interferon alfa-2b plus ribavirin therapy. J Transl Med.**4,** 10-25.

[38] Li Y, Boehning DF, Qian T, Popov VL, Weinman SA. 2007. Hepatitis C virus core protein increases mitochondrial ROS production by stimulation of Ca2+ uniporter activity. The FASEB Journal. **21(10),** 2474-2485.

[39] Liu P, Jamaluddin M, Li K, Garofalo RP, Casola A, Brasier AR. 2007. Retinoic Acid-inducible gene I mediates early antiviral response and toll-like receptor 3 expression in respiratory syncytial virus-infected airway epithelial cells. J Virol. **81,** 1401-1411.

[40] Loginov AS, Matiushin BN, Tkachev VD. 1991. Enzyme system for dismutation of active forms of oxygen in the liver during chronic impairment of hepatobiliary system. Vapor Med Khim. **37,** 31-33.

[41] Malik A, Qureshi MS, Manan A, Saleem S, Munir M, Fatima A, Arooj M, Qazi MH. 2013. Assessment of Oxidative Stress in Hepatitis C Patients Receiving Interferon Therapy. Pakistan Journal of Medicine and Dentistry. **2,** 04.

[42] Marra M, IM Sordelli IM, Lombardi A. 2011. Molecular targets and oxidative stress biomarkers in hepatocellular carcinoma: an overview. Journal

of Translational Medicine. **9(1),** 171.

[43] Mendes AF, Caramona MM, Carvalho AP, Lopes MC. 2003. Hydrogen peroxide mediates interleukin-1beta-induced AP-1 activation in particular chondrocytes: implications for the regulation of iNOS expression. Cell Biol Toxicol. **19,** 203-214.

[44] Osman HG, Gabr OM, Lotfy S, Gabr S. 2007. Serum levels of bcl-2 and cellular oxidative stress in patients with viral hepatitis. Indian J Med Microbiol. **25(4),** 323-329.

[45] Paradis V, Mathurin P, Kollinger M, Imbert-Bismut F, Charlotte F, Piton A. 1997. In situ detection of lipid peroxidation in chronic hepatitis C:correlation with pathological features. J Clin Pathol. **50,** 401-406.

[46] Pearlman BL and Traub N. 2011. Sustained Virologic Response to Antiviral Therapy for Chronic Hepatitis C Virus Infection: A Cure and So Much More, Clinical Infectious Diseases. **52(7),** 889-900.

[47] Pearl-Yafe M, Halperin D, Halevy A, Kalir H, Bielorai B, Fabian I. 2003. An oxidative mechanism of interferon induced priming of the Fas pathway in Fanconi anemia cells. Biochem Pharmacol. **65,** 833-842.

[48] Pearl-Yafe M, Halperin D, Scheuerman O, Fabian I. 2004. The p38 pathway partially mediates caspase-3 activation induced by reactive oxygen species in Fanconi anemia C cells. Biochem Pharmacol. **67,** 539-546.

[49] Piccoli C, Scrima R, Quarato G. 2007. Hepatitis C virus protein expression causes calcium-mediated mitochondrial bioenergetic dysfunction and nitro-oxidative stress," Hepatology. **46 (1),** 58-65.

[50] Poynard T, Marcellin P, Lee SS, Niederau C, Minuk GS, Ideo G, Bain V, Heathcote J, Zeuzem S, Trepo C. 1998. Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International

Hepatitis Interventional Therapy Group (IHIT) Lancet. **352,** 1426-1432.

[51] Qadri I, Iwahashi M, Capasso JM. 2004. Induced oxidative stress and activated expression of manganese superoxide dismutase during hepatitis C virus replication: role of JNK, p38 MAPK and AP-1. Biochemical Journal**. 378 (3),** 919-928.

[52] Reshi ML, Su YC, Hong JR. 2014. RNA viruses: ROS-mediated cell death. International Journal of Cell Biology. **2014(467452),** 16.

[53] Romero MJ, Bosch-Morell F, Romero B, Rodrigo JM, Sera MA, Romero FJ. 1998. Serum Malondialdehyde: Possible use for the clinical management of chronic hepatitis C patients. Free Radic Biol Med.**25,** 993-997.

[54] Rosser BG, Gores GJ. 1995. Liver cell necrosis: Cellular mechanisms & clinical implications. Special reports & reviews. Gastroenterology.**108,** 250-256.

[55] Schroder HC, Suhadolnik RJ, Pfleiderer W, Charubala R, Muller WE. 1992. 2'-5') Oligoadenylate and intracellular immunity against retrovirus infection. Int J Biochem. **24,** 55-63.

[56] Schulze-Osthoff K, Bakker AC, Vanhaesebroeck B, Beyaert R, Jacob WA, Fiers W. 1992. Cytotoxic activity of tumor necrosis factor is mediated by early damage of mitochondrial functions. Evidence for the involvement of mitochondrial radical generation. J Biol Chem. **267,** 5317-5323.

[57] Schulze-Osthoff K, Beyaert R, Vandevoorde V, Haegeman G, Fiers W. 1993. Depletion of the mitochondrial electron transport abrogates the cytotoxic and gene inductive effects of TNF. EMBO J.**12,** 3095-3104.

[58] Stewart TA, Hultgren B, Huang X, Pitts-Meek S, Hully J, MacLachlan NJ. 1993. Induction of type diabetes by interferon-alpha in transgenic mice. Science. **260,** 1942-1946.

[59] Tardif KD, Waris G, Siddiqui A. 2005. Hepatitis C virus, ER stress,

and oxidative stress," Trends in Microbiology. **13(4),** 159-163.

[60] Thévenot T, Regimbeau C, Ratziu V, Leroy V, Opolon P, Poynard T. 2001. Meta-analysis of interferon randomize Viral Hepat. **8,** 48-62.

[61] Valgimigli L, Valgimigli M, Gaiani S, Pedulli GF, Bolondi L. 2000. Measurement of oxidative stress in human liver by EPR spin-probe technique. Free Radic Res. **33(2),** 167-178**.**

[62] Valgimigli M, Valgimigli L, Trere D, Gaiani S, Pedulli GF, Gramantieri L, Bolondi L. 2002. Oxidative stress EPR measurement in human liver by radical-probe technique. Correlation with etiology, histology and cell proliferation. Free Radic Res. **36(9),** 939-948.

[63] Wang T, Weinman SA. 2013. Interactions between hepatitis C virus and mitochondria: impact on pathogenesis and innate immunity. Current Pathobiology Reports. **1(3),** 179-187.

[64] Wang T, Campbell RV, Yi MK, Lemon SM, Weinman SA. 2010. Role of Hepatitis C virus core protein in viral-induced mitochondrial dysfunction. Journal of Viral Hepatitis. **17(11),** 784-793.

[65] Wasley A and Alter M. 2000. Epidemiology of hepatitis C: geographicdifferences and temporal trends. Semin Liver Dis**. 20,** 1-16.

[66] Watanabe Y, Suzuki O, Haruyama T, Akaike T. 2003. Interferon-gamma induces reactive oxygen species and endoplasmic reticulum stress at the hepatic apoptosis. J Cell Biochem. **89,** 244-253.

[67] Weiss T, Klingel K, Hildt E. 2000. Hepatitis C virus impairs the induction of cytoprotective Nrf2 and their involvement in liver disease. Curr Pharm Des **14,** 969-972.

[68] Wichers MC, Koek GH, Robaeys G, Verkerk R, Scharpe S, Maes M. 2005. IDO and interferon-α-induced depressive symptoms: a shift in hypothesis from tryptophan depletion to neurotoxicity. Molecular Psychiatry. **10,** 538-544.

[69] Witko-Sarsat V, Friedlander M, Capeillere-Blandin C, Nguyen-Khoa T, Nguyen AT, Zingraff J, Jungers P, Descamps-Latscha B. 1996. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. Kidney. Int. **49,** 1304-1313.

[70] Witko-Sarsat VT, Nguyen-Khoa P, Jungers Drueke T, Descamps-Latscha B. 1999. Advanced oxidation protein products as a novel molecular basis of oxidative stress in uremia. Nephrol. Dial. Transplant**. 14,** 76-78**.**

[71] World Health Organization. 2000. Hepatitis C- global prevalence (update) (2000). Wkly Epidemiol. Rec**. 75,** 18-9.

[72] Yasuyama T, Inoue K, Kojima T, Sasaki H. 1988. Activities, electrophoretic profiles and immunolocalization of superoxide dismutase in human liver specimens. Jpn J Med. **27,** 34-41.

[73] Zuwała-Jagiełło J, Maria W, Monika PS. 2012. Ischemia-modified albumin (IMA) is increased in patients with chronic hepatitis C infection and related to markers of oxidative stress and inflammation. **59(4),** 661-667.