A Study of the Effect of the Use of Antioxidants in Patients with Hepatitis C Receiving Interferon/Ribavirin Therapy on the Response to Therapy

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Background and study aim: Treatment of HCV with interferon takes a long duration and has many side effects. The use of antioxidants with interferon/ribavirin therapy is believed to minimize the side effects and improves adherence and hence improves response to therapy. Reactive oxygen species are part of the human defense mechanisms towards infection and they increase due to hepatitis C virus infection. In this study we aim to study the impact of the concomitant use of antioxidants with interferon/ribavirin combination therapy for HCV on response as regards enzymes level, rate of viral clearance as well as liver histopathology..

Patients and methods: 240 patients on interferon/ribavirin therapy for chronic hepatitis C divided in two groups. The test group received concomitant antioxidant combination while the control group received only interferon/ribavirin. Follow up of liver function tests, complete blood count, viral load by PCR and post treatment histopathology by liver biopsy were performed.

Results: Liver enzymes level in test group achieved a larger and faster decline than in control group. Hematological parameters were significantly higher in the test group all through period of follow up. Viral load and histopathology showed no significant difference between the two groups.

Conclusion: concomitant use of antioxidants with interferon/ ribavirin therapy minimizes complications of therapy and rapidly normalizes the liver enzymes level without affecting the rate of response to therapy or histopathology of the liver.

INTRODUCTION

Pegylated interferon α (peginterferon α, peg-IFNα) in combination with weight-based doses of ribavirin (RBV) is currently recommended as the first-line "standard-of-care" treatment for chronic hepatitis C virus (HCV) infection. [1]

A recent trend in the treatment strategy of chronic HCV infection is the development of individualized treatment regimens based on strong predictors of SVR to IFN-based treatment, such as HCV genotype [2] and the initial virologic response to treatment.[3] Meanwhile, alternative options, such as modified regimens with currently available medications, novel modified IFNα and RBV or combinations with specifically targeted antiviral therapy for HCV (STAT-C) agents, are currently being investigated for the growing number of patients for whom current "standard-of-care" treatment has failed. For the foreseeable future, however, peg-IFNα and RBV appear to remain the backbone of "standard-of-care" treatment. [4]

Dose: Peg INF alpha 2a 180ug/week subcutaneous injection + ribavirin 800 mg /day oral may be increased to 1000-1200mg daily according to the

body weight.Peg INF alpha 2b 1-1.5mg/kg weekly subcutaneous injection + ribavirin at same previous dose. [5].

So many studies were done to evaluate the beneficial effects of the use of antioxidants in hepatitis C virus. Schizandrae chinensis, a potent anti-oxidant, lowers ALT levels in patients with chronic viral hepatitis**.** [6] A combination of three potent antioxidants (alpha-lipoic acid, silymarin, and selenium) induced marked clinical, laboratory and histologic improvement in chronic HCV patients. [7]

Another study observed that high vitamin E supplementation improves the aminotransferase status in patients who have chronic HCV. [8] A retrospective study examining the effects of stronger neo-minophagen C (SNMC), which contains glycyrrhizin as an active component, revealed that treatment with this agent reduces the long term relative risk of developing hepatocellular carcinoma by a factor of 2.49. [9] A randomized double-blind trial of thioctic acid (alpha-lipoic acid) in chronic hepatitis patients showed that 55% patients have significant improvements in mean ALT levels, and 77% patients have histological improvements on liver biopsy.[10] Intravenous glycyrrhizin was tested in patients with chronic HCV infection, and lowered ALT levels (26% ***vs*** 6% with placebo) within 4 wk were noted. The effect disappears after cessation of therapy. [11]

Administration of glutathione to patients with chronic hepatitis significantly decreases GSH-Px activity of catalase (CAT), and increases superoxide dismutase (SOD) activity. [12] A Cochrane systematic review of trials of medicinal herbs in HCV, reported that silybinin significantly reduced serum AST and GGT levels in only one trial, with no firm evidence for the use of herbal medicines in this condition. [13]

It was demonstrated that antioxidant vitamin (E and C) supplementation during interferon alfa-2b prevented decrease in eicosapentaenoic acid of mononuclear cell phospholipids. [14] The results of another more recent study showed a modest reduction in liver enzymes at the end of 24 wk of treatment in patients receiving the combined intravenous and oral protocol. [15]

So many studies also evaluated the concomitant use of antioxidants with interferon. It was reported that a combination therapy of interferon (IFN) with glycyrrhizin induces normalization of serum ALT levels in 64.3% of non-responders with serum HCV RNA disappeared in 38.5%. [9] It has been demonstrated that vitamin E-treated patients have a 2.4 times higher chance of obtaining a complete response and a more significant reduction in viral load than patients not treated with vitamin E. [16]

The use different antioxidants regimens prior and with interferon therapy lead to decline in liver enzymes. The liver histology wasn't affected and liver enzymes levels re-rise after stoppage of therapy in non-responders to interferon. This was confirmed by many studies which used different and complex antioxidant combination of oral and intravenous preparations. These studies also declared that antioxidants improved patients' quality of life during interferon therapy. [17, 18] The efficacy of antioxidants in European studies was less than that reported on Japanese subjects, which can be explained by the genetic polymorphism in drug metabolism. [19]

Side effects of interferon are so many but most important of them are the hematologic effects. They are the most recurrent abnormal laboratory values that can lead to dosage reductions and premature treatment termination. [20] Neutropenia is defined as an absolute neutrophil count less than 500 cells/mm^3 when using pegylated interferon α-2a. [21]

In most clinical trials, neutropenia is treated with dose modification. Interferon dose reduction occurs in about 17% to 20% of patients and treatment termination in 2% to 3% of patients**.** [2] Another option for patients who develop neutropenia from interferon therapy is the use of granulocyte colony-stimulating factor (GCSF)**.** [22] Another interferon-induced hematologic adverse effect is thrombocytopenia. It has been shown that platelet count can fall up to 50% of pretreatment count. [23] Eltrombopag, a thrombopoietin receptor agonist, to effectively increase platelet counts to greater than 250,000/mm^3 in thrombocytopenic patients with hepatitis C virus. [24]

Ribavirin also has so many serious side effects most important of them is the hematologic effects. The signature adverse effect of ribavirin is anemia, occurring in up to 30% of treated individuals. Ribavirin-related anemia is one the most common reasons for dosage reduction or discontinuation of the drug, resulting in 9% to 22% of patients requiring dosage reduction. [4] The mechanism of ribavirin-associated hemolytic

anemia is unclear, but is believed to be related to impaired antioxidant defenses and red blood cell oxidative damages through its metabolites .[25] Erythropoietin use in early-onset anemia minimized treatment discontinuation and led to higher sustained viral response rates. [26]

PATIENTS AND METHODS

The study was conducted in Tropical Medicine Department, Zagazig University Hospitals and patients were selected randomly from patients attending the hepatitis viruses out patient clinic of Alahrar General Hospital.

Total number of 240 patients, all having chronic HCV infection, were included in the study and were randomly divided on two groups.

Test Group: consists of 120 patients received:

1- Pegylated INF-α2a 180µg/wk SC.

2- Ribavirin 15 mg/kg/day orally.

3- Antioxidant combination regimen (vitamin E 400 mg, silymarin 420 mg, N-acetylcysteine (NAC) 600mg, vitamin C 500mg) orally daily.

Control group: consists of 120 patients received:

1- Pegylated INF-α 2a 180µg/wk SC.

2- Ribavirin 15mg/kg/day orally.

All patients were subjected to the following: Careful history taking and thorough clinical examination with calculation of the body mass index (BMI).The following laboratory tests: complete blood count, liver function tests, coagulation profile, kidney function tests, thyroid function tests, serologic markers for hepatitis B virus, anti-nuclear antibodies, quantitative polymerase chain reaction for HCV RNA, fasting blood glucose level and glycosylated hemoglobin A1C if fasting blood glucose is elevated.

Liver biopsy was performed to all patients before start of their therapy protocol in Tropical Medicine Department. Biopsies were prepared and examined in the Pathology Department in Faculty of medicine, Zagazig University and were interpreted according to METAVIR score.

Follow up:

Patients repeat liver function tests, serum creatinine level, complete blood count and PCR for HCV RNA at 4 weeks, 12 weeks, 24 weeks, and 48 weeks. Post-treatment liver histopathology was performed by number of patients after they stopped therapy ie after being declared non-responders or at end of therapy at 48 weeks.

RESULTS

Table (1): Demographic data

	Test group (n=120)		Control group (n=120)		X^2	P	Significance
	No.	%	No.	%			
Gender							
Male	84	70.0	85	70.8	0.02	0.88	NS
Female	36	30.0	35	29.2			
Diabetes	1.6	13.3	18	15	0.14	0.75	NS
Age (years)							
$\overline{X}$ ±SD	35.0±6.8		33.3±7.8		t=1.88	0.06	NS
Range	21-49		20-50				

NS: non-significant

Table (2): Rates of responses throughout period of therapy.

	Test group (n=120)		Control group (n= 120)		X^2	P	Significance
	No.	%	No.	%			
RVR	56/120		51/120				
Negative PCR	27	49.1	26	51	0.04	0.81	NS
EVR							
Negative PCR	95	79.16	92	76.6	0.22	0.64	NS
Incomplete responders (>2log decline)	5	4.16	7	5.8	0.35	0.55	NS
24wks							
Negative PCR	83	69.1	82	68.3	0.02	0.88	NS
48wks							
Negative PCR	80	66.6	80	66.6	0	1	NS

NS: non-significant

Table (3): Incidences of breakthroughs through out the period of treatment.

Items	Test group (n=120)		Control group (n=120)		X^2	P	Signif.
	No.	%	No.	%			
Total	16	13.3	14	11.6	0.15	0.69	NS
24wks	Test n = 100/120		Control n = 99/120				
	13	13	12	12.1	0.03	0.85	NS
48wks	Test n = 83/120		Control n = 82/120				
	3	3.6	2	24	0.001	0.98	NS

NS: non-significant

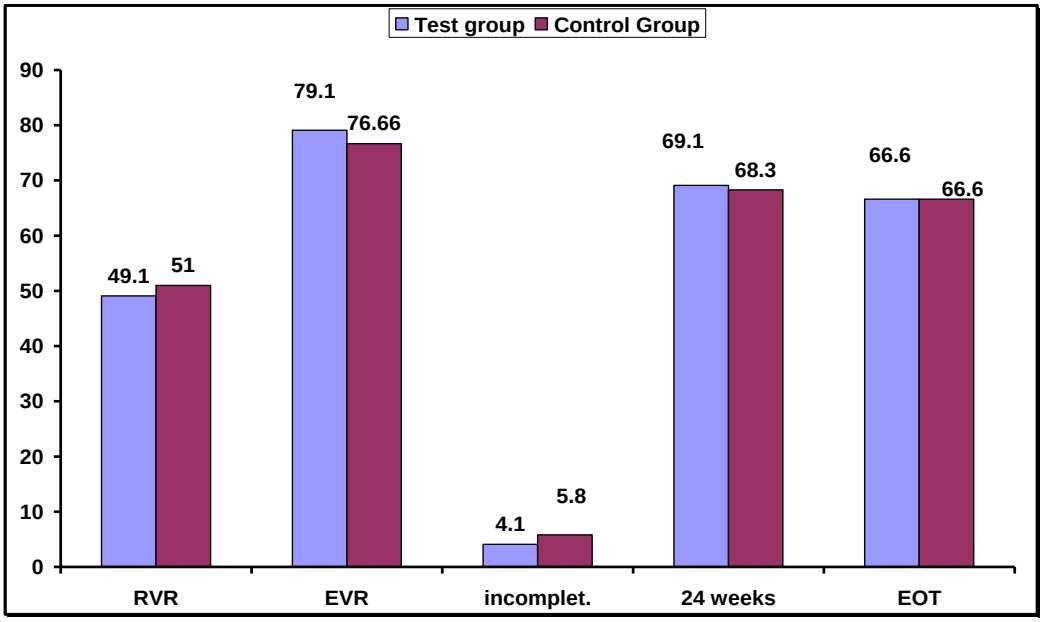

Figure (1): Represents the rates of responders all through the period of therapy.

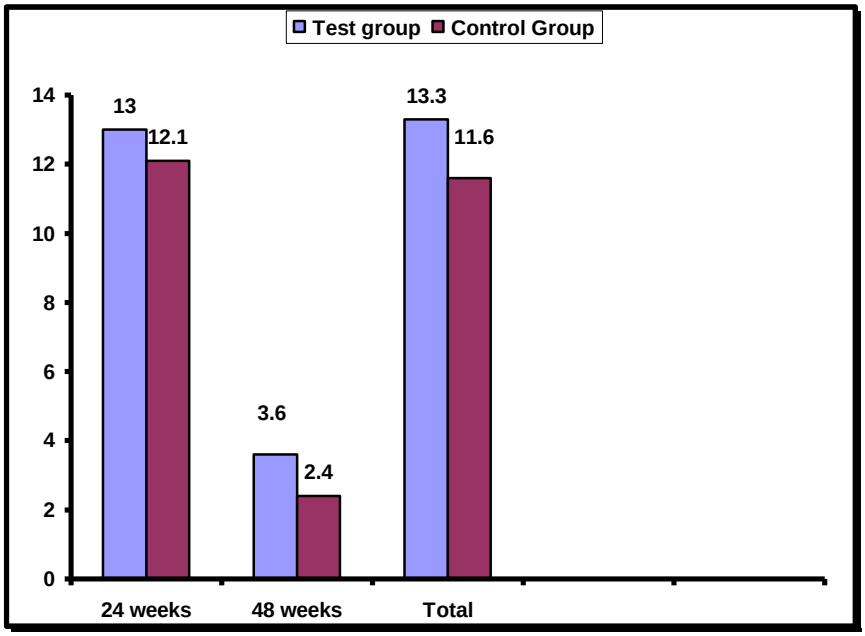

Figure (2): Represents the rates of break through all through the period of therapy

Table (4): Test groups liver function tests, creatinine and hematological parameters all through period of follow up.

Items	Base line	4 weeks	12 weeks	24 weeks	48 weeks	Weekly rate of change
ALT (IU/ml)						
$\overline{X}$ ±SD	61.9±31.4	56.3±27.5	54.8±27.6	41.1±18.2	24.4±11.5	0.78 IU/ml/wk
AST (IU/ml)						
$\overline{X}$ ±SD	50.2±23.9	49±22.3	41.1±17.3	37.2±15.4	25.9±14.7	0.50 IU/ml/wk
Alkaline phoshpatase (IU/ml)						
$\overline{X}$ ±SD	89.3±44.1	89.6± 58	83±22.4	77.2±17.5	75.1±13.3	0.29 IU/ml/wk
Bilirubin (mg/dl)						
$\overline{X}$ ±SD	0.6±0.17	0.9 ± 0.2	1.3±0.3	1.5±0.2	1.9±0.5	0.03 mg/dl
Albumin (g/dl)						
$\overline{X}$ ±SD	4.5±1.2	4.4±1.2	4.1±0.7	3.9±0.6	3.8±0.7	0.014 g/dl/wk
Cr (mg/dl)						
$\overline{X}$ ±SD	0.83±0.2	0.85±0.15	0.86±0.2	0.9±0.1	0.9±0.9	
WBC (cells/ml)						
$\overline{X}$ ±SD	6.1±1.6	5.5±1.7	4.6±1.6	4±1.4	3.5±1.6	54 cells/ml/wk
Hb (g/dl)						
$\overline{X}$ ±SD	13.9±1.1	13.1±1.4	12.4±1.2	11.8±1.3	11.7±1.3	0.045 g/dl/wk
PLT (cells/ml)						
$\overline{X}$ ±SD	177.8±41	162.2±45.2	14.8±46	137.1±43	121.1±47	1181 platelets/ml/wk

Table (5): Control group liver function tests, creatinine and hematological parameters all through period of follow up.

Items	Base line	4 weeks	12 weeks	24 weeks	48 weeks	Weekly rate of change
ALT (IU/ml)						
$\overline{X}$ ±SD	68.7±45.4	51.8±27.9	50.6±36	53.4±27	31.4±10	0.77 IU/ml/wk
AST (IU/ml)						
$\overline{X}$ ±SD	49.7±23.4	53.6±32	47.5±20.5	46.8±20.6	28.1±9.3	0.45 IU/ml/wk
Alkaline phoshpatase (IU/ml)						
$\overline{X}$ ±SD	85.6±39.2	91.2 ± 62.1	86.5±30.2	82±12.3	80.3±10.1	0.11 IU/ml/wk
Bilirubin (mg/dl)						
$\overline{X}$ ±SD	0.7±0.23	1.6± 0.4	2.1±0.5	2.7±1	2.7±0.6	0.04 mg/dl/wk
Albumin (g/dl)						
$\overline{X}$ ±SD	4.4±1.1	4.5±1.5	4±1.02	3.8±0.8	3.5±0.5	0.02 g/dl/wk
Cr (mg/dl)						
$\overline{X}$ ±SD	0.86±0.14	0.89±0.2	0.87±0.14	0.9±0.12	0.87±0.15	
WBC (cells/ml)						
$\overline{X}$ ±SD	6.5±1.74.6±1.2	3.7±1	3.2±1	2.7±1	79	79 cells/ml/wk
Hb (g/dl)						
$\overline{X}$ ±SD	14.2±1.5	12.5±1.4	11.8±1.4	11.1±1.36	11±1.3	0.066 g/dl/wk
PLT (cells/ml)						
$\overline{X}$ ±SD	180.7±50.4	156.4±39.7	142.5±37	123.7±38	105.6±31	1560 cells/ml/week

Table (6): The percent of change that occurs in liver function tests and hematological parameters.

Items	Test group (n=120)	Control group (n=120)	P	Significance
ALT	-56.6%	-40.0%	0.035	S*
AST	-41.02%	-38.0%	0.7	NS
Alk.phos.	-15.9%	-6.2%	<0.001	HS**
Bilirubin	+192%	+285%	<0.001	HS**
Albumin	-22%	-20.4%	0.7	NS
WBC	-38.6%	-56.6%	<0.001	HS**
HB	-15.8%	21.4%	0.012	S*
PLT	-34.3%	-39.8%	0.25	NS

NS: non-significant S: significant HS: highly significant

Table (7): Comparison between pre-treatment and post-treatment biopsy of test group.

Items	Pre-treatment (N=120)		Post-treatment (N=26)		*McNemar's test of Significance*	
	No.	%	No.	%		
A						
0	0	0	1	3.8	1	NS
1	41	34.2	12	46.1	0.24	NS
2	58	48.3	11	42.3	0.57	NS
3	21	17.5	2	7.6	0.3	NS
4	0	0	0	0	1	NS
F						
0	4	3.3	0	0	0.7	NS
1	46	38.3	11	42.3	0.68	NS
2	44	36.7	10	38.4	0.78	NS
3	26	21.7	5	19.2	1	NS
4	0	0	0	0	1	NS

NS: non-significant **NB:** McNemar's test ignores statistically all the subjects with missing data.

Table (8): Comparison between pre-treatment and post-treatment biopsy of control group.

Items	Pre-treatment (n=120)		Post-treatment (n=32)		McNemar's test of Significance	
	No.	%	No.	%		
A						
0	1	0.8	0	0	0.77	NS
1	38	31.7	11	34.4	0.67	NS
2	65	54.2	16	50	0.96	NS
3	16	13.3	5	15.2	1	NS
4	0	0	0	0	1	NS
F						
0	0	0	0	0	1	NS
1	35	29.2	5	15.2	0.12	NS
2	53	44.2	18	56.2	0.22	NS
3	32	26.6	9	28.1	0.86	NS
4	0	0	0	0	1	NS

NS: non-significant

DISCUSSION

The response to interferon that we evaluate in our study has three parameters: biochemical; level of ALT and AST, virological; measured at 4 weeks (RVR), 12 weeks (EVR), 24 weeks, 48 weeks (EOT),(the rate of SVR isn't assessed in this study), and histopathology using liver biopsy to assess the degree of activity and fibrosis.

The biochemical response: We found out as regards ALT levels; they fall gradually over the period of follow up so by the end of treatment most patients had normal ALT level. The level of ALT showed non significant difference between the two groups in the early period of follow up but became significantly lower in test group starting from week 24 to end of therapy. Although the weekly rate of ALT levels decline was calculated in both groups [.78 IU/ml/week in group I and .77 IU/ml/week in group II] and there was no significant difference between two groups, the percent of change that occurred in ALT levels was calculated in both groups at end of therapy and showed that test group I achieved a higher percent of decline than the control group. Our results disagree with Seeff et al, 2008 and Par et al, 2009 who used silymarin with long term interferon therapy found that it had no favorable effects on the liver enzymes or on viral load. In our study we used other antioxidants (vitamin E and C) with silymarin which have superoxide-scavenging activity so the results for liver enzymes were favorable. [27, 28] On the contrary, our results are supported by the study of Emerit et al, 2005 who used a phenol-rich processed grain food with superoxide-scavenging properties. They found a manifest decline in liver enzymes but no effect on viral load or biopsy was noted. [29] Our results also agree with Murakami et al, 2006, who found that vitamin E and C supplementation during INF therapy prevented the drop in level of Ecosapentaenoic acid in the cell membrane phopholipids in monomuclear cells. This keeps the integrity in those cell membranes and the level of ecosapentaenoic acid level was inversely co-related to the ALT level. [14]

AST level is different from ALT level being less specific to hepatocyte injury; AST is elevated also in hemolysis. The drop in AST level expressed as a weekly decline rate and as percent of change showed non-significant difference between the two groups. However, AST level was significantly lower in a specific point of time which is 24th week. Being non-specific to liver injury, AST doesn't seem to follow ALT strictly; AST achieves a slower decline than ALT.

In our study we threw light at other biochemical parameters such as; alkaline phosphatase level, albumin and bilirubin. As regards alkaline phosphatase level it showed no significant differences between the two groups until the 24th week when it became significantly lower in test group and then highly significantly lower in 48th week. When we calculated its weekly rate of decline and percent of change, we found a highly significant difference between the two groups. This means that, like the other liver enzymes, alkaline phosphatase achieves a faster and greater decline with antioxidants added to INF/RBV therapy than with INF/RBV therapy alone.

The bilirubin level seamed to be affected the most by the antioxidants. It became highly significantly higher in the control group starting from week 4 to the end of therapy. This can easily be explained on the background that antioxidants abolish the ribavirin-induced hemolysis. The rise in bilirubin was also associated with a decline in Hb concentration in both groups. This emphasizes, without doubt, that the hyperbilirubinemia is due to hemolysis. The weekly rate of rise as well as the percent of change of bilirubin level was highly significantly higher in the control group.

Among all the biochemical parameters monitored in our study, the albumin level seams to be the least affected by addition of antioxidants to therapy. This can be easily rationalized by the facts that albumin has a long half life, the synthetic function of the liver is good and the dyspepsia associated with interferon therapy is not severe enough to affect nutritional status of the patients.

The virological response: There was no significant difference between two groups as regards the rate of virological responses; RVR, EVR, 24 weeks and EOT. There were also no significant differences between two groups as regards rates of incomplete response and rate of breakthroughs at 24 weeks and at end of therapy. Most studies that studied the impact of use of antioxidants on the viral load with or without interferon therapy came to a common result that antioxidants don't affect the viral load [15, 17, 27, 28, 29] Our results disagree with Kalantari et al, 2011 who found that using an antioxidant (silymarin alone) use can decrease the viral load. This study used the antioxidant without interferon and involved a small number of patients. The period of follow up was shorter 24 weeks only in both studies. [30]

The histological response: There was non significant difference between two groups as regards post-treatment biopsy. There was no significant difference between pre and post-treatment biopsy as regards both degrees of fibrosis and activity in each group. Hence, we can say that the decline in liver enzymes doesn't mean an actual decline in the degree of activity in biopsy. This also means that the degree of fibrosis showed neither progression nor regression during the period of follow up. Here, we can mention that the period between the pretreatment and the post treatment biopsies ranged from 3 months in null-responders to 12 months in responders (EOT), we can also mention that 3-month period may not be enough to manifest the change. The previous studies that recorded histological changes ranged from 12 to 26 weeks and also found no evidence of histological changes. [15, 18, 31, 32] We recommend a longer duration up to 2 years to manifest if there will ever be significant histological changes with antioxidant use vs placebo.

Complications of interferon therapy are so many, and they are not the main topic that this thesis was designed to study, however we managed to describe and evaluate some of them, being difficult to totally ignore. Most important of these complications that we studied are; hematological complications, seen clearly in the CBC. The mean Hb concentration was non-significantly different in two groups before treatment began, then became significantly higher in test group all through the period of follow up. The weekly rate of decline of Hb concentration was significantly higher in control group; the percent of change was also significantly higher in the control group. Our results are in agreement with DeFranceschi et al, 2000 who demonstrated the central role of oxidative stress in the ribavirin-induced hemolysis. [25] Our results also agree with Murakami et al, 2006 and Hino et al, 2006 who concluded that vitamin E and C supplementation helped stopping RBC's premature destruction through keeping the ecosapentaenoic acid level in their cell membranes from falling with interferon therapy. Hino et al, also assured that the oxidative damage in erythrocyte membrane plays an important role in ribavirin induced anaemia. [14, 33]

Mean WBC count was also non-significantly different in two groups before treatment began, then became highly significantly higher in test group all through the period of follow up, the weekly rate of decline and percent of change were significantly higher in control group.

Mean platelet count was non-significantly different in two groups before treatment began and all through period of therapy, however the weekly rate of decline was significantly higher in control group. Our results are comparable to those of Rustgi et al, 2002 who found that platelet count falls over a longer period than the other hematological parameters. [34]

Conclusion: We ascertain that antioxidants slowed the deterioration of hematological parameters that occurs with INF/RBV therapy. This effect is central in the maintaining the patients adherence to therapy. They also helped to achieve a faster decline in liver enzymes without affecting the virological response to therapy or liver histopathology.

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Conflicts of interest: The authors declare that there is no conflict of interest.

Ethical approval: approved.

REFERENCES

1. Ghany MG, Strader DB, Thomas DL, Seeff LB, Shuhart MC, Davis GL, et al. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; 49:1335–1374.
2. Hadziyannis SJ, Sette H, Morgan TR, Balan V, Diago M, Marcellin P, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004;140:346–355.
3. Tsubota A, Arase Y, Someya T, Suzuki Y, Suzuki F, Saitoh S, et al. Early viral kinetics and treatment outcome in combination of high-dose interferon induction vs. pegylated interferon plus ribavirin for naive patients infected with hepatitis C virus of genotype 1b and high viral load. *J Med Virol* 2005;75:27–34.
4. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçales FL. et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002;347:975–982.
5. Poynard T, McHutchison J, Goodman Z, Ling MH, and Albrecht J. Is an "a la carte" combination interferon alfa-2b plus ribavirin regimen possible for the first line treatment in patients with chronic hepatitis C? The ALGOVIRC Project Group. *Hepatology* 2000;31:211–218.
6. Liu GT. Pharmacological actions and clinical use of fructus schizandrae. *Chin Med J (Engl)* 1989;102: 740-749.
7. Buzeelli G, Moscarella S, Giusti A, Duchini A, Marena C, and Lampertico M. A pilot study on the liver protective effect of silybin-phosphatidylcholine complex (IdB1016) in chronic active hepatitis. *Int J Clin Pharmacol Ther Toxicol* 1993;31:456-460.
8. Von Herbay A, Stahl W, Niederau C, and Sies H. Vitamin E improves the aminotransferase status of patients suffering from viral hepatitis C: a randomized, double-blind, placebo-controlled study. *Free Radic Res* 1997;27:599-605.
9. Arase Y, Ikeda K, Murashima N, Chayama K, Tsubota A, Koida I, et al. The long term efficacy of glycyrrhizin in chronic hepatitis C patients. *Cancer* 1997; 79: 1494-1500.
10. Bustamante J, Lodge JK, Marcocci L, Tritschler HJ, Packer L, Rihn BH et al. Alpha-lipoic acid in liver metabolism and disease.*Free Radic Biol Med* 1998;24: 1023-1039.
11. Van Rossum TG, Vulto AG, Hop WC, Brouwer JT, Niesters HG, Schalm SW, et al. Intravenous glycyrrhizin for the treatment of chronic hepatitis C: a double-blind, randomized, placebo-controlled phase I/II trial. *J Gastroenterol Hepatol* 1999;14:1093-1099.
12. Sun F, Hayami S, Ogiri Y, Haruna S, Tanaka K, Yamada Y, et al. Evaluation of oxidative stress based on lipid hydroperoxide, vitamin C and vitamin E during apoptosis and necrosis caused by thioacetamide in rat liver. *Biochim Biophys Acta* 2000;1500: 181-185.
13. Liu J, Manheimer E, Tsutani K, Gludd C. Medicinal herbs for hepatitis C virus infection: a Cochrane hepatobiliary systematic review of randomized trials. *Am J Gastroenterol* 2003;98: 538-544.
14. Murakami Y, Nagai A, Kawakami T, Hino K, Kitase A, Hara Y et al. Vitamin E and C supplementation prevents decrease of eicosapentaenoic acid in mononuclear cells in chronic hepatitis C patients during combination therapy of interferon α-2b and ribavirin. *Nutrition* 2006;22:114-122.
15. Gabby E, Zigmond E, Pappo O, Hemed N, Rowe M, Zabrecky G et al. Antioxidant therapy for chronic hepatitis C after failure of interferon: Results of phase randomized, double-blind placebo controlled clinical trial. *World J Gastroenterol* 2007;13(40): 5317-5323.
16. Meydani SN, Meydani M, Blumberg JB, Leka LS, Siber G, Loszewski R, et al. Vitamin E supplementation and in vivo immune response in healthy elderly subjects. A randomized controlled trial. *JAMA* 1997;277: 1380-1386.
17. Look MP, Gerard A, Rao GS, Sundhop T, Fischer HP, Sauerbruch et al. Interferon/antioxidant combination therapy for chronic hepatitis C-a controlled pilot trial. *Antiviral Res* 1999;43: 113-122.
18. Melhem A, Stern M, Shibolet O, Israeli E, Ackerman Z, Pappo O et al. Treatment of chronic hepatitis C virus infection via antioxidants: results of a phase I clinical trial. *J Clin Gastroenterol* 2005;39:737-742.

19. Van Rossum TG, Vul to AG, Hop WC, and Schalm SW. Pharmacokinetics of intravenous glycyrrhizin after single and multiple doses in patients with chronic hepatitis C infection. *World J Gastroenterol* 2007;13(40).

20. Fried MW. Side effects of therapy of hepatitis C and their management. Hepatology 2007;36(5 Suppl 1):S237–44.

21. Soza A, Everhart JE, Ghany MG, Doo E, Heller T, Promrat K, et al. Neutopenia during combination therapy of interferon alpha and ribavirin for chronic hepatitis C. *Hepatology* 2002;36(5):1273-9

22. Sharvadze L, Karchava M, Bolokadze N, Gatserelia L, Tsertsvadze T. Safety and efficacy of systematic administration of Filgrastim to prevent neutropenia and infections with hepatitis C. *Georgian Med News* 2009;175:32–5.

23. Yamane A, Nakamura T, Suzuki H, Ito M, Ohnishi Y, Ikeda Y et al. Interferonalpha2b-induced thrombo-cytopenia is caused by inhibition of platelet production but not proliferation and endomitosis in human megakaryocytes. *Blood* 2008;112:542–50.

25. DeFranceschi L, Fattovich G, Turrini F, Ayi K, Brugnara C, Manzato F, et al. Hemolytic anemia induced by ribavirin therapy in patients with chronic hepatitic C virus infection: role of membrane oxidative damage. *Hepatology* 2000;31:997–1004.

26. Jia L, Chopp M, Zhang L, Lu M, Zhang Z. Erythropoietin in combination of tissue plasminogen activator exacerbates brain hemorrhage when treatment is initiated 6 h after stroke. *Stroke* 2010;41(9):2071–6.

27. Seeff LB, Curto TM, Szabo G, Everson GT, Bonkovsky HL, Dienstag JL et al. Herbal products use by persons enrolled in the hepatitis C antiviral long-term treatment against cirrhosis (HALT-C) trial. *Hepatology* 2008;47(2):605-12.

28. Par A, Roth E, Miseta A, Hegedus G, Par G, Hundyady B et al. Effects of supplementation with the antioxidants flavonoid, silymarin, in chronic hepatitis C patients treated with peg-interferon + ribavirin. A placebo-controlled double blind study. *Orv Hetil* 2009;150(2):73-9.

29. Emerit I, Huang CY, Serejo F, Filipe P, Fernandes A, Costa A, et al. Oxidative stress in chronic hepatitis C: a preliminary study on the protective effects of antioxidant flavonoids. *Hepatogastroentrology* 2005;52(62):530-6.

30. Kalantari H, Shahshahan Z, Hejazi SM, Ghafghazi T, and Sebghatolahi V. Effects of silybum marianum on patients withhepatitis C. *J Res Med Sci* 2011;16(3):287-90.

31. Abe Y, Ueda T, Kato T, and Kohil Y. Effectiveness of interferon, glycyrrhizin combination therapy in patients with chronic hepatitis C. *C Nippon Rinsho* 1994;52:1817-1822.

32. Orient H, Hansen BE, Willems M, Brouwer JT, Huber R, Kullak-Ublick GA et al. Biochemical and histological effects of 26 weeks of glycyrrhizin treatment in chronic hepatitis C: a randomized phase II trial. *J Hepatol* 2006;45(4):539-46.

33. Hino K, Murakami Y, Nagai A, Kitase A, Hara Y, Furutani T et al. Alpha tocopherol [corrected] and ascorbic acid [corrected] attenuates the ribavirin [corrected] induced decrease in ecosapentaenoic acid in erythrocyte membrane in chronic hepatitis C patients. *J Gastroentrol Hepatol* 2006;21(8):1269-75.

34. Rustgi VK, Lee P, Finnegan S, and Ershler W. Safety and efficacy of recombinant IL-11 (oprelvekin) in combination with interferon/ribavirin in hepatitis C patients with thrombocytopenia. *Hepatology* 2002;36(4 Pt 2): 361A.