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ROLE OF OXIDIZED LOW-DENSITY LIPOPROTEIN IN HEPATITIS C RELATED CHRONIC LIVER DISEASE

By
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Abstract

Hepatitis C virus(HCV) lifecycle is closely connected to host cell lipid metabolism, inducing oxidative stress and oxidation reaction induce chemical modification of proteins and lipids in plasma LDL transforming it to abnormal oxidized-LDL (Ox-LDL). Ox-LDL is more stable marker molecule with longer half-life than free radicals. This study assessed its contribution to the pathogenesis of liver diseases, and liver cirrhosis. This work studied serum level of ox-LDL to evaluate its role in chronic hepatitis C patients and its association with liver cirrhosis. Also, the level of serum total antioxidant status was determined to evaluate their role in the treatment. The study included 20 chronic hepatitis C patients, 20 chronic hepatitis C patients with liver cirrhosis and 20 controls (age, sex & anthropometric measures were matched). All patients and controls were males. Serum was used to estimate Ox-LDL total direct bilirubin, albumin, aminotransferases, total antioxidant capacity, Urea and creatinine.

Keywords: Egypt, Patients, HCV, Chronic liver disease, Ox-LDL,TAC.

Introduction

The hepatitis C virus (HCV) lifecycle is closely connected to host cell lipid metabolism, from cell entry, through viral RNA replication to viral particle production and formation/assembly (Pécheur 2012). HCV causes oxidative stress by activation of prooxidant enzymes, and weakening of antioxidant defenses. Oxidative stress and oxidation reaction induce chemical modification of the proteins and lipids in plasma LDL transforming it to the abnormal oxidized-LDL (ox-LDL). Ox-LDL is more stable marker molecule with longer half-life than free radicals. It can be assessed to study its contribution to the pathogenesis of liver diseases, and liver cirrhosis (Schaefer and Chung, 2013)

HCV appears to make use of the host lipid metabolism and one common feature of chronic hepatitis C is the steatosis, characterized by excessive accumulation of triglycerides and lipid content in the liver. HCV induces condition of oxidant / antioxidant disequilibrium where there is overproduction of ROS on one side and a deficiency of enzymatic and non-enzymatic antioxidants on the other side (Liochev, 2013). Oxidation reaction & ROS induces chemical modification of proteins and lipids in plasma LDL transforming it to abnormal oxidized-LDL (ox-LDL). Ox-LDL is not recognized by the liver LDL receptors but is taken up by lectin-like ox-LDL receptor-1 (LOX-1) present in macrophages, natural killer cells, and vascular endothelial cells (Xu *et al*, 2010).

This work aimed is determine serum level of Ox-LDL and evaluate its association with different clinically parameters of liver disease in patients with chronic hepatitis C.

Subjects and Methods

This study included 40 male patients from Al-Hussain and Sayed Galal, Al Azhar University Hospitals with chronic hepatitis C & 20 male controls (age, sex & BMI matched).

The protocol of the work was approved by the Ethical Committee of Al-Azhar University. The protocol of the work was explained to all participants and a written medical consent was signed. Male subjects participating in the procedures of this study were divided into: GA: 20 HCV patient with liver cirrhosis with signs and symptom of cirrhosis diagnosed by Sonography, and gave positive HCV/Ab. GB: 20 HCV patients with neither liver cirrhosis nor signs or symptom of cir-

rhosis, and gave positive HCV/Ab. GC: 20 normal controls with negative HCV/Ab. Age & BMI of all were cross-matched

Exclusion criteria: a- Positive HBV, b- Suffering from any systemic disease like diabetes mellitus, hypertension, cardiovascular system diseases, and renal dysfunction, c- Suffering from autoimmune hepatitis, d- Smokers, alcoholics and drug addicts, and e- Suffering from hepatic cellular carcinoma or other malignancies. Inclusion criteria: HCV hepatitis patients, but schistosomiasis free.

All patients were taking liver support: 1- Glutathione; 50mg daily orally, 2-Silymarin; 420 mg daily orally, c- Zinc (as zinc oxide; 11mg/day oral), d-Vitamin A as retinyl acetate (700mgm orally once daily), vitamin C (0.5gm orally once daily), and vitamin E as α-tocopherol (1mgm/day oral), e- Selenium as sodium selenite,100µg/day oral, & f- Vitamin K (Phytomenadione) 10mg IM./day for cirrhotic patients. Patients started anti-viral therapy after enrollment in the study.

Patients and controls were subjected to: a- Anthropometric body measurements (weig ht, height, BMI, triceps skin fold, mid arm circumference and waist circumference), b- Full history taking, c- Full clinical examination, d- Abdominal Sonography, & e- Blood samples (8ml) 12 hours fasting withdrawn; 2ml with an anticoagulant for prothrombin time; 6ml added to polypropylene to clot for 20min. at 37˚C, centrifuged at 3000xg for 10 min. and serum was used to estimate liver function tests, oxidized low-density lipoprotein and total antioxidant capacity.

The following laboratory tests were done: 1- Urine, stool and serology to exclude schistosomiasis 2-Liver function tests performed on Roche/Hitachi 902 auto analyzer, Roche Diagnostic, Germany. 3- Anti-HCV antibodies in sera ELISA assayed using kit supplied by Biocare Diagnostics. 4- HBsAg in sera was ELISA assayed by kit supplied by Biocare Diagnostics, 5- Blood urea and creatinine performed on Roche/Hitachi 902 auto analyzer (Roche Diagnostic, Germany). 6- Total antioxidative capacity measured colorimetrically using a kit supplied from Biodiagnostic. 7- Ox-LDL measured by WKEA human oxidized LDL ELISA kit (WKEA MED SUPPLIES CORP, NY 10123, USA). The specific murine monoclonal antibody 4E6 was used (Holvoet *et al*, 2001).

Statistical analysis; Data were expressed as Mean± standard deviation. Comparison between groups was done using Student's t test with significance defined as $p \leq 0.05$.

Results

The results are shown in tables (1 to 12) and figures (1, 2, 3, 4, 5 & 6)

Table 1: Age, weight, height, BMI, triceps skin fold, mid arm circumference and waist circumference of HCV cirrhotic tient.

	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	Skin fold (cm)	Mid arm circumference (cm)	Waist circumference (cm)
1	57	45	160	17.5	0.7	17.5	75
2	55	49	174	16.18	0.7	17.5	88
3	54	79	172	26.77	1.1	20.5	111
4	42	54	165	19.83	1.4	21	82
5	65	64	163	24.09	1	18.5	93
6	67	57	170	19.72	1	20	91
7	60	67	168	23.74	1.1	20	114
8	53	65	173	21.72	1.4	23	65
9	70	50	160	19.53	0.7	18	96
10	60	71	177	22.66	0.7	21	107
11	54	84	177	26.81	2.6	27	100
12	65	75	173	25.06	1.5	22	113
13	60	82	170	28.37	1.5	25	117
14	60	86	171	29.41	1.3	23	99
15	58	65	167	23.31	0.9	21	100
16	61	70	168	24.8	1	19	95
17	44	78	165	28.65	2.1	23	115
18	45	77	162	29.34	0.7	22	111
19	62	75	162	28.58	1.9	25	83
20	60	78	165	28.65	1.5	24	99
Mean	57.6	68.55	168.1	24.24	1.24	21.4	97.7
± SD	7.44	12.25	5.3	4.13	0.52	2.65	14.22

Table 2: Age, weight, height, BMI, triceps skin fold, mid arm and waist circumference of HCV non-cirrhotic patient

	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	Skin fold (cm)	Mid arm circum-ference (cm)	Waist circum-ference (cm)
21	36	65	167	23.31	2.2	26	92
22	54	89	170	30.79	2	25	95
23	35	60	161	23.15	1.5	23	81
24	38	57	162	21.72	1.8	21	85
25	55	85	167	29.421	2.6	27	98
26	46	80	175	26.12	1.5	25	97
27	27	75	168	26.57	1.7	24	84
28	35	72	169	25.21	1.8	22.5	86
29	43	83	170	28.72	1.9	23	90
30	50	79	173	26.4	1.5	22	84
31	41	80	168	28.34	2.1	23	90
32	54	83	169	29.06	2.2	25	93
33	31	71	170	24.57	1.6	22	78
34	38	72	167	25.82	1.7	21	84
35	48	65	162	24.77	1.8	20	80
36	32	72	165	26.45	1.9	21	86
37	55	83	169	29.6	2.5	24	93
38	36	60	164	22.31	1.6	21	80
39	40	75	168	26.57	2	22	86
40	37	65	168	23.03	1.7	21	88
Mean	41.55	73.55	167.6	26.1	1.88	22.92	87.5
± SD	8.64	9.26	3.52	2.63	0.32	1.94	5.83

Table 3: Age, weight, height, BMI, triceps skin fold, mid arm circumference and waist circumference of control group.

	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	Skin fold (cm)	Mid arm circumfer-ence (cm)	Waist circumference (cm)
41	49	84	170	29.07	2.3	25	86
42	55	90	176	29.05	2.1	23	88
43	63	79	172	26.77	1.8	20.5	85
44	38	82	169	28.71	2	24	89
45	42	70	173	23.39	1.7	21	78
46	37	72	168	25.51	1.9	21	79
47	60	67	167	24.02	1.8	20	75
48	53	87	173	29.07	2.5	23	89
49	48	82	175	26.78	2	24	79
50	37	75	177	23.94	1.6	22	74
51	54	84	176	27.12	2.1	24.5	87
52	62	78	173	26.06	2	22	79
53	45	82	170	28.37	2.4	24	84
54	56	86	170	29.76	2.1	26	90
55	47	68	166	24.68	1.9	22	80
56	62	74	168	26.22	2.2	24	86
57	47	79	165	29.02	2.6	23	91
58	49	82	169	28.77	1.9	24	81
59	51	75	171	25.68	2	22	74
60	43	68	165	24.98	1.8	21	76
Mean	49.9	78.2	170.65	26.85	2.04	22.8	82.5
± SD	8.23	6.81	3.66	2.03	0.26	1.63	5.64

Table 4: t-test and p- values of serum albumin (g/dl) in different groups.

	Control	HCV cirrhotic patients	HCV without cirrhosis
Mean	3.97	2.53	3.84
±SD	0.23	0.50	0.26
Control	t-test P-value	2.5 0.02	0.099 >0.2

Table 5: t-test and p- values of INR in different groups.

	Control	HCV cirrhotic patients	HCV without cirrhosis
Mean	1.04	1.43	1.05
±SD	0.06	0.38	0.06
between patients and control group	t-test P-value	5.46787 0.001	0.0602 >0.20

Table 6: t-test and p- values of serum AST (U/L) in different groups in u/dl

	Control	HCV cirrhotic patients	HCV without cirrhosis
Mean	24.65	71.7	36
±SD	4.97	34.38	9.56
between patients and control group	t-test P-value	6.67 <0.001	5.83 <0.001

Table 7: t-test and p- values of serum ALT (U/L) in different groups

	Control	HCV cirrhotic patients	HCV without cirrhosis
Mean	22.7	55.4	39.55
±SD	3.44	27.54	11.44
between patients and control group	t-test P-value	3.96 <0.001	2.2 <0.05

Table 8: TAC in serum (mM/L)

	Control	Cirrhotic	Noncirrhotic
1	1.85	0.52	1.82
2	1.35	0.91	2.64
3	1.67	1.04	2.33
4	1.46	0.63	1.97
5	1.24	0.62	1.64
6	1.19	0.63	1.81
7	1.52	0.59	1.93
8	2.04	1.18	2.51
9	1.92	1.13	1.94
10	1.87	0.43	1.95
11	1.51	0.29	1.96
12	1.43	0.56	2.66
13	2.1	0.83	2.24
14	1.42	0.74	1.79
15	1.26	0.83	2.34
16	1.62	0.93	2.18
17	1.53	0.87	1.44
18	1.32	1.1	1.91
19	1.27	0.66	2.46
20	1.24	0.82	1.48
Mean	1.54	0.77	2.05
±SD	0.28	0.24	0.36

Table 9: t-test and p- values of serum TAC (mM/L) in different groups

	Control	HCV cirrhotic patients	HCV without cirrhosis
Mean	1.54	0.77▼	2.05▲
±SD	0.28	0.24	0.36
between patients and control group	t-test P-value	2.72 <0.02	1.48 <0.2

Table 10: t-test and p- values of Oxidized LDL (µg/L) in different groups

	Control	HCV cirrhotic patients	HCV without cirrhosis
Mean	51.4	77.49	71.24
SD	6.68	7.26	5.63
between patients and control group	t-test P-value	6.45 <0.001	3.95 <0.001

Table 11: Serum levels of ox-LDL (µg/L).

	Control	Cirrhotic	Noncirrhotic
1	53.3	76.4	80
2	61.5	80.9	75
3	58.6	88.7	65.5
4	57.4	68.7	69.2
5	62.3	81.3	70.3
6	71.3	68.5	81.1
7	59.4	77.2	65.4
8	63.6	72.7	69.1
9	57.8	69.5	71.2
10	48.4	82.4	64.2
11	61.5	70.6	76.9
12	55.4	75.7	63.1
13	48.8	90.2	68
14	60.0	71.6	74.4
15	46.3	78	73.3
16	59.4	87.2	79.1
17	63.1	73	62.8
18	70.1	68.6	76
19	48.4	79.5	71.9
20	52.9	89.1	68.2
Mean	51.4	77.49	71.235
±SD	6.68	7.26	5.63

Table 12: Serum levels of aminotransferases, total bilirubin, indirect bilirubin, albumin, TAC, ox-LDL, urea, creatinine and INR in groups.

Variable	GA (n=20)	GB (n=20)	GC (n=20)
Albumin (g/dL)	*2.53±0.49	3.84±0.26	3.98±0.25
Total bilirubin (mg/dL)	1.78±1.06	0.83±0.09	0.77±0.09
Unconjugated bilirubin (mg/dL)	1.1±0.43	0.72±0.10	0.7±0.09
ALT (U/L)	55.4±27.54▲▲	39.55±11.44▲	22.7±3.44
AST (U/L)	71±34.4▲▲	36±9.6▲▲	24.65±4.97
INR	1.42±.38▲▲	1.05±0.06	1.04±0.06
Urea(mg/dl)	52.55±38.01	30.4±5.32	26.65±6.66
Creatinine(mg/dl)	0.995±0.45	0.89±0.15	0.87±0.18
TAC(mM/L)	0.77±0.24*	2.05±0.36▲	1.54±0.28
OxLDL(µg/L)	77.49±7.26 ▲▲	71.24±5.63 ▲▲	51.4±6.68

* Significant compared to control, ▲ Significant high levels compared to control, ▲▲ Highly significant compared to control.

Discussion

In the present study, the mean age, height and weight were49.9± 8.23 years, 170.65 ± 3.66cm and 78.2±6.81 kg, respectively. The mean BMI of control group was 26.85±2.03. The mean triceps skin fold, mid arm circumference and waist circumference were 2.04± 0.26 cm, 22.8±1.63 cm and 82.5 cm, respectively. The HCV patients showed mean age of cirrhotic patients was 57.6±7.44 years while the mean age of HCV patients without cirrhosis was 41.55±8.64 years. The mean height of the HCV cirrhotic patients was 168.1±5.3 cm, while the mean height of HCV patients without cirrhosis was 167.6 ± 3.5 cm. The mean weight of HCV cirrhotic patients was 68.55±12.25 kg, while the mean weight of HCV patients without cirrhosis was 73.55±9.26. The mean BMI of HCV cirrhotic patients was 24.24±4.13, while the mean BMI of HCV patients without cirrhosis were 26.1± 2.63.

Also, the mean triceps skin fold of HCV cirrhotic patients was 1.24±0.52 cm, while the mean skin fold of HCV patients without cirrhosis was 1.88± 0.32 cm**.** The mean mid arm circumference of HCV cirrhotic patients was 21.4±2.65 cm while the mean mid arm circumference of HCV patients without cirrhosis was 22.92 ± 1.94 cm. The mean waist circumference of HCV cirrhotic patients was 97.7±14.22 cm while the mean waist circumference of HCV patients without cirrhosis was 87.5±5.83 cm

In the present study, the mean serum albumin level in control group was 3.97±0.23 g/dl, while in HCV cirrhotic patients were 2.53±0.5 g/dl. Serum level of albumin was significantly lower in HCV cirrhotic patients than those of other groups. The mean serum

albumin level in HCV non-cirrhotic patients was 3.84±0.26 g/dl

In the present study, the international normalized ratio (INR) was a calculation based on results of a prothrombin time. The INR is the ratio of the sample's prothrombin time, to the prothrombin time of a normal sample of blood measured at the same time. The mean levels of INR values in control group, the HCV cirrhotic patients and HCV non-cirrhotic patients were 1.04, 1.43 and 1.05 respectively. INR was significantly higher in HCV patients with cirrhosis than those of the control group.

In the present study, the mean levels of total serum bilirubin in control group, HCV cirrhotic patients and HCV non cirrhotic patients were 0.77±0.09mg/dl, 1.78±1.06mg/dl and 0.83±0.09mg/dl**,** respectively**.** The mean levels of serum indirect bilirubin in controls, the HCV cirrhotic patients and HCV non-cirrhotic ones were 0.70±0.09, 1.1±0.43 and 0.72 ±0.1mg/dl respectively. Serum levels of tot-al bilirubin & indirect bilirubin did not show any significant differences among the patients and controls groups

In the present study, the mean levels of serum urea in control group, HCV cirrhotic patients and HCV non-cirrhotic patients were 26.65±6.66 mg/dl, 52.55±38.11 mg/dl and 30.4±5.32 mg/dl**,** respectively

In the present study, the mean levels of serum creatinine in controls, HCV cirrhotic patients and HCV non-cirrhotic ones were 0.86±0.18mg/dl, 0.99±0.45mg/dl & 0.89± 0.15mg/dl**,** respectively**.** Serum levels of urea and creatinine did not show any significant differences between all groups studied.

In the present study, the mean levels of serum AST in control group, HCV cirrhotic patients and HCV non cirrhotic patients were 24.65 ±4.97 U/L, 71.7±34.38 U/L and 36±9.56 U/L, respectively. The mean levels of serum ALT in control group, HCV cirrhotic patients and HCV non cirrhotic patients were 22.7±3.44 U/dl, 55.4±27.54 U/L & 39.55±11.44 U/L, respectively.

In the present study, the serum levels of ALT and AST were significantly higher in HCV patients than those of the control group. The mean levels of TAC in control group, HCV cirrhotic patients and HCV non-cirrhotic patients were 1.54, 0.77 and 2.05 mM/L, respectively. TAC was significantly lower in HCV patients with cirrhosis (▼) than those of the control group. TAC was significantly higher in HCV (▲) non-cirrhotic patients.

In the present study as to Ox-LDL the mean levels of serum in control group, HCV cirrhotic patients and in noncirrhotic patients were 51.4±6.68 µg/L, 77.49±7.26 µg/L and 71.24±5.63µg/L respectively. Serum levels of ox-LDL were significantly high in non-cirrhotic patients, and in cirrhotic patients as compared to control group. Antioxidants supplementations did not affect the levels of ox-LDL in all HCV hepatitis patients.

Generally speaking, body measurement data (Anthropometric data) in adults are used to evaluate health and dietary status, disease risk, and body composition changes that occur over the adult lifespan. In this study the anthropometric data of all subjects were similar to the age group of American women (Fryar *et al,* 2010). Anthropometric data of the patients were matching with the control group. Obese persons were excluded from the study. Obesity is linked with a state of increased oxidative stress. Obesity is a principal causative factor in the development of metabolic syndrome. The metabolic syndrome was a common and complex disorder combining obesity, dyslipidemia, and hypertension and insulin resistance. It is associated with a high cardiovascular risk that could only partially be explained by its components. There was evidence that low-grade inflammation and high oxidative stress add to this risk. Oxidized LDL, a marker of lipoprotein-associated oxidative stress, was an emerging cardiovascular risk factor. Therefore, changes in serum levels of ox-LDL in this study were not due increased body weight (Tumova *et al,* 2013).

No doubt, the oxidative stress may play a role in persistence of liver damage. Values of alanine aminotransferase and aspartate aminotransferase positively correlate with various serum markers of oxidative stress. Moreover, an ALT increase in patients who previously had persistently normal levels of transaminases was preceded by a burst in oxidative stress markers (Li *et al,* 2015).

In the present study, the serum levels of TAC were significantly lower in HCV patients with cirrhosis (0.77 mM/L) than those of the control group (1.54mM/L). Serum levels of TAC were significantly higher in HCV non-cirrhotic patients (2.05mM/L). TAC assay (used in this study) measured a complex of non-enzymatic antioxidants present in blood, which include exogenous antioxidants such as ascorbic acid, α tocopherol, β carotene and polyphenols. It also measures the endogenous antioxidants such as reduced glutathione, uric acid, and bilirubin

Generally, the antioxidants work in a network to exert protective effects, no single antioxidant could represent overall antioxidant status *in vivo*. For example, GSH regenerates ascorbic acid, which then regenerates α-tocopherol from their radical forms. Plasma antioxidant status is the result of interaction and cooperation of various antioxidants. The concept of TAC considers the synergistic role of those antioxidants rather than the simple sum of individual antioxidants (Wang *et al*, 2013).

Protection against ROS mediated environmental pollutants can generally occur at two levels: (I) physiochemical protection to lower the dose of exposure, which typically cannot be accomplished by individuals living in polluted areas, or (II) physiological protection to increase the antioxidative defense of the organism. Low molecular-weight antioxidants are involved in the prevention of or the decrease in the damage caused by certain polyphenol antioxidant is known to be rapidly absorbed. Silymarin is widely used in the management of chronic liver diseases and cirrhosis as hepatoprotective agent (Zhou *et al,* 2014). Silymarin displays anti-inflam-matory effects on T lymphocytes in vitro. Yet, high-dose oral silymarin exerts modest nonspecific immunomodulatory effects in vivo. Ascorbic acid is superior to silymarin in inhibiting intracellular ROS generation (Adeyemo *et al,* 2013).

The significant low levels of TAC in patients with liver cirrhosis may be either due to decrease in the level of plasma albumin or in the synthesis of glutathione and other endogenous antioxidants. In addition, it may be due to increased oxidative stress and decreased capacity of the body to scavenge free radicals. Supplementation of exogenous antioxidants did not increase antioxidant status in cirrhotic patients (Péter *et al,* 2015).

Serum levels of ox-LDL were significantly high in non-cirrhotic patients (71.24 µg/L), and in cirrhotic patients (77.49 µg/L) as compared to control group (51.4µg/L). Antioxidants supplementations did not affect the levels of ox-LDL in all HCV hepatitis patients. Ox-LDL was used as a marker of oxidative stress in this study. Ox-LDL is a stable marker molecule with longer half-live than free radicals. Ox-LDL can potentially contribute to the pathogenesis of liver diseases, kidney diseases, uremia, cardiovascular disease, and inflammation (Russ *et al.,* 2015).

LDL lipoperoxidation leads to modifications in apolipoprotein B-100 (apo B-100) and lipids. Ganini and Mason (2014) reported the lack of protection of α-tocopherol on the Apo B-100 and lipid free radical formation by LPOx. This explained the failure of vitamin E as a cardiovascular protective agent for humans. In addition, it may explain the high levels of ox-LDL in hepatitis patients although they are on vitamin E as a supplement. Vitamin E works as a free radical scavenger, protecting polyunsaturated fatty acids (PUFA), a major structural component of the cell membranes, from peroxidation (Traber, 2000). Woestenenk *et al.*

(2015) stated that Serum vitamin E levels are strongly influenced by concentration of serum lipids, and do not accurately reflect tissue vitamin levels. Therefore, effective vitamin E levels are calculated as the following ratio: Effective serum vitamin E level= Alpha-tocopherol / (cholesterol + triglycerides), and a normal ratio is >0.8 mg alpha-tocopherol/gram total lipids. Besides, Vitamin A is a subclass of a family of lipid-soluble compounds referred to as retinoic acids. These consist of four isoprenoid units joined in a head to tail fashion (Ross, 2000). Graham-Maar, *et al*. (2006) stated that individuals with chronic liver disease were typically treated with a standard multivitamin which includes vitamin A. If vitamin A deficiency is detected in these patients, standard replacement doses must be given. High levels of alcohol consumption appeared to potentiate the hepatotoxic effects of vitamin A. El-Tawdy *et al*. (2017) reported that vitamin D potentially regulates many cellular functions. Its deficiency was implicated as a risk factor for many diseases. However, a causal association between poor vitamin D status and nearly all major diseases (infections, autoimmune disorders, cardiovascular and metabolic diseases) was not yet established.

The significant high levels of ox-LDL reported in this study, may be a factor in the pathogenesis HCV hepatitis and HCV liver cirrhosis. Ox-LDL in serum of HCV patients may be a result of HCV induced oxidative stress in infected cells or it may be due to decreased endogenous production of antioxidants. The mechanisms of HCV oxidative stress induction include; alteration of functioning of the respiratory chain complex I, and induction of NADPH oxidases 1 and 4. NADPH oxidases contribute to production of H_2O_2 and O_2^-. H_2O_2 induces phosphorylation of eIF2α resulting in inhibition of global (including that of the viral) protein synthesis and constitutes an important defense against virus infection (Lozano-Sepulveda *et al*, 2015).

ROS induced by the HCV inhibit virus replication without affecting the stability of its RNA genome. ROS can induce viral genome heterogeneity, which facilitates viral escape during treatment and probably escape from the immune system. ROS induce fixed chemical modification of the proteins and lipids in plasma LDL transforming it to the abnormal ox-LDL. Oxidative changes in amino acids as well as proteolysis and cross-links of apoprotein B (apo B) occur that result in extensive alteration in the protein composition and structure. A significantly high level of carbonylated proteins in HCV cirrhotic patients was reported and These protein oxidations should be treated before they are initiated at the stage of HCV hepatitis (Abou-El-Makarem *et al*, 2014).

Oxidative cell damage plays an important role in HCV physiopathology. Oxidative stress is triggered when the concentration of oxygen species in the extracellular or intracellular environment exceeds antioxidant defenses. Cells are protected and modulate oxidative stress through the interplay of intracellular antioxidant agents, mainly glutathione system (GSH) and thioredoxin; and antioxidant enzyme systems such as superoxide dismutase, catalase, GSH peroxidase, and heme oxygenase-1. Also, the use of natural and synthetic antioxidants (vitamin C & E, N-acetylcysteine, glycyrrhizin, polyenylphosphatidyl choline, mitoquinone, quercetin, S-adenosylmethionine and silymarin) has already shown promising results as co-adjuvants in HCV therapy (Lozano-Sepulveda *et al*, 2015).

The Ox-LDL is not always harmful. Late-outgrowth of the endothelial progenitor cells (EPCs) are stress-resistant and responsible for reparative functions in the cardiovascular system. Ox-LDL has concentration-dependent bi-phasic effects on late-outgrowth EPC tube formation in vitro and in vivo. Serum ox-LDL in low concentration enhanced neovasculogenic EPCs function. Whether it has similar effects on hepatocytes and kupher cells needs further studies (Watt *et al*, 2016).

No doubt, the liver fibrosis, in particular, is

the major pathogenic consequence of HCV infection. Liver fibrosis is a hepatic dysregulation induced by oxidative stress in the HCV-infected liver. It was shown that increase in oxidative stress markers such as malonaldehyde and Trx in serum or 8-isoprostane in urine of HCV chronic hepatitis patients correlated with fibrosis score. ROS levels, measured in liver using Electron Paramagnetic Resonance Imaging correlated with histologic disease activity, without sera transaminases levels (Ivanov *et al.* 2013).

In this study the significant high levels of ox-LDL in HCV cirrhotic patients is another confirmation that ROS plays an important role in liver cirrhosis. Treatment with antioxidants in use does not alter the liver cirrhosis. Ox-LDL is a permanent modification of lipids and proteins in LDL. Permanent modification of serum proteins and high levels of carbonylated proteins were reported (Di Rosa *et al*, 2009). HCV infection induces an immune response characteristically mediated by Th1-cells. These lymphocytes secrete γ-interferon as the predominant cytokine, which is able to enhance the production of TNF-α by macrophages. At the same time, HCV directly causes liver fat deposition. All these events could be potential risk factors for liver fibrosis. Ox-LDL activates the NF-kB complex, responsible for the regulation of genes involved in inflammation and cell survival (Scazzocchio *et al*, 2009).

Conclusion

Serum levels of Ox-LDL were significantly high in non-cirrhotic patients and in cirrhotic patients as compared to controls. Antioxidants supplementations did not affect the levels of Ox-LDL in all HCV hepatitis patients. TAC (total antioxidant capacity) was significantly low in the HCV patients with the cirrhosis than in controls

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Explanation of figures

GA: (HCV patients with liver cirrhosis), GB: (HCV patients without liver cirrhosis), Group C: (Control group).
Figure 1: Mean of serum albumin (g/dl) in all studied groups
Figure 2: Mean of serum AST (U/L) in all studied groups
Figure 3: Mean of serum ALT (U/L) in all groups
Figure 4: Mean of INR values in all groups.
Figure 5: Mean of serum TAC (mM/L) in all groups
Figure 6: Mean of serum Oxidized LDL (µg/L) in all groups