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Do oxidized low-density lipoproteins link to extra hepatic manifestations in chronic, non-cirrhotic HCV patients?

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ABSTRACT

Background: Tissue damage by viral hepatitis is a major cause of morbidity and mortality worldwide. Oxidation reactions and reactive oxygen species (ROS) transform proteins and lipids in plasma low-density lipoproteins (LDL) into the abnormal oxidized LDL (ox-LDL). Hepatitis C virus (HCV) infection induces oxidative/nitrosative stress from multiple sources, including the inducible nitric oxide synthase (iNOS), the mitochondrial electron transport chain, hepatocyte NAD(P)H oxidases (NOX enzymes), and inflammation. Further, HCV decreases reduced glutathione (GSH) synthesis and regeneration.
Design: Cross-section.
Objective: to quantify ox-LDL in serum of chronic non-cirrhotic HCV patients, and to assess ox-LDL association with HCV-induced extra hepatic manifestations.
Patients and methods: Twenty chronic, non-cirrhotic HCV female patients with extra hepatic manifestations, twenty chronic, non-cirrhotic female HCV patients without extra hepatic manifestations and twenty healthy age, sex matched controls.
Methods: Serum was used for determination of liver function tests, ox-LDL and the extracellular antioxidant enzyme Superoxide Dismutase EC CuZn-SOD.
Results: Patients with extra hepatic manifestations had statistically higher ox-LDL (76.63 ± 6.86 μg/L) than patients without extra hepatic manifestations (63.05 ± 6.6 μg/L) $p < 0.001$, and both patient groups had higher ox-LDL levels than the control group (44.1 ± 4.1 μg/L) $p < 0.001$. EC CuZn-SOD correlated negatively with ox-LDL in HCV patients with extra hepatic manifestation only.
Conclusion: Extra hepatic manifestations were not risk for anthropometric changes seen with HCV infection. Extra hepatic manifestations were associated with high serum ox-LDL. High serum levels of ox-LDL associated with- or were due to deregulated expression of serum EC CuZn-SOD in chronic HCV patients.

List of Abbreviations

ALT	Alaminne transaminase
AST	Aspartate transaminase
EC CuZn-SOD	Extracellular superoxide dismutase-containing Cu and Zn
DAAs	Direct acting antivirals
RT-qPCR	Real-time quantitative polymerase chain reaction
BMI	Body Mass Index
PT	Prothrombin time

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DB	Direct bilirubin
TB	Total bilirubin
ELISA	Enzyme – linked immunosorbent assay
GSH	Glutathione (reduced form)
H_2O_2	Hydrogen peroxide
$O_2^{\bullet-}$	Superoxide radical
O_2	Molecular Oxygen
HCC	Hepatocellular carcinoma

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NO	Nitric oxide
HCV	Hepatitis C Virus
TMB	Tetramethylbenzidine
Alb	Albumin
IFNα	Interferon alpha
IFNγ	Interferon gamma
IL-18	Interleukin −18
IL-6	Interleukin – 6
INR	International normalized ratio
LDL	Low density lipoprotein
LOX-1	Lectin-like ox-LDL receptor-1
NADPH	Nicotinamide adenine dinucleotide phosphate reduced form
SPSS	Statistical package for social sciences
SD	Standard deviation
ANOVA	Analysis of variance
iNOS	Inducible nitric oxide synthase
NOX	NADPH oxidase
OD	Optical density
Ox-LDL	Oxidized low density lipoprotein
ROS	Reactive oxygen species
SOD	Superoxide dismutase enzyme
TNF-α	Tumor necrosis factor - alpha
NK	Natural Killer cells
IgG	Immunoglobulin G
IgM	Immunoglobulin M
cxcl 10	C-X-C motif ligand 10 gene

1. Introduction

Chronic HCV infection has been a major global health issue [1]. Globally, the peak annual number of patients with compensated cirrhosis (n = 36210), decompensated cirrhosis (n = 3380), hepatocellular carcinoma (HCC), (n = 2220) and liver-related deaths (n = 1880) are expected to occur between 2031 and 2035 [2]. Before direct acting antivirals (DDAs) have become available, Egypt has had the highest prevalence of HCV in the world, estimated about 14.7 % [3]. Even-though with the DDAs been available, HCV complications such as liver cirrhosis and HCC are still sources of considerable morbidity/mortality among millions of Egyptians with chronic HCV history [4]. Extra hepatic organs which may be affected by chronic HCV infection include muscles, bones, joints, neural, gastrointestinal and the skin. HCV promotes atherogenesis and its complications through several direct and indirect mechanisms [5], particularly in the aged people [6]. Frequently associated dermatological manifestations are mixed cryoglobulinemia, lichen planus and porphyria cutanea tarda. Thyroid dysfunction has been reported during HCV hepatitis [7,8].

HCV induces oxidative stress by a variety of processes [9]. The metalloenzymes SODs catalyze the dismutation of the toxic superoxide radical ($O_2^{\bullet-}$) into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) [10,11]. Three SOD isoenzymes are expressed in humans. The intracellular SOD1 binds Cu and Zn ions (CuZn-SOD) primarily in the cytoplasm, though small amounts have been identified in the mitochondrial intermembrane space [12]. The extracellular SOD3 (EC CuZn-SOD) is secreted into plasma, lymph, synovial fluid and is highly expressed in specific tissues including blood vessels, heart, lungs, kidney and placenta to dismutate the $O_2^{\bullet-}$ produced in the extracellular space and to counteract the inflammatory response mounted by activated macrophages [13,14]. EC CuZn-SOD is induced in a nitric oxide (NO)-dependent manner in skeletal muscles, and is highly induced by exercise training [15].

Versatile proteomic and metabolomic studies demonstrated the role of LDL receptor in mitigating HCV infection and tackled the host factors involved in lipid metabolism and HCV replication, assembly, and production. HCV makes use of the host lipid metabolism, in particular, lipoproteins, a common feature of steatosis. Specific infectivity and fusion of low-density particles are greater than those of high-density particles, which renders lipids-viral lipoproteins interaction axial processes in HCV life cycle [16]. Viral invasion has been thought of as key target for antiviral strategies [17]. Serum ox-LDL has been reported as an inhibitor of HCV cell entry [18]. This study aims to determine ox-LDL in serum of chronic, non-cirrhotic HCV patients and to assess the association of ox-LDL with HCV-induced extra hepatic manifestations.

2. Patients and Methods

2.1. Patients

This cross sectional study was approved by the Ethical Committee of Al-Azhar University, Cairo, Egypt. All patients were referred from the outpatient clinics of Damietta Tropical Hospital, Al-Azhar University, Damietta, Egypt as potential candidates for DAAs therapy. All patients had positive HCV RNA confirmed by Real-Time quantitative Polymerase Chain Reaction (RT-qPCR). The study included 40 female non-cirrhotic patients with chronic hepatitis C, and 20 healthy controls (age, sex-matched with the experimental groups.All subjectss had BMI< 30 kg/m^2. Patients were divided into twenty chronic HCV patients with extra hepatic manifestations; twenty chronic HCV patients without extra hepatic manifestations. The study protocol was explained to all participants before enrollment. A written medical consent was signed by all participants including the attending physician. All subjects underwent physical examination, abdominal sonography and history intake. All patients had daily oral routine liver supporter containing: 50 mg GSH; 420 mg silymarin; 11 mg Zn as Zn oxide; 700 μg vitamin A as retinyl acetate; 0.5 g vitamin C; and 15 μg vitamin E as α-tocopherol; 100 μg selenium as sodium selenate; 5 mg vitamin K as phytonadione. The DAAs therapy was initiated after enrollment in the study. Patients who were suffering from extra hepatic manifestations received treatments according to the manifestations of each case.

2.2. Inclusion criteria

Female patients with chronic HCV without cirrhosis were included in this study.

2.3. Exclusion criteria

Pregnant women, subjects with positive Hepatitis B virus (HBV), autoimmune hepatitis, type 1 and 2 diabetes mellitus, cigarette smokers, subjects diagnosed with alcoholic HCV, subjects who consume >20 g alcohol/day [19], and drug addicts. Subjects suffering from HCC or any other malignancies. Subjects with obesity (BMI >30 kg/m^2).

3. Methods

3.1. Body Mass Index

BMI was calculated as weight/height^2 (kg/m^2) for all subjects.

3.2. Blood samples

Determination of prothrombin time (PT) was done using plasma from 12 h fasting subjects after Horsti (2002) [20]. The International Normalized Ratio (INR) is a ratio of patient's PT to PT of a normal sample measured simultaneously. Serum was used for determination of liver function tests i.e., Total bilirubin, Direct bilirubin (TB and DB) and Indirect bilirubin after Wahlefeld et al. (1972) [21]; plasma Albumin (Alb) after Doumas et al. (1971) [22]; Alanine transaminase (ALT) and Aspartate transaminase (AST) after Bergmeyer et al. (1986) [23]. Additionally, serum was used for identifying patients positive for HCV antibodies and quantifying their HCV RNA titer by RT-qPCR using kit from Qiagen by Applied Biosystem 7500, USA. As well, serum was used to determine levels of ox-LDL and EC-CuZn-SOD. All blood chemistry protocols are described in details in supplementary data.

3.3. EC CuZn-SOD

EC CuZn-SOD levels in serum were determined using kit supplied from Biodiagnostic Research Agents, Cairo, Egypt. The assay is based on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye. Change in absorbance was measured at wavelength 560 nm after 5 min for control and samples. Purified EC CuZn-SOD was shown to inhibit the initial rate of photo activated phenazine methosulphate-mediated reduction of O_2, then reduced nitroblue tetrazolium. 1.5 U/assay of the purified enzyme produced 80 % inhibition. SOD activity U/ml = % inhibition × 3.75.

3.4. Ox-LDL by competitive enzyme-linked immunosorbent assay (ELISA)

Ox-LDL in serum was quantitated by competitive ELISA technique, using WKEA human's ox-LDL kit supplied from WKEA MED SUPPLIES CORP, NY, USA. Microwell plates are coated with anti-human ox-LDL antibodies. Ox-LDL competitive ELISA is based on the monoclonal antibody specific to human's ox-LDL. Ox-LDL in the sample competes with a fixed amount of ox-LDL bound to the microtiter well for binding to biotin-labeled specific antibodies. Washing removes unreactive sample components, followed by biotin-labeled antibody binding to the well, which is then detected by Horse Radish protein -conjugated streptavidin. After a second incubation and an additional washing step, the bound conjugate is detected by reaction with 3,3′,5,5′-Tetramethylbenzidine (TMB). The reaction is stopped by adding acid to yield a colorimetric endpoint that is read spectrophotometrically [24]. The optical density (OD) of all wells was read within 15 min by a microplate reader (Tecan) at 450 nm. Samples, standard curve preparations and absorbances are described in details in supplementary data.

3.5. Statistical analysis

Statistical package for social sciences (SPSS) version 23.0 for Windows 8 was used for data analysis. Quantitative variables were demonstrated as mean ± standard deviation (SD), whereas qualitative variables as frequency and percentage (%). A two tailed Student's *t-test* was used to compare quantitative variables among different groups. Simple correlation between EC CuZn-SOD (U/ml) and ox-LDL (μg/L) in serum was demonstrated using Pearson's correlation. ANOVA was used to analyze variance within and among the studied groups. Significant *p* values were <0.05.

4. Results

4.1. Extra hepatic manifestations in HCV patients

Eight patients presented with lichen planus; two patients with psoriasis; two patients with hypothyroidism; eight patients with rheumatoid arthritis.

4.2. Clinical and anthropometric characteristics of patients

The anthropometric measures were similar among all groups. The control group had higher BMI than both patient groups, although such differences were insignificant. Table 1 and Supplementary Tables 1–3.

4.3. Liver functions

The control group had significantly higher plasma Alb (4.32 ± 0.46) g/dl than both patient groups (3.79 ± 0.26 (Group 1), 4.01 ± 0.44 (Group 2), $p < 0.001$. Patient groups had comparable plasma Alb, AST and ALT values, plasma PT presented as an INR values, serum TB, DB and Indirect bilirubin Table 2 and Supplementary Tables 4–6. Patients with extra hepatic manifestations had significantly higher AST levels than the control group (89.8 ± 14.7, 25.2 ± 5.09) $p < 0.001$ respectively. Both patient groups had significantly higher ALT values (80.5 ± 14.95 Group 1, $p < 0.001$; 84.8 ± 8.3 Group 2, $p < 0.001$) than the control group (23.7 ± 4.53), Table 2 and Supplementary Tables 4–6. Serum values of ALT and AST in patients correlated with markers of oxidative stress, as shown in Table 3.

Table (1)
Anthropometric measurements of the studied subjects.

Variable	Control group (n = 20)	HCV hepatitis with extra hepatic (n = 20)	*p*	HCV hepatitis without extra hepatic (n = 20)	*p*
	Mean ± SD	Mean ± SD		Mean ± SD	
Age (y)	55.5 ± 5.1	52.8 ± 6.39	>0.05	51.7 ± 8.07	>0.05
Height (cm)	177.85 ± 6.71	172.9 ± 8.25	>0.05	172.7 ± 6.61 cm	>0.05
Weight (kg)	88.75 ± 7.53	79.35 ± 9.64	>0.05	82.4 ± 6.01	>0.05
BMI weight/ height^2 (kg/m^2)	28.01 ± 1.83	26.50 ± 2.18	>0.05	27.65 ± 1.89	>0.05

Significant when $p < 0.05$.

Table 2
Biochemical parameters of the studied groups.

Variable	Control group (n = 20)	HCV hepatitis with extra hepatic (n = 20)	*p*	HCV hepatitis without extra hepatic (n = 20)	*p*
	Mean ± SD	Mean ± SD		Mean ± SD	
Alb (g/dL)	4.3 ± 0.46	3.8 ± 0.26	**	4.01 ± 0.44	*
TB (mg/dL)	0.74 ± 0.15	0.85 ± 0.16	▲	0.80 ± 0.11	
Indirect bilirubin (mg/dL)	0.5 ± 0.15	0.59 ± 0.16	▲	0.55 ± 0.12	
INR	1.04 ± 0.05	1.07 ± 0.07		1.03 ± 0.051	
ALT (U/L)	23.7 ± 4.53	80.5 ± 14.95	▲▲	84.8 ± 8.3	▲▲
AST (U/L)	25.2 ± 5.09	89.8 ± 14.7	▲▲	103.95±	▲▲
AST/ALT	1.1 ± 0.30	1.14 ± 0.19		1.23 ± 0.12	
EC CuZn-SOD (U/ml)	14.1 ± 4.1	8.6 ± 1.1	**	10.33 ± 1.6	**
Ox-LDL (μg/L)	44.1 ± 4.1	76.63 ± 6.86	▲▲	63.05 ± 6.6	▲▲
RT-qPCR (IU/ml)	-ve	684367.4 ± 351581.9		114675 ± 122146	

▲Significant difference from the control, when $p<$ (0.05); ▲▲Significant difference from the control when p (<0.001).
*Significant difference from the control when $p<$ (0.05). **Significant difference from the control when p (<0.001).

4.4. EC CuZn-SOD

Patients of either groupshad significantly lower serum EC CuZn-SOD levels(8.6 ± 1.1 Group 1), (10.33 ± 1.6 Group 2)than the control (14.1 ± 4.1), $p < 0.001$ respectively. Table 2 and Supplementary Table 7.

4.5. Serum ox-LDL

Serum levels of ox-LDL were significantly higher in patient groups (76.63 ± 6.86 Group 1 $p < 0.001$, 63.05 ± 6.6 Group 2), $p < 0.001$, respectively than the control (44.1 ± 4.1). Patients in Group 1 had higher ox-LDL than those in Group 2, Table 2. Such increase was significant and correlated negatively with decreased serum EC CuZn-SOD

Table 3
Correlation between serum ALT, AST and oxidative stress markers EC CuZn-SOD (U/ml) and ox-LDL (μg/L) among the studied subjects.

		AST	ALT	EC CuZn-SOD	Ox-LDL
AST	R				
	Sig. (2-tailed)				
	N				
ALT	R	0.951[a]			
	Sig. (2-tailed)	0.0001			
	N	60			
EC CuZn-SOD	R	−0.583[a]	−0.606[a]		
	Sig. (2-tailed)	0.0001	0.0001		
	N	60	60		
Ox-LDL	R	0.708[a]	0.745[a]	−0.641[a]	
	Sig. (2-tailed)	0.0001	0.0001	0.0001	
	N	60	60	60	

[a] Pearson correlation is significant at $p < 0.01$, (2-tailed).

levels in Group 1 patients $p < 0.05$. Table 2, 4 and 5; Supplementary Table 8. Figs. 1 and 2,

5. Discussion

The aim of this work was to determine the serum level of ox-LDL, as to evaluate the role of ox-LDL in chronic hepatitis C patients and its association with HCV-induced extra hepatic manifestations. The study amounts to a large background knowledge needed to explore if ox-LDL has a role in the pathophysiology of HCV with extra hepatic manifestations, either directly as an inflammatory molecule or indirectly through inhibition of EC CuZn-SOD expression.

5.1. Clinical and anthropometric characteristics of subjects

Table 1 shows clinical and anthropometric characteristics of all subjects. All subjects enrolled in this study were above 50 years, in menopause. Therefore, changes in ox-LDL were not due to hormonal effects. Subjects with obesity were excluded from our study as obesity is linked to a state of increased oxidative stress. An accumulating evidence shows that older women who were overweight or obese were more likely to have higher lipoprotein oxidation reactions [25]. Ox-LDL is implicated in the pathogenesis of metabolic syndrome, truncal obesity, and dyslipidemia. HCV infection is associated with insulin resistance and increased risk of type 2 diabetes mellitus incidence, independently of liver disease stage [26]. Our study reveals similar anthropometric measures in all patient groups, regardless of themanifested extra hepatic changes.

5.2. Extra hepatic manifestations in HCV patients

The extra hepatic manifestations included eight patients with lichen planus; eight patients with rheumatoid arthritis; two patients with hypothyroidism; and two patients with psoriasis. This study found an association of serum biomarkers with oxidative stress and increased ROS

Table 4
Correlation between serum EC CuZn-SOD (U/ml) and serum ox-LDL (μg/L) in HCV with extra hepatic manifestation.

		EC CuZn-SOD	ox-LDL
EC CuZn-SOD	Pearson Coefficient (R)	1	−0.491*
	P		0.028
	N	20	20

Pearson correlation is significant at $p < 0.05$, (2-tailed).

Table 5
Correlation between serum EC CuZn-SOD (U/ml) and serum ox-LDL (μg/L) in HCV without extra hepatic manifestation.

		EC CuZn-SOD	Ox-LDL
EC CuZn-SOD	Pearson Coefficient (R)	1	−0.303
	p		0.193
	N	20	20

* Pearson correlation is significant at $p < 0.05$, (2-tailed).

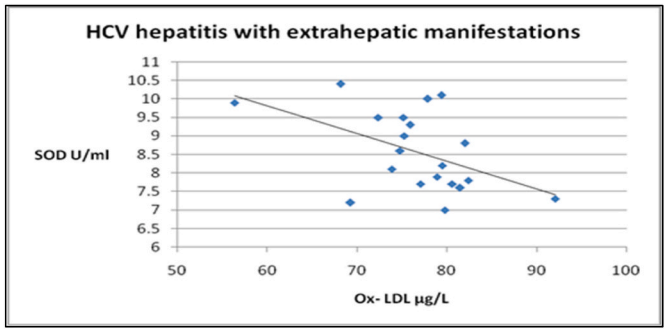

Fig. 1. Correlation between serum EC CuZn-SOD (U/ml) and serum ox-LDL (μg/L) in HCV hepatitis with extra hepatic manifestation.

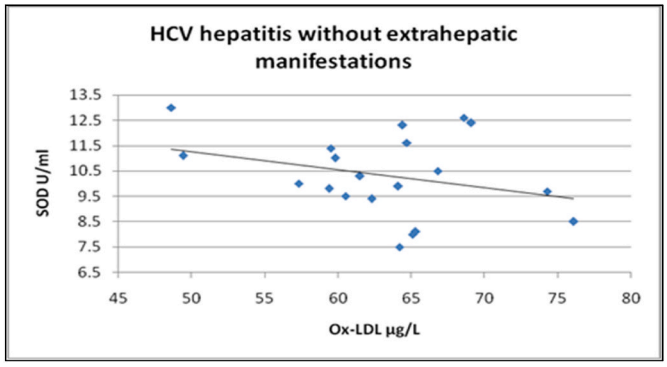

Fig. 2. Correlation between serum EC CuZn-SOD (U/ml) and serum ox-LDL (μg/L) in HCV hepatitis without extra hepatic manifestation.

production in such patients. I.e., a significant reduction of plasma Alb was associated with the extra hepatic manifestations. In humans, Alb is the most abundant plasma protein, presenting about 50 % of the total protein content. Alb is exclusively synthesized by hepatic cells, which release it directly into the blood stream without storage. Under physiological conditions, only 20–30 % of hepatocytes produce 9–12 g of Alb/day, therefore, the liver has a large functional reserve, and it can increase the synthesis of this protein by three-four times, if necessary. In HCV infection, the production of Alb is inhibited by acute phase cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) [27].

HCV cirrhotic patients were excluded from the study. Vitamin K deficiency was seen in decompensated liver cirrhosis secondary to various complex mechanisms, which include bile salts deficiency, bile salts secretory failure, and the use of broad-spectrum antibiotics. The use of vitamin K in our patients was not cause of normalized INR levels observed in them, similarly to other studied subjects [28]. Serum TB and Indirect bilirubin did not change among all groups studied. Bilirubin is the final degradation product of heme. Normal levels of serum bilirubin indicates that the degradation of heme was within normal range while hepatocytes metabolism and excretion of bilirubin are unaffected at this stage of the disease.

Patients of both groups had higher AST and ALT levels than the control, indicating that damage of liver cells and/or oxidative stress has been persistent. Values of ALT and AST positively correlated with various serum markers of oxidative stress, Table 3. Our data was similar to a study who found that the increase in ALT in patients who previously had persistent abnormal levels of transaminases was preceded by a burst in oxidative stress markers [29]. HCV is one of the risk factors for HCC. Our study revealed a ratio of AST/ALT (AAR) < 1.4. Such ratio when >1.4 may be a useful surrogate marker to identify candidates at high risk for developing HCV-induced HCC. Similarly to our data, this ratio in HCV patients with or without extra hepatic manifestations was lower than the published cut-off value [30,31].

5.3. Serum EC CuZn-SOD

This study reveals a significant decrease in serum CuZn-SOD in all HCV patients from the control. Antioxidant enzymes play crucial role in regulating the oxidative status. Single nucleotide polymorphism in genes encoding antioxidant enzymes may directly implicate modulation of oxidative stress. Polymorphisms in FeMn-SOD and EC CuZn-SOD genes were reported as risk factor of diabetic nephropathy [32]. In this study, changes in the levels of EC CuZn-SOD in patients were not due to decreased intake of antioxidants in their diet. All patients were on multi-vitamins and antioxidants therapy regimen since they were diagnosed. These antioxidants contain silymarin, vitamins A, C, and E, selenium and Zn. Zn was used because it acts as a prosthetic group for EC CuZn-SOD. Selenium acts as a prosthetic group for glutathione peroxidase. In a similar study, patients have used the multi-vitamins and antioxidants for about three months before they were enrolled in this study [33].

Using a mouse model of viral hepatitis that induces early transcriptional changes in the redox pathways in the liver, including down-regulation of *sod1*, *sod1*$^{-/-}$ null mice exhibited increased inflammation and aggravated liver damage upon viral infection, independent of T-helper cells and natural killer cells (NK) which was ameliorated by antioxidant treatment. Interferon alpha (IFN-α) down-regulates *sod1* and causes oxidative liver damage in *sod1*$^{-/-}$ and wild type mice. IFN-α mediated oxidative stress is key mediator of virus-induced liver damage [16].

Chronic HCV induces oxidative/nitrosative stress from multiple sources, including iNOS, the mitochondrial electron transport chain, hepatocyte NOX, and inflammation. In addition, HCV decreases GSH synthesis and regeneration [34,35]. HCV core is the main regulator of NOX4 expression [36]. ROS production in HCV-infected cells might also arise from the endoplasmic reticulum-residing cytochrome P450 [37]. Oxidation reactions can mediate intra- and intermolecular crosslinking of peptides and proteins and fragmentation of polypeptide chains [38].

5.4. Serum ox-LDL

In this study, patients in either group presented with higher ox-LDL levels than the control. Such increase was even higher in patients with extra hepatic manifestations compared to those without extra hepatic manifestations. A significant negative correlation exists between serum EC CuZn-SOD and ox-LDL in HCV patients with extra hepatic manifestations, i.e. high serum levels of ox-LDL was associated with or due to low levels of EC CuZn-SOD. Free radicals have a very short half-life (in seconds), hence, their evaluation in clinical setting is difficult. However, there are more stable biomolecular markers with longer half-lives, ranging from hours to weeks, which can be used to assess oxidative stress associated with diverse diseases. Some of those are oxidation by-products, such as ox-LDL. Ox-LDL is taken up by LOX-1 and not by the conventional LDL receptor. Oxidative changes in amino acids as well as proteolysis and crosslinks of apolipoprotein B occur and result in extensive alteration in the protein composition and structure. A significantly high levels of carbonylated proteins in HCV cirrhotic patients was reported [31]. These protein oxidation products may contribute to the pathogenesis of HCV [39].

Chronic HCV infection induces magnificent oxidative stress. ROS inhibit virus replication without affecting stability of its genome RNA. ROS can induce viral genome heterogeneity, which facilitates viral escape during treatment and probably viral escape from the immune system. Hepatitis C virus replication is associated with the endoplasmic reticulum, where the virus can induce cellular stress. Cells are protected and modulated against oxidative stress through the interplay of intracellular antioxidant agents, mainly GSH, thioredoxin, and antioxidant enzyme systems such as SOD, catalase, GSH peroxidase, and heme oxygenase-1. Further, the use of natural and synthetic antioxidants (Vitamin C, E, N-acetylcysteine, glycyrrhizin, polyenylphosphatidyl choline, mitoquinone, quercetin, s-adenosylmethionine and silymarin) has shown promising results as co-adjuvants in HCV therapy [40].

ROS produced by endothelial cells and macrophages play important roles in atherogenesis because they promote the formation of ox-LDL. The present study suggests that HCV-induced oxidative stress played magnificent role in downregulating the expression of EC CuZn-SOD mRNA and protein levels. Such imbalance in the oxidative/anti-oxidative status increased lipoprotein oxidation, thus promoted ox-LDL binding to LOX-1, and the resultant pathogenic extra hepatic manifestations. Our finding agrees with another study of ox-LDL that accelerated the development of atherosclerosis by suppressing the expression of EC CuZn-SOD. The higher ox-LDL associated with and/or due to decreased expression of EC CuZn-SOD in chronic HCV is plausible target for treatment of extra hepatic manifestations associated with oxidative stress as atherosclerosis. Makino et al. (2016) suggested luteolin: a polyphenol antioxidant to have a counteracting pharmacological effect to the later conditions [41].

EC CuZn-SOD is expressed by vascular smooth muscles. Ox-LDL binding to LOX-1 induces endothelial inactivation and dysfunction, supports the recruitment of circulating leukocytes, triggers foam cell formation, and sustains migration and proliferation of smooth muscle cells, thus contributes to the development of the atherosclerotic plaque. Furthermore, ox-LDL-LOX-1 interaction may also contribute to plaque destabilization by inducing smooth muscle cell apoptosis and the release of matrix degrading enzymes matrix metalloproteinases [42].

5.5. Psoriasis

In this study, among patients with extra hepatic manifestations, two patients with psoriasis had higher serum ox-LDL levels than the control. Autoantibodies against ox-LDL were found in psoriasis, and their level correlates with disease severity [43]. Psoriatic patients have increased risk of cardiovascular mortality. Chronic HCV infection is characterized by both hepatic and systemic inflammation through activation of several pathways, resulting in cytokine release i.e. IL-6 and IL-18 that accompany oxidative stress; an immune-mediated process; and/or by inducing metabolic derangement [44]. HCV infection induces an immune response mediated by T1-cells. These lymphocytes secrete IFNγ as the predominant cytokine, which is able to enhance the production of TNF-α by macrophages.

5.6. Rheumatoid arthritis

Eight patients with rheumatoid arthritis were included in this study. Serum levels of ox-LDL were (56.4, 69.3, 73.9, 72.4, 77.1, 78.9, 82.4, 68.2, μg/L, mean value 72.33 μg/L). The mean level of ox-LDL in such patients was higher than the control group. The relationship of ox-LDL with the presence or absence of subclinical atherosclerosis has been evaluated and a positive association of ox-LDL levels with intima-media thickness has been demonstrated [45]. Further, ox-LDL leads to the formation of foam cells and atherosclerotic plaques of arthritis patients [46]. Our study finding was similar to Dai et al. (1996) who detected ox-LDL in the synovium and synovial fluids of rheumatoid arthritis

patients in a significantly higher manner than age and sex matched controls [47].

Oxidative modification can alter the structure and function of affected proteins. Such post-translational modifications of proteins and modifications of lipid/carbohydrate components of the body can result in the formation of "altered self" and have the potential to trigger an autoimmune response. An example of diagnostically relevant post-translational modification in rheumatoid arthritis is the citrullination of proteins [48]. Oxidative modifications have the capacity to trigger autoimmune reactions. For example, oxidative damaged immunoglobulin G (IgG) and - (IgM) can form advanced glycation end-products. Serum autoantibodies against these products are associated with rheumatic arthritis [49]. Anti-ox-LDL levels have a strong positive correlation with C reactive protein levels: an acute phase reactant in rheumatoid arthritis patients that may both depend on the degree of inflammation and/or the presence of a prooxidative status [50]. Further, the process of lipid peroxidation releases aldehydic products of lipid peroxidation that can form adducts with amino acids. Aldehyde-modified proteins are highly immunogenic [51].

5.7. Hypothyroidism

Two patients with hypothyroidism were enrolled in this study. Serum ox-LDL levels were 92.1 and 80.6 μg/L, that were higher than the control group. Ages of female patients in this study were 49- and 56 years. Hypothyroidism, among other thyroid disorders, is common in patients with chronic HCV, particularly women. Antithyroid antibodies exist in 5–17 % of patients with HCV infection. The highest prevalences of both thyroid antibodies and thyroid disease occur in older women. HCV thyroid infection could act by upregulating (C-X-C motif) ligand 10 (*cxcl* 10) gene expressions and secretion in thyrocytes that recruits T1 lymphocytes, the latter secrete IFNγ and TNF-α. These cytokines might induce CXCL10 secretion by thyrocytes, thus perpetuating the immune cascade, which may lead to the appearance of autoimmune thyroid disorders in genetically predisposed subjects [52].

Pastore et al. (2016) conducted a study and found that the standard IFN-based treatment was associated with an increase of the immune-mediated thyroid damage. They suggested mechanisms of thyroid damage to be disturbances in the balance between the environment and the host. Loss of tolerance was the main mechanism that promoted autoimmune thyroid disorders, with autoantibodies or T lymphocytes (humoral or cellular response) reacting with self-antigens [53]. Contradictory to their study, our study patients did not receive IFN therapy before their enrollment.

Clinical and subclinical hypothyroidism are independent risk factors for atherosclerosis. However, this connection cannot be entirely explained by dyslipidemia accompanied by subclinical hypothyroidism. Lipid peroxidation also plays an important role in the development of atherosclerosis. Lipid peroxidation is higher in subclinical hypothyroidism patients than in the euthyroid subjects [54].

5.8. Lichen planus

Eight patients with lichen planus were enrolled in this study. Serum levels of ox-LDL were (79.5, 74.8, 75.9, 77.9, 79.8, 82.0, 81.5, 79.4 μg/L, mean value 78.8 μg/L). The mean level of ox-LDL in lichen planus patients was higher than the control group. Lichen planus disturbs lipid metabolism causing dyslipidemia, and highly predisposes for cardiovascular pathogenesis. The increase in oxidative stress and the imbalance in the antioxidant defense mechanisms may play a role in the pathogenesis of Lichen planus [55].

5.9. Conclusion and significance

The increased ox-LDL has a magnificent role in the pathophysiology of HCV with extra hepatic manifestation, either directly as an inflammatory molecule or indirectly, secondary to the inhibition of EC CuZn-SOD expression. The role played by ox-LDL in the development of extra hepatic manifestations may be via its uptake by specific receptors i. e. (LOX-1). Increased serum levels of ox-LDL may be due to the property of HCV to induce oxidative stress or due to the inflammatory cytokine associated with HCV pathogenesis. Exercise training is the most effective intervention against complex chronic diseases. Development of novel strategies for treatment of patients with psoriasis with antioxidants activity may be useful [56].

5.10. Limitations

The small sample size limited the validation of data obtained. Further, eliminating apparent causes of oxidative stress such as HCV patients comorbid with obesity, type-1 and -2 diabetes millitus, HCC, malignancies and alcohol-induced HCV was limiting factor. Lack of resources and study design did not allow for long-term follow up on the consistency of the association of high serum level of ox-LDL with deregulated EC CuZn-SOD in patients. An *in vitro* study shall evaluate the interaction between ox-LDL and EC CuZn-SOD at the histopathological, molecular, and epigenetic levels, and made a correlation between severity of disease and the aggressiveness of such interaction and/or extra hepatic manifestations.

5.11. Future research

Further studies on the effect of HCV antiviral drugs that may reduce ox-LDL levels are required, i.e. DAAs. The effects of HCV eradication on oxidative alterations of proteins and lipids (ox-LDL and carbonylated proteins) needs further investigations as they may be responsible for complications of HCV, such as liver cirrhosis and HCC. Future investigations should better define the boundaries of HCV pathogenesis, along with the actual etiologic and genetic features of this virus in different disorders. The role of exercise and determined doses of antioxidative therapy should be available as standard intervention.

CRediT authorship contribution statement

Dina Johar: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Ahmed Hamed Bedair El-Assal:** Writing – review & editing, Writing – original draft, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Mahmoud Mohamed Abou-El-Makarem:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Conceptualization. **Essam Foad A. Hammouda:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Conceptualization. **Mohamed Soliman Hegazy:** Conceptualization, Supervision, Validation, Visualization, Writing – review & editing. **Samy Zaky:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Conceptualization.

Ethical approval and consent to participate

All procedures, involving human participants, were performed in accordance with the Declaration of Helsinki 1964 and its later amendments. This prospective study was approved by the Ethical Committee of Al-Azhar University, Cairo, Egypt. The study protocol was explained to all participants before enrollment. A written medical consent was signed by all participants including the attending physician.

Consent for publication

N/A.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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Conflict of interest

The authors declare that no conflict of interest exists.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metop.2024.100339.

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