Saudi Pharmaceutical Journal 29 (2021) 1120–1128

Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Original article

The effect of direct acting antiviral agents on vascular endothelial function in Egyptian patients with chronic hepatitis C virus infection

Dalia A Freekh [a], Maged W Helmy [b], Mohamed Said [c], Noha M El-khodary [d,*]

[a] Clinical Pharmacy & Pharmacy Practice Department, Faculty of Pharmacy, Damanhour University, Damanhour City, Egypt
[b] Professor of Pharmacology & Toxicology, Pharmacology & Toxicology Department, Faculty of Pharmacy, Damanhour University, Damanhour City, Egypt
[c] Professor of Endemic Medicine & Hepatology, Endemic Medicine & Hepatology Department, Cairo University, Cairo City, Egypt
[d] Lecturer of Clinical Pharmacy, Clinical Pharmacy & Pharmacy Practice Department, Faculty of Pharmacy, Damanhour University, Damanhour City, Egypt

ARTICLE INFO

Article history:
Received 11 March 2021
Accepted 1 August 2021
Available online 4 August 2021

Keyword:
Endothelial function
Chronic hepatitis C
Direct-acting antiviral agents

ABSTRACT

Chronic hepatitis C virus (HCV) infection is correlated with cerebrovascular and cardiovascular disease (CVD). This study aimed to assess the effect of treatment with DAAs on vascular endothelial function in cirrhotic and non-cirrhotic HCV infected patients without any CVD risk factors. Fifty chronic HCV genotype 4 infected patients, without cardiovascular risks who have been listed to receive sofosbuvir/daclatasvir with ribavirin combination as triple therapy for 3 months were prospectively recruited. Endothelial dysfunction markers as soluble vascular cell adhesion molecule-1 (sVCAM-1) and Von willebrand factor (vWf) and inflammation marker (IL6) were estimated at baseline and 3 months post the end of therapy (SVR). All patients achieved SVR. VCAM1 level was significantly improved after HCV clearance with DAA in cirrhotic HCV patients (P = 0.002) compared to patients with mild liver fibrosis (P = 0.006). Levels of vWF also decreased significantly in cirrhosis and non-cirrhosis groups after SVR (P < 0.001 and P = 0.011, respectively). Systemic inflammatory marker (IL6) showed significant decrease in cirrhotic patients (P = 0.001). While, IL6 level did not change significantly in non-cirrhotic group (P = 0.061). Also at SVR, noninvasive liver fibrosis indices have been reduced significantly in the two groups (P < 0.001). HCV clearance by new DAA treatment improves the vascular endothelial dysfunction in Egyptian HCV infected patients with different levels of liver fibrosis and with no risk factors for endothelial dysfunction or CVD.

1. Introduction

Globally, chronic hepatitis C virus (HCV) infection affects over 71 million individuals about 1% of world population (Lombardi et al., 2019). Egypt has the highest incidence of HCV infection globally, with an estimated 11.9% of population where genotype 4 constitutes 94.1% of HCV infection (Kouyoumjian et al., 2018).

HCV is the primary contributor of chronic hepatic disease, including cirrhosis and hepatocellular carcinoma. HCV infection is a systemic disease that not only causes liver injury but damages other organs as well. Many HCV patients have extra hepatic complications, as cryoglobulinemia, kidney disease, insulin resistance, type 2 diabetes mellitus, cerebrovascular, cardiovascular disease (CVD), non-Hodgkin lymphoma, erectile dysfunction and venous thromboembolism (van der Meer 2015; Ambrosino et al. 2016; Gentile et al. 2018). A recent meta-analysis found that patients with positive HCV infection are at increased risk for cardiovascular (CV)-related morbidity and mortality (Roed et al. 2012; Petta et al. 2016). Infection with HCV has been linked with an elevated risk of sub-clinical atherosclerosis, coronary artery disease, peripheral artery disease, cerebrovascular and CV incidents (Babiker et al. 2017; Adinolfi et al. 2014). The main cause of an elevated incidence of cerebro- and CV events in HCV patients is a proatherogenic effect of HCV infection because of immune response activation, chronic subclinical inflammation and endothelial dysfunction induced by the virus (Moucari et al. 2008; Ampuero and Romero-Gómez 2015).

Generally, endothelial dysfunction was established as the earliest stage in atherosclerosis process (Sitia et al. 2010), and is devel-

* Corresponding author.
E-mail address: noha.mahmoud@pharm.dmu.edu.eg (N.M El-khodary).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jsps.2021.08.006
1319-0164/

oping as a new risk factor for CVD on its own (Hinderliter and Caughey 2003). All the typical CV risk factors as hyperlipidemia, elevated blood pressure, and diabetes are linked with endothelial dysfunction (Sitia et al. 2010). Endothelial dysfunction includes release of inflammatory mediators, cytokines, adhesion molecules and pro-thrombotic factors (Hansson 2005). Von Willebrand factor (vWF: Ag), a glycoprotein mainly derived from endothelium, is released by activated endothelial cells into the circulation. This biomarker has a role in more cellular activation as well as in promoting activation of platelet and coagulation, and can be easily assessed (Ridker et al. 2004; Antonova et al. 2013). Soluble vascular adhesion molecule 1 (sVCAM-1) has been found to be associated with endothelial dysfunction as well as the risk of CVD (Antonova et al. 2013).

Endothelial dysfunction has been examined in HCV patients with human immunodeficiency virus (HIV) co-infection (de Castro et al. 2010; Masiá et al. 2011), patients infected with HCV on haemodialysis (Oyake et al. 2008; Zaki 2017) and in people with liver cirrhosis (Barone et al. 2015; Chen et al. 2012). However, there are insufficient data for HCV mono-infected patients. In the above populations, HCV infection is consistent with elevated serum endothelial dysfunction markers (de Castro et al. 2010; Antonova et al. 2013).

Optimal treatment for chronic HCV genotype 4 infected patients is progressing quickly; the optimal therapy for long period was interferon-based therapy as pegylated interferon and ribavirin combination, with reasonable response rates and more side effects (Abdel-Razek and Waked 2015). After the investigation of direct acting antiviral drugs (DAAs) which target non-structural proteins essential for viral RNA replication, as daclatasvir (DCV) and sofosbuvir (SOF), the length of therapy has been significantly reduced with very high rates of sustained virological response (SVR) and fewer side effects (Elsharkawy et al. 2017). Finally, recent studies showed a SVR in HCV infected patients reduced mortality rate, progression of decompensated cirrhosis, and hepatocellular carcinoma appearance (Romero-Gómez et al. 2005), and decreased significantly risk of CVD and stroke (Li et al. 2019). In addition, recent studies suggest that HCV eradication with DAAs treatment has a direct positive vascular effect, which enhances endothelial function and reduces serum markers of endothelial dysfunction (Schmidt et al. 2018). Therefore, the objective of this study was to assess the impact of DAAs therapy on vascular endothelial function in cirrhotic and non-cirrhotic patients with chronic HCV infection.

2. Patients and methods

A single–center prospective study was conducted to evaluate the effect of new HCV therapy (DAAs) on vascular endothelial dysfunction. Fifty patients with chronic HCV genotype 4 infection aged 18 to 75 years were recruited from viral hepatitis center, Damanhour Fever Hospital, affiliated to National Committee for Control of Viral Hepatitis, January 2019 to January 2020. Patients were listed for 3 months regimen of sofosbuvir/daclatasvir with ribavirin combination as triple therapy. Patients were classified into two groups: Group I has 25 cirrhotic HCV infected patients, and Group II has 25 non cirrhotic HCV infected patients. The classification was done according to the severity of hepatic fibrosis, which was evaluated by an abdominal ultrasonography signs of liver cirrhosis as recorded elsewhere (Gentile et al. 2009), fibrosis score 4 (FIB-4), a noninvasive procedure, using 4 factors according to this equation: Age (years) × aspartate aminotransferase (AST) (IU/L) / platelet count (10^9 /L) x$\sqrt{}$alanine aminotransferase (ALT) (IU/L), where FIB-4 score is>3.25 = F3–F4 (severe fibrosis or cirrhosis) and FIB-4 score<1.45 = F0–F1 (mild fibrosis) (Sterling et al. 2006; Xu et al. 2014), and APRI score. APRI score is the index of AST to platelet ratio, a non-invasive procedure, where APRI score is $\leq$ 0.5, it is a good predictor that there is very little or no fibrosis present, and whether the APRI score is $\geq$ 1.5, it is a strong predictor of cirrhosis.

2.1. Selection of patients

Patients included in this study who had positive HCV RNA PCR and received oral fixed dose of SOF and DCV with ribavirin combination as triple therapy for 3 months. Exclusion criteria included co-infection with hepatitis B virus (HBV) or HIV or presence of other hepatic diseases as autoimmune liver disease, cholestasis, Wilson disease, hemochromatosis, metabolic disease, patients who underwent a liver transplantation, cryoglobulinemic vasculitis, patients who<18 or>75 years old, pregnant female, decompensated cirrhosis, hepatocellular carcinoma or extra hepatic malignancy, chronic renal failure, history of cerebrovascular disease or CVD or obese patients whose body mass index (BMI) equal to or above 30. Patients with history of previous treatment with anti HCV medicine, diabetes mellitus, chronic autoimmune diseases or treated with immunosuppressant drugs and/or drugs-induced steatosis or statins are also excluded from the study.

2.2. Clinical assessments

Before treatment, a complete history was taken from all patients, and physical examination including general and abdominal examination was done to check inclusion and exclusion criteria. Data was collected from each patient including age, gender, smoking status, height, weight. BMI was calculated for each patient.

2.3. Definitions

Body mass index was measured as weight (kg)/squared meter of height (m^2). If BMI was $\geq$ 30, patient was categorized as obese. Hypertension was defined as systolic blood pressure equal to or above 130 mm Hg and/or diastolic blood pressure equal to or above 85 mm Hg or use of blood pressure lowering drugs. SVR was described as undetectable HCV-RNA levels 3 months post the termination of antiviral therapy.

2.4. Laboratory procedures

From each patient, blood samples were collected after fasting overnight (12-h) into serum gel separator tubes (SST) containing EDTA in order to investigate complete blood count (CBC), hemoglobin, platelet count, white blood cells (WBCs), anti-HCV antibody, HBsAg, routine liver biochemistry as ALT, AST, serum albumin, serum total bilirubin, international randomized ratio (INR), alpha fetoprotein (AFP), serum creatinine, fasting blood glucose concentration, glycated hemoglobin percent (Hb A1C%), and pregnancy test (for female). All parameters were measured at hospital laboratories. HCV RNA testing was done with use of a real-time HCV RNA PCR (Cobas Ampliprep, Cobas Taqman 48, Roche) according to the instructions of manufacturer. Abdominal ultrasound was performed using the Toshiba Aplio XG ultrasound machine (Toshiba). Electrocardiograms (ECG) and echocardiography were used to diagnose any CV abnormalities prior to therapy.

2.5. Patients' follow up

After initiation the regimen of DAAs therapy, patients were followed up every month until completion of the regimen and 3 months after the end of treatment to evaluate SVR12. All participants were followed up to 3 months after the end of treatment by

the following: liver function test: AST, ALT, serum total bilirubin, fasting blood glucose concentration, hemoglobin A1C, serum creatinine, hemoglobin, platelet count, white blood cells, PCR assay for HCV-RNA.

2.6. Assessment of endothelial dysfunction

Endothelial dysfunction was assessed by circulating markers as vWF Ag and sVCAM-1 that reflect the degree of endothelial damage and correlate with liver fibrosis in chronic HCV infection (Antonova et al. 2013). Assessment of markers had been done at two time points at baseline (pre-treatment) and at 3 months post-treatment (at SVR point). At each time point blood samples were collected into SSTs and kept at room temperature 10–20 min to coagulate, and centrifuged twenty minutes at the speed of 2000–3000 r.p.m. and then serum was frozen immediately in aliquots at −80 °C until analysis. VCAM-1 and vWF: Ag levels were analyzed in serum using the human VCAM-1 and human vWF Ag ELISA kits, respectively (Shanghai Sunredbio (SRB) Technology Co., Ltd.), according to the instructions of manufacturer. Interleukin 6 (IL6) level was evaluated using human IL6 ELISA kit (Shanghai Sunredbio (SRB) Technology Co., Ltd.) as systemic inflammatory parameter.

2.7. Liver fibrosis assessment

Liver fibrosis was evaluated in all patients using FIB-4 score, and APRI Score at two time points at baseline (pre-treatment) and at 3 months post-treatment

2.8. Antiviral therapy

Patients participated in the study received regimen of DAAs for 3 months in accordance with the national treatment protocol in Egypt, AASLD (Panel et al. 2015). All patients in the study received the same regimens of therapy which included combination of SOF, DCV and ribavirin as a triple therapy in oral fixed dose of 400 mg and 60 mg for SOF and DCV respectively, once daily however the dose of ribavirin not fixed as it is weight –based dose and calculated as following, for patients weighing < 75 kg, daily dose was 1000 mg or 1200 mg for patients weighing > 75 kg divided depending on tolerability of patient. According to NCCVH (National Committee for Control of Viral Hepatitis) ribavirin given for cirrhotic patients and difficult to treat non cirrhotic patients who have one of these criteria: total serum bilirubin < 1.2 mg/dl, serum albumin < 3.5 g/dl, INR > 1.2, platelet count < 150,000/mm^3.

2.9. Ethics

This study was conducted in compliance with Helsinki Declaration of the World Medical Association. Ministry of Health and the Research Ethics Committee of the Faculty of Pharmacy, Damanhour University approved the study protocol (Ref.no. 1118 PP 7). Each participant signed informed consent.

2.10. Statistical analysis

Data analysis was done using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Numbers and percentages (%) were used for expressing qualitative data. To determine if variables follow a normal distribution, Kolmogorov-Smirnov tests were used. Continuous variables were presented using mean, range, standard deviation, median and interquartile range (IQR). Statistically significant results were determined at P-value < 0.05. The comparison between non-parametric categorical variables was done with the Chi-square test ($\chi 2$). For non-parametric quantitative data, the comparison between pre- and post-DAAs therapy was determined with the Wilcoxon-Rank test, and the comparison between means was determined with Mann–Whitney *U* test. Comparison between the means of two studied groups was performed with Student *t*-test for normally distributed quantitative variables. For normally distributed quantitative variables, comparison between two means from the same group at different times was done with Paired *t*-test.

3. Results

3.1. Patients' characteristics

Fifty chronic HCV patients aged between 32 and 70 years were included in the study, where 25 patients were allocated in the group 1 "HCV patients with cirrhosis" and 25 in the group 2 "HCV patients with mild fibrosis". Patients' baseline demographic and clinical characteristics are showed in Table 1. Patients with HCV cirrhosis showed significant increase in mean age (57.52 ± 8.68 years vs 49.16 ± 9.23 years; P = 0.002) compared to non cirrhotic patients with male prevalence (52% in cirrhosis patients vs 36% in non-cirrhosis patients; P = 0.254). Cirrhotic patients had significantly higher baseline AST, ALT (P < 0.001) and AFP (P < 0.001) and showed significant decrease in platelets and WBCs (P < 0.001, <0.001, respectively) compared to non cirrhotic patients. There was no difference in total bilirubin, serum creatinine, serum albumin, and INR (P = 0.152, 0.883, 0.868, and 0.979 respectively) was found between the two studied groups as well as in the average of BMI (P = 0.210), and systolic (P = 0.936) and diastolic blood pressure (P = 0.400). Of the 25 HCV cirrhotic patients, 11 patients (44%) had platelet count > 100.000/mm^3. All patients were naïve to antiviral treatment. Patients participated in the study had less comorbidities: no patients in both groups had DM, hypertension or was smoker. All 50 patients were compliant with the regimen of DAAs and completed the study.

3.2. Clinical assessments

All 50 patients (100%) achieved SVR after DAAs. Biochemical data changes in both groups are shown in Table 2. All data were evaluated at 2 points: before treatment, 3 months post treatment (SVR) in cirrhosis and non-cirrhosis groups. At SVR, a significant decrease in ALT, AST level was observed in both groups (P < 0.001). Platelet levels increased significantly in cirrhosis and non-cirrhosis groups (P = 0.001, P < 0.001, respectively), and bilirubin levels showed a significant reduction in cirrhotic and non-cirrhotic patients (P = 0.001, P = 0.036, respectively). No statistically significant improvement in fasting glucose levels was observed while HbA1C% decreased significantly in cirrhosis and non-cirrhosis groups (P = 0.049 and P = 0.001, respectively). Blood hemoglobin has been decreased significantly in the two groups after SVR.

3.3. Endothelial function assessment

At baseline, cirrhotic patients showed vWF-Ag level of 184.2 ± 42.37 ng/ml and sVCAM-1 of 45.58 ± 12.47 ng/ml, while levels of vWF-Ag and sVCAM-1 were 131.8 ± 27.01 ng/ml and 25.0 ± 6.85 ng/ml, respectively in non-cirrhotic patients.

At SVR, a significant improvement in vWF-Ag and sVCAM-1 levels from [184.2 ± 42.37 to 134.3 ± 44.28 ng/ml (P < 0.001), and from 45.58 ± 12.47 to 36.58 ± 14.88 ng/ml (P = 0.002), respectively] in cirrhotic patients was found. Further, a significant improvement in vWF-Ag and sVCAM-1 levels from [131.8 ± 27.01 to 103.9 ± 32.97 ng/ml (P = 0.011), and from 25.0 ± 6.85 to 18.

Table 1
Patients' baseline characteristics and clinical features in studied groups.

	Total (n = 50)		Group I: Cirrhosis (n = 25)		Group II: Non Cirrhosis (n = 25)		p
	No.	%	No.	%	No.	%	
Sex							
Male	22	44	13	52	9	36	0.254
Female	28	56	12	48	16	64	
Age (years)	53.34 ± 9.82		57.52 ± 8.68		49.16 ± 9.23		0.002*
Weight (kg)	67.08 ± 8.33		68.28 ± 8.72		65.88 ± 7.91		0.313
Height (cm)	165 ± 7.54		165 ± 7.88		165 ± 7.35		1.000
BMI (kg/m^2)	24.61 ± 2.16		25 ± 1.93		24.23 ± 2.35		0.210
Systolic	121.8 ± 8.69		121.92 ± 8.04		121.72 ± 9.45		0.936
Diastolic	78.98 ± 6.81		79.80 ± 6.51		78.16 ± 7.13		0.400
Smoking	0	0	0	0	0	0	–
Previous antiviral treatment	0	0	0	0	0	0	–
HBs Ag	0	0	0	0	0	0	–
Q HCV RNA(x10^3/IU/ml)	858.5(184 – 1560)		380(158.2 – 1210)		1060(232 – 1570)		0.222
Hb (g/dl)	13.62 ± 1.33		13.95 ± 1.41		13.29 ± 1.17		0.077
WBCs (x10^3/mm^3)	6.01 ± 1.95		4.75 ± 1.05		7.27 ± 1.81		<0.001*
AST (IU/L)	64.28 ± 41.73		94.96 ± 36.64		33.60 ± 15.89		<0.001*
ALT (IU/L)	46.0 (32 – 86)		82.0 (40–103)		32.0 (30 – 53)		<0.001*
AFP (ng/ml)	2.88 ± 1.42		3.53 ± 1.46		2.24 ± 1.06		0.001*
S. albumin (g/dl)	3.72 ± 0.44		3.71 ± 0.45		3.73 ± 0.43		0.868
Total bilirubin (mg/dl)	0.98 ± 0.41		1.06 ± 0.39		0.89 ± 0.43		0.152
Platelet count (*10^9/l)	148 (110 – 250)		110 (88–131)		250 (177 – 264)		<0.001*
INR	1.23 ± 0.18		1.23 ± 0.18		1.23 ± 0.18		0.979
Serum fasting glucose mg/dl	89.90 ± 11.01		88.44 ± 10.21		91.36 ± 11.79		0.354
HbA1C%	5.62 ± 0.75		5.66 ± 0.79		5.57 ± 0.72		0.677
Serum Creatinin (mg/dl)	0.72 ± 0.18		0.73 ± 0.19		0.72 ± 0.17		0.883
Platelet count (⟨100) (*10^9/l)	11	22	11	44	0	0	<0.001*

BMI: body mass index. HBs Ag: hepatitis B antigen, Hb: hemoglobin concentration, WBCs: white blood cells, AST: aspartate aminotransferase, ALT: alanine aminotransferase, AFP: Alpha-fetoprotein, INR: international normalized ratio, HbA1C%: glycated hemoglobin percent.
*: Statistically significant at P ≤ 0.05.

Table 2
Biochemical data of patients in both groups at baseline and SVR.

	Cirrhosis group (n = 25)		P value	Non Cirrhosis (n = 25)		P value
	Baseline	SVR		Baseline	SVR	
Hb (g/dl)	13.95 ± 1.41	13.12 ± 1.47	^{t}p = 0.003*	13.29 ± 1.17	12.40 ± 1.32	^{t}p < 0.001*
WBCs (x10^3/mm^3)	4.75 ± 1.05	4.78 ± 0.97	^{t}p = 0.913	7.27 ± 1.81	6.89 ± 2.02	^{t}p = 0.135
AST (IU/L)	94.96 ± 36.64	29.68 ± 6.47	^{t}p < 0.001*	33.60 ± 15.89	20.32 ± 4.57	^{t}p < 0.001*
ALT (IU/L)	82 (40 – 103)	26 (19 – 35)	^{Z}p < 0.001*	32 (30 – 53)	22 (18 – 25)	^{Z}p < 0.001*
Total bilirubin (mg/dl)	1.06 ± 0.39	0.80 ± 0.23	^{t}p = 0.001*	0.89 ± 0.43	0.70 ± 0.32	^{t}p = 0.036*
Platelet count (x10^9/l)	110 (88 – 131)	139 (102 – 176)	^{Z}p = 0.001*	250(177 – 264)	275 (247 – 360)	^{Z}p < 0.001*
Serum fasting glucose mg/dl	88.44 ± 10.21	88.0 ± 7.93	^{t}p = 0.608	91.36 ± 11.79	91.32 ± 8.20	^{t}p = 0.962
HbA1C%	5.66 ± 0.79	5.49 ± 0.57	^{t}p = 0.049*	5.57 ± 0.72	5.31 ± 0.43	^{t}p = 0.001*
Serum Creatinine (mg/dl)	0.73 ± 0.19	0.85 ± 0.11	^{t}p < 0.001*	0.72 ± 0.17	0.77 ± 0.16	^{t}p = 0.003*

Hb: hemoglobin concentration, WBCs: white blood cells, AST: aspartate aminotransferase, ALT: alanine aminotransferase, HbA1C%: glycated hemoglobin. SVR: Sustained viral response.
*: Statistically significant at P ≤ 0.05.
^{t}: Paired *t*-test.
^{Z}: Wilcoxon signed ranks test.

76 ± 8.92 ng/ml (P = 0.006), respectively] was found in non-cirrhotic patients (Figs. 1, 2).

Serum IL6 was also determined as a marker of systemic inflammation in both groups at baseline and SVR points. At baseline, cirrhotic patients showed IL6 level of 258.7 ± 72.60 ng/ml, while in non-cirrhotic patients the level was 235.0 ± 53.77 ng/ml. At SVR, a significant reduction in IL6 level with cirrhotic group from 258. 7 ± 72.60 to 212.9 ± 86.78 ng/ml (P = 0.001). While, there was insignificant change in IL6 level in non-cirrhotic group (P = 0.061) was found (Fig. 3).

3.4. Noninvasive liver fibrosis assessment

At baseline, cirrhotic patients had FIB4 score with a mean 5.78 ± 1.82 and APRI score with a mean 2.19 ± 0.90 and non-cirrhotic patients had FIB4 with a mean 1.17 ± 0.42 and APRI score with a mean 0.39 ± 0.19. At SVR, a significant improvement in FIB4 and APRI score from [5.78 ± 1.82 to 2.83 ± 1.66, and from 2.19 ± 0.90 to 0.62 ± 0.31, P < 0.001), respectively] in cirrhotic patients was observed. Also, a significant improvement in FIB4 and APRI score from [1.17 ± 0.42 to 0.79 ± 0.22, and from 0.39 ± 0.19 to 0.19 ± 0.07, P < 0.001, respectively] was found in non-cirrhotic patients (Table 3).

No safety issues were identified, with no serious side effects, and no participant required to discontinue or modify SOF or DCV doses. Six patients (12%) needed ribavirin dose adjustment because of anemia (by 800 mg daily in each case). No patient had any infectious or other disease that could affect inflammation or endothelial function at baseline and during treatment periods.

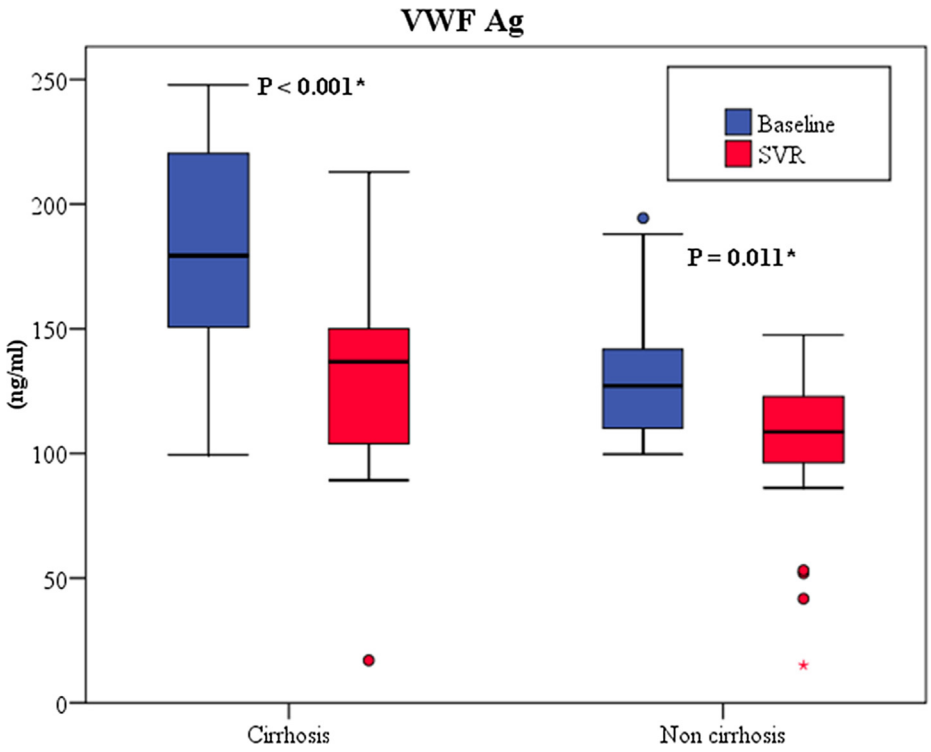

Fig. 1. VWF Ag levels in patients with and without cirrhosis, at baseline (before therapy) and at SVR. *: Statistically significant at P ≤ 0.05. SVR: sustained virological response, VWF Ag: vonwillebrand factor antigen.

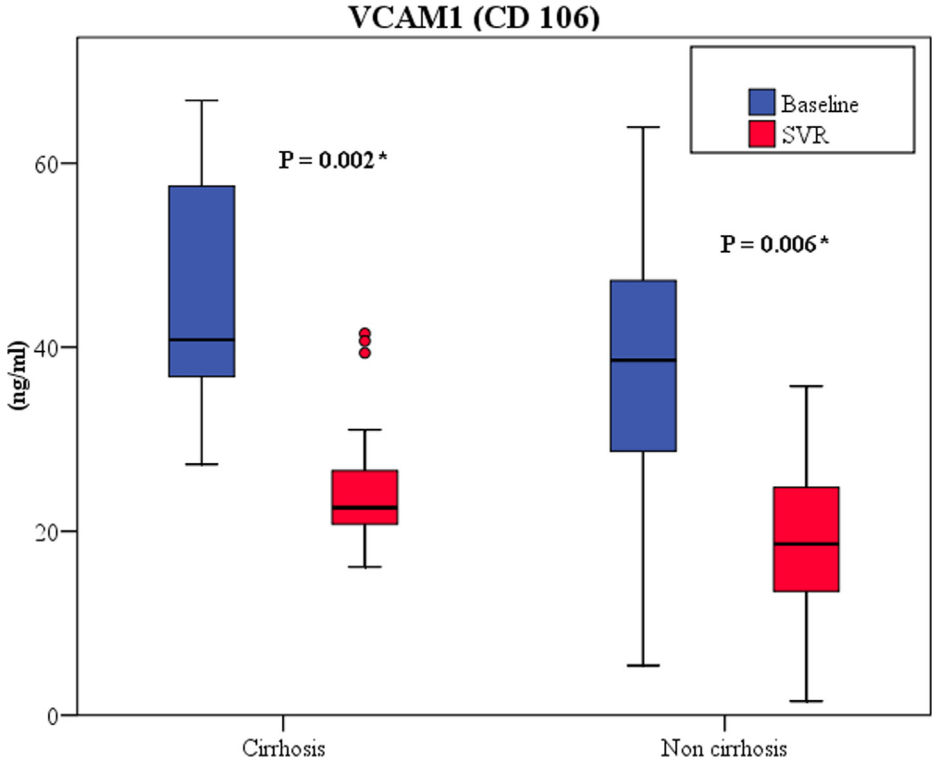

Fig. 2. Levels of VCAM-1 in patients with and without cirrhosis, at baseline (before therapy) and at SVR. SVR: sustained virological response, VCAM: vascular cell adhesion molecule. *: Statistically significant at P ≤ 0.05.

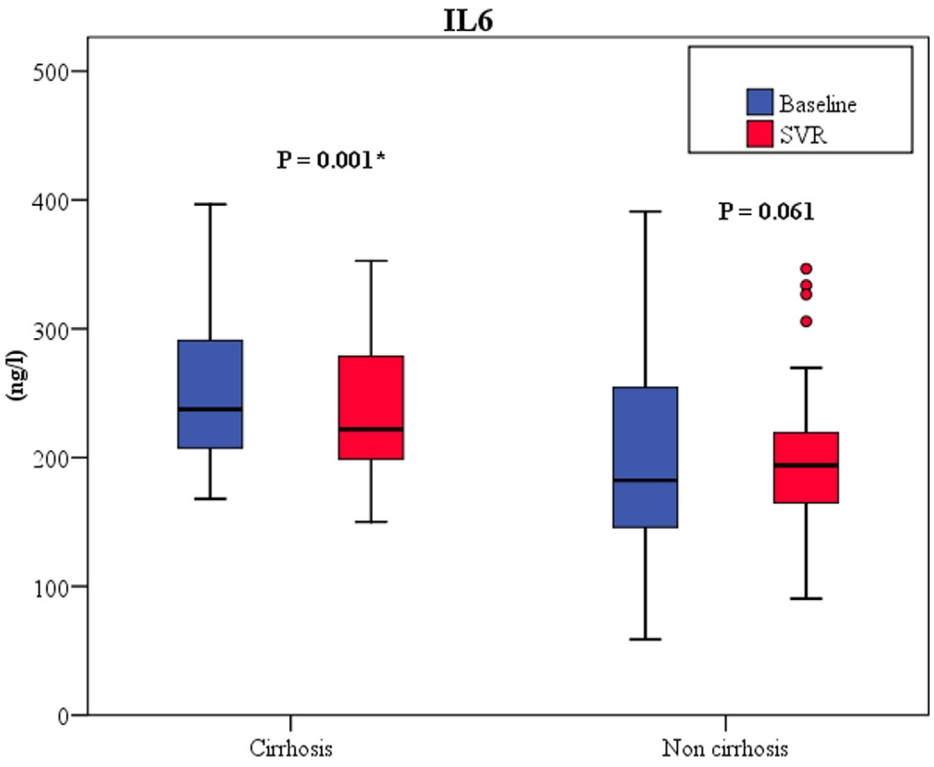

Fig. 3. Levels of IL6 in patients with and without cirrhosis, at baseline (before therapy) and at SVR. SVR: sustained virological response, IL6: interleukin 6. *: Statistically significant at P ≤ 0.05.

Table 3
Comparison between baseline and SVR according to noninvasive liver fibrosis indices in each group.

	Cirrhosis group (n = 25)		P value	Non Cirrhosis (n = 25)		P value
	Baseline	SVR		Baseline	SVR	
FIB4						
Mean ± SD.	5.78 ± 1.82	2.83 ± 1.66	^{Z}p < 0.001*	1.17 ± 0.42	0.79 ± 0.22	^{Z}p < 0.001*
Median (IQR)	5.64 (4.60 – 6.06)	2.33 (1.39 – 3.40)		1.17 (1.0 – 1.26)	0.72 (0.61 – 0.97)	
APRI score						
Mean ± SD.	2.19 ± 0.90	0.62 ± 0.31	^{t}p < 0.001*	0.39 ± 0.19	0.19 ± 0.07	^{t}p < 0.001*
Median (IQR)	1.95 (1.46 – 2.80)	0.58 (0.39 – 0.91)		0.29 (0.27 – 0.56)	0.18 (0.14 – 0.21)	

FIB4: fibrosis score 4, APRI score: the index of aspartate aminotransferase to platelet ratio, SVR: Sustained viral response.
*: Statistically significant at P ≤ 0.05.
t: Paired *t*-test,
Z: Wilcoxon signed ranks test.

4. Discussion

Treatment of HCV patients with DAA has been proven to be very efficient in improvement of liver manifestations and extra hepatic complications (Tada et al. 2017; Mohanty et al. 2019).

HCV, besides hepatic manifestations, has been linked with endothelial dysfunction and chronic inflammation as an independent risk factor for cerebrovascular and CV events (Roed et al. 2014; Rafieian-Kopaei et al. 2014).

Recent studies have been reported that HCV eradication with DAAs enhanced vascular endothelial function and therefore decrease atherosclerosis and CV events (Di Minno et al. 2020; Nahon et al. 2017). Our study is the first that conducted to assess the effect of HCV eradication with new DAAs on endothelial dysfunction in Egyptian HCV monoinfected patients at different degrees of hepatic fibrosis (mild fibrosis and compensated cirrhosis) and without risk factors for endothelial dysfunction or CVD.

Our study showed that HCV clearance and achieving SVR following DAAs treatment improved endothelial dysfunction and coagulation abnormalities, and therefore decrease subclinical atherosclerosis through a decrease of VCAM-1, vWF: Ag and IL6 levels to a variable degree in both studied groups suffering from baseline endothelial dysfunction.

Our results were in line with the recent multicenter and prospective study (HEPCAR Study) of HCV patients treated by DAAs with long follow-up period which demonstrated significant reduction in levels endothelial dysfunction markers VCAM-1 and e-selectin at SVR point in all participants and continued in reduction during follow-up for one year (Muñoz-Hernández et al. 2020).

Our findings were also in consistent with J.S. Davis *et al.* (Davis et al. 2018) who observed that serum markers of endothelial dysfunction reduced during and post DAAs and no significant improvement in systemic inflammation as estimated by IL6, although J.S. Davis *et al.* limited their research to patients without significant fibrosis only and used different DAAs regimens to those included in our study. In addition, J.S. Davis *et al.* used serum Angiopoietin-2 and E-selectin as markers of endothelial dysfunction whereas our study used VCAM-1 and vWF: Ag.

The improvement of endothelial function after HCV eradication with new DAAs has been also documented by F.P. Schmidt *et al.* (Schmidt et al. 2018), who observed a significant decrease in circulating levels of the endothelial cell-adhesion molecules (VCAM-1, ICAM-1, E-Selectin) in 20 HCV non-cirrhotic patients treated with DAAs.

Our findings showed that endothelial dysfunction markers (VCAM-1, vWF) were significantly higher ($P > 0.001$) in cirrhotic patients compared to patients with mild fibrosis before treatment. These results supported by Michele Barone *et al.* (Barone et al. 2015) who showed that endothelial dysfunction links with liver fibrosis in chronic HCV infection and reported that developed endothelial dysfunction in patients with liver cirrhosis was 6.9 higher compared to patients with mild liver fibrosis. The findings of this study are in agreement with previous trails which demonstrated that the higher level of sVCAM-1 and vWF: Ag was associated with the degree of liver fibrosis in chronic HCV infected patients (Antonova et al 2013).

The improvement in endothelial dysfunction markers (sVCAM-1 and vWF: Ag), which is the initial step for subclinical atherosclerosis after SVR with DAAs in patients with cirrhosis, was observed in this study. These results agreed with the study which showed that viral clearance with DAAs improved carotid atherosclerosis in patients with advanced fibrosis/compensated cirrhosis (Petta et al. 2018). While other trials are in contrast with these results (Ichikawa et al. 2019; Di Minno et al. 2020).

We had expected that the improvement in the endothelial function and vascular healing is more significant in patients without cirrhosis than cirrhotic patients as these patients had lower degree of endothelial dysfunction than the other group and this expectation was concluded from similar results in previous study (Di Minno et al. 2020). In contrast, we reported a more significant decrease in VCAM1 and vWF levels at SVR in cirrhotic patients who had higher degree of endothelial dysfunction than the other group. This is in agreement with Muñoz-Hernandez *et al.* (Muñoz-Hernández et al. 2020) who found that successful DAA treatment in HCV patients without a history of CV disease has been shown to decrease the risk of CV disease by improving endothelial function, which showed more improvement in patients with worse baseline status. These results supported by Nicola Coppola *et al.* (Coppola et al. 2019) who found that the achievement of SVR12 by DAAs regimens has a significant improvement in renal dysfunction as extrahepatic manifestation of HCV infection more in patients with CKD or cirrhosis than patients without cirrhosis or CKD at baseline.

From our findings, we suggest that HCV infection is the main factor directly affect the vascular endothelium and this assumption is confirmed by the significant improvement in endothelial function markers occurred after HCV clearance by DAAs, regardless to a significant change in systemic inflammation in both groups. Further, these findings are confirmed by a continuous decrease in endothelial dysfunction markers after the end treatment. The same was reported by Muñoz-Hernandez *et al.* (Muñoz-Hernández et al. 2020) where a continuous improvement in vascular function markers at SVR time and 1 year after treatment was observed. Therefore, the effect is unlikely to be associated with the pleiotropic effect of DAA.

Proteins of HCV can stimulate endothelial cells activation and elevate oxidative stress in these cells (Urbaczek et al. 2014). Moreover, the virus invaded carotid plaques indicating its local atherogenic role in plaque tissue (Boddi et al. 2007). However, if endothelial cells are not affected by HCV, proteins derived from HCV tend to cause endothelial cell inflammation probably by increasing IL8 production by NF-Kappa B dependent pathways (Balasubramanian et al. 2005). As a consequence, HCV infected patients have a pro-coagulant state (Papatheodoridis et al. 2003; Poujol-Robert et al. 2004) as vascular endothelial damage leading to lack of a healthy barrier between vessel and blood components.

vWf is a glycoprotein (GP) that is necessary for normal hemostasis. Serum vWf concentrations are elevated after injury to endothelial cells (Boneu et al. 1975). Interestingly, by estimating vWf level we found a significant decrease in vWf concentrations in both groups after antiviral treatment, supporting a vascular healing and improvement of coagulation state in our patients after successful treatment. As elevated vWf levels increased risk, not only of portal vein thrombosis, but also venous thromboembolism, pulmonary embolism, cerebrovascular and CV ischemic events, so this may have an important role in reducing morbidity and mortality in these patients.

There is no evidence of all the pathways causing the recovery of endothelial function after treatment with DAAs. Certain medications affect endothelial function through modifying metabolic pathways. Some studies revealed that infection with HCV could be linked with increased risk of insulin resistance (Moucari et al. 2008) and the achievement of SVR with DAAs therapy is related to risk reduction of insulin resistance (Delgado–Borrego et al. 2010) and a reduction of type 2 diabetes mellitus development (Arase et al. 2009). Interestingly, our results confirmed such findings as compared to baseline data, both groups showed significant reduction in HbA1C after SVR.

Regarding liver fibrosis assessment, the present study revealed that 3 months after DAAs therapy a significant reduction in noninvasive values of APRI and FIB-4 indices was reported in both groups compared to baseline data. These results have verified that DAAs could reduce liver inflammation and fibrosis in patients with HCV in a little period following SVR achievement. Our findings are in matching with past researches which showed a significant decrease in APRI and FIB-4 after DAA therapy (Haseltine et al. 2015; Alem et al. 2018).

The study has some limitations include the lack of vascular endothelial function examination by brachial artery flow–mediated dilation assessment at each time-point (pretreatment with DAA and at SVR), and was dependent only on the assessment of circulating markers of endothelial dysfunction. Relatively small sample size and limited time of follow-up period are considered another limitation of our study.

5. Conclusion

In conclusion, DAAs used for treatment of chronic HCV infection has been proven to be very efficient in improvement the degree of endothelial function and therefore decrease risk of atherosclerosis and CV events in Egyptian HCV infected patients with different levels of hepatic fibrosis and with no risk factors for endothelial dysfunction or CVD. Besides the improvement of liver inflammation and function.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

We thank all of the patients for their participation in this study and Viral Hepatitis Center, Damanhour Fever Hospital for collecting patient data.

Funding

The authors did not receive a grant from any organizations.

References

Abdel-Razek, W., Waked, I., 2015. Optimal therapy in genotype 4 chronic hepatitis C: finally cured? Liver Int. 35, 27–34. https://doi.org/10.1111/liv.12724.

Adinolfi, L.E., Zampino, R., Restivo, L., Lonardo, A., Guerrera, B., Marrone, A., Nascimbeni, F., Florio, A., Loria, P., 2014. Chronic hepatitis C virus infection and atherosclerosis: clinical impact and mechanisms. World J. Gastroenterol. 20 (13), 3410–3417. https://doi.org/10.3748/wjg.v20.i13.3410.

Alem, S.A., Said, M., Anwar, I., Abdellatif, Z., Elbaz, T., Eletreby, R., AbouElKhair, M., El-Serafy, M., Mogawer, S., El-Amir, M., El-Shazly, M., Hosny, A., Yosry, A., 2018. Improvement of liver stiffness measurement, acoustic radiation force impulse measurements, and noninvasive fibrosis markers after direct-acting antivirals for hepatitis C virus G4 recurrence post living donor liver transplantation: Egyptian cohort. J. Med. Virol. 90 (9), 1508–1515. https://doi.org/10.1002/jmv.25210.

Ambrosino, P., Tarantino, L., Criscuolo, L., Nasto, A., Celentano, A., Di Minno, M.N.D.J. T., 2016. The risk of venous thromboembolism in patients with hepatitis C. Thromb Haemost. 116 (11), 958–966. https://doi.org/10.1160/TH16-03-0185.

Ampuero, J., Romero-Gómez, M., 2015. Assessing cardiovascular risk in hepatitis C: An unmet need. World J. Hepatol. 7 (19), 2214–2219. https://doi.org/10.4254/wjh.v7.i19.2214.

Antonova T, Romanova M, Lymar I.V, 2013. Endothelial dysfunction markers (VCAM-1, vWF) in chronic hepatitis C. Ter Arkh. 2013;85(12):86-9. URL: https://ter-arkhiv.ru/0040-3660/article/view/31399.

Arase, Y., Suzuki, F., Suzuki, Y., Akuta, N., Kobayashi, M., Kawamura, Y., Yatsuji, H., Sezaki, H., Hosaka, T., Hirakawa, M., Ikeda, K., Kumada, H., 2009. Sustained virological response reduces incidence of onset of type 2 diabetes in chronic hepatitis C. Hepatology. 49 (3), 739–744. https://doi.org/10.1002/hep.22703.

Babiker, A., Jeudy, J., Kligerman, S., Khambaty, M., Shah, A., Bagchi, S., 2017. Risk of cardiovascular disease due to chronic hepatitis C infection: a review. J. Clin. Transl. Hepatol. 5 (4), 343–362. https://doi.org/10.14218/JCTH.2017.00021.

Balasubramanian, A., Munshi, N., Koziel, M.J., Hu, Z., Liang, T.J., Groopman, J.E., Ganju, R.K., 2005. Structural proteins of Hepatitis C virus induce interleukin 8 production and apoptosis in human endothelial cells. J. Gen. Virol. 86 (12), 3291–3301. https://doi.org/10.1099/vir.0.81056-0.

Barone, M., Viggiani, M.T., Amoruso, A., Schiraldi, S., Zito, A., Devito, F., Cortese, F., Gesualdo, M., Brunetti, N., Di Leo, A., Scicchitano, P., Ciccone, M.M., 2015. Endothelial dysfunction correlates with liver fibrosis in chronic HCV infection. Gastroenterol. Res. Pract. 2015, 1–7. https://doi.org/10.1155/2015/682174.

Boddi, M., Abbate, R., Chellini, B., Giusti, B., Solazzo, V., Sofi, F., Pratesi, G., Pratesi, C., Gensini, G., Zignego, A.L., 2007. HCV infection facilitates asymptomatic carotid atherosclerosis: preliminary report of HCV RNA localization in human carotid plaques. Dig. Liver Dis. 39, S55–S60. https://doi.org/10.1016/S1590-8658(07)80012-0.

Boneu, B., Abbal, M., Plante, J., Bierme, R., 1975. Factor-VIII complex and endothelial damage. Lancet. 305 (7922), 1430. https://doi.org/10.1016/s0140-6736(75)92650-1.

Chen, C.-H., Chang, L.-T., Tung, W.-C., Chen, Y.-L., Chang, C.-L., Leu, S., Sun, C.-K., Tsai, T.-H., Tsai, I.-T., Chang, H.-W., Yip, H.-K., 2012. Levels and values of circulating endothelial progenitor cells, soluble angiogenic factors, and mononuclear cell apoptosis in liver cirrhosis patients. J. Biomed. Sci. 19 (1), 66. https://doi.org/10.1186/1423-0127-19-66.

Coppola, N., Portunato, F., Buonomo, A.R., Staiano, L., Scotto, R., Pinchera, B., De Pascalis, S., Amoruso, D.C., Martini, S., Pisaturo, M., Coppola, C., Gentile, I., 2019. Interferon-free regimens improve kidney function in patients with chronic hepatitis C infection. J. Nephrol. 32 (5), 763–773. https://doi.org/10.1007/s40620-019-00608-z.

Davis, J.S., Young, M., Lennox, S., Jones, T., Piera, K., Pickles, R., Oakley, S., 2018. The effect of curing hepatitis C with direct-acting antiviral treatment on endothelial function. Antivir. Ther. 23 (8), 687–694. https://doi.org/10.3851/IMP3257.

de Castro, I.F., Micheloud, D., Berenguer, J., Guzmán-Fulgencio, M., Catalán, P., Miralles, P., Álvarez, E., López, J.C., Cosín, J., Lorente, R., Muñoz-Fernández, M.A., Resino, S., 2010. Hepatitis C virus infection is associated with endothelial dysfunction in HIV/hepatitis C virus coinfected patients. AIDS. 24 (13), 2059–2067. https://doi.org/10.1097/QAD.0b013e32833ce54d.

Delgado-Borrego, A., Jordan, S.H., Negre, B., Healey, D., Lin, W., Kamegaya, Y., Christofi, M., Ludwig, D.A., Lok, A.S., Chung, R.T., 2010. Reduction of insulin resistance with effective clearance of hepatitis C infection: results from the HALT-C trial. Clin. Gastroenterol. Hepatol. 8 (5), 458–462. https://doi.org/10.1016/j.cgh.2010.01.022.

Di Minno MND, Ambrosino P, Buonomo AR ,Pinchera B, Calcaterra I,Crispo M, Scotto R, Borgia F, Mattia C, Gentile I, 2020. Direct-acting antivirals improve endothelial function in patients with chronic hepatitis: a prospective cohort study. Intern Emerg Med. 2020;15(2):263–71.doi: 10.1007/s11739-019-02163-8.

Elsharkawy A, Fouad R, El Akel W, El Raziky M, Hassany M, Shiha G, Said M, Motawea I, El Demerdash T, Seif S, Gaballah A, El ShazlyY , Makhlouf M A M, Waked I, Abdelaziz A O, Yosry A, El Serafy M, Thursz M, Doss W, Esmat G, 2017. Sofosbuvir-based treatment regimens: real life results of 14 409 chronic HCV genotype 4 patients in Egypt. Aliment Pharmacol. Ther. 2017;45(5):681–7. https://doi.org/10.1111/apt.13923.

Gentile, I., Viola, C., Graf, M., Liuzzi, R., Quarto, M., Cerini, R., Piazza, M., Borgia, G., 2009. A simple noninvasive score predicts gastroesophageal varices in patients with chronic viral hepatitis. J Clin. Gastroenterol. 43 (1), 81–87. https://doi.org/10.1097/MCG.0b013e318157464b.

Gentile, I., Fusco, F., Buonomo, A.R., Scotto, R., Zappulo, E., Pinchera, B., Persico, F., Califano, G., Borgia, G., Longo, N., 2018. Prevalence and risk factors of erectile dysfunction in patients with hepatitis B virus or hepatitis C virus or chronic liver disease: results from a prospective study. Sex Health. 15 (5), 408–412. https://doi.org/10.1071/SH17168.

Hansson, G.K., 2005. Inflammation, atherosclerosis, and coronary artery disease. N Engl. J. Med. 352 (16), 1685–1695. https://doi.org/10.1056/NEJMra043430.

Haseltine, E., Penney, M., George, S., Kieffer, T., 2015. Successful treatment with telaprevir-based regimens for chronic hepatitis C results in significant improvements to serum markers of liver fibrosis. J. Viral. Hepat. 22 (9), 701–707. https://doi.org/10.1111/jvh.12382.

Hinderliter, A.L., Caughey, M., 2003. Assessing endothelial function as a risk factor for cardiovascular disease. Curr. Atheroscler Rep. 5 (6), 506–513. https://doi.org/10.1007/s11883-003-0042-x.

Ichikawa, T., Miyaaki, H., Miuma, S., Motoyoshi, Y., Narita, S., Toda, S., Takahashi, Y., Honda, T., Yajima, H., Uehara, R., Hino, N., Hori, T., Hirata, R., Taura, N., Nakao, K., 2019. Carotid intima-media thickness and small dense low-density lipoprotein cholesterol increase after one year of treatment with direct-acting antivirals in patients with hepatitis C virus infection. Intern. Med. 58 (9), 1209–1215. https://doi.org/10.2169/internalmedicine.1514-18.

Kouyoumjian, S.P., Chemaitelly, H., Abu-Raddad, L.J., 2018. Characterizing hepatitis C virus epidemiology in Egypt: systematic reviews, meta-analyses, and meta-regressions. Sci. Rep. 8 (1), 1661. https://doi.org/10.1038/s41598-017-17936-4.

Li, J., Gordon, S.C., Rupp, L.B., Zhang, T., Trudeau, S., Holmberg, S.D., Moorman, A.C., Spradling, P.R., Teshale, E.H., Boscarino, J.A., Schmidt, M.A., Daida, Y.G., Lu, M., 2019. Sustained virological response to hepatitis C treatment decreases the incidence of complications associated with type 2 diabetes. Aliment Pharmacol. Ther. 49 (5), 599–608. https://doi.org/10.1111/apt.15102.

Lombardi A, Mondelli MU, Hepatitis ESGfV,2019. Hepatitis C: Is eradication possible? Liver Int.39(3):416-–6. https://doi.org/10.1111/liv.14011.

Masiá, M., Padilla, S., Robledano, C., Ramos, J.M., Gutiérrez, F., 2011. Evaluation of endothelial function and subclinical atherosclerosis in association with hepatitis C virus in HIV-infected patients: a cross-sectional study. BMC Infect. Dis. 11 (1), 265. https://doi.org/10.1186/1471-2334-11-265.

Mohanty, A., Salameh, S., Butt, A., 2019. Impact of direct acting antiviral agent therapy upon extrahepatic manifestations of hepatitis C virus infection. Curr HIV/AIDS Rep. 16 (5), 389–394. https://doi.org/10.1007/s11904-019-00466-1.

Moucari, R., Asselah, T., Cazals-Hatem, D., Voitot, H., Boyer, N., Ripault, M.P., Sobesky, R., Martinot-Peignoux, M., Maylin, S., Nicolas-Chanoine, M., Paradis, V., Vidaud, M., Valla, D., Bedossa, P., Marcellin, P., 2008. Insulin resistance in chronic hepatitis C: association with genotypes 1 and 4, serum HCV RNA level, and liver fibrosis. Gastroenterology. 134 (2), 416–423. https://doi.org/10.1053/j.gastro.2007.11.010.

Muñoz-Hernández, R., Ampuero, J., Millán, R., Gil-Gómez, A., Rojas, Á., Macher, H.C., Gallego-Durán, R., Gato, S., Montero-Vallejo, R., Rico, M.C., Maya-Miles, D., Sánchez-Torrijos, Y., Soria, I.C., Stiefel, P., Romero-Gómez, M., 2020. Hepatitis C Virus Clearance by Direct-Acting Antivirals Agents Improves Endothelial Dysfunction and Subclinical Atherosclerosis: HEPCAR Study. Clin. Transl. Gastroenterol. 11 (8), e00203. https://doi.org/10.14309/ctg.0000000000000203.

Nahon P, Bourcier V, Layese R, Audureau E, Cagnot C, Marcellin P, Guyader D, Fontaine H, Larrey D, Lédinghen VD, Ouzan D, Zoulim F, Roulot D, Tran A, Bronowicki J, Zarski J, Leroy V, Riachi G, Calès P, Péron J, Alric L, Bourlière M, Mathurin P, Dharancy S, Blanc J, Abergel A, Serfaty L, Mallat A, Grangé J, Attali P, Bacq Y, Wartelle C, Dao T, Benhamou Y, Pilette C, Silvain C, Christidis C, Capron D, Bernard-Chabert B, Zucman D, Di Martino V, Thibaut V, Salmon D, Ziol M, Sutton A, Pol S, Roudot-Thoraval F, ANRS CO12 CirVir Group, 2017 . Eradication of hepatitis C virus infection in patients with cirrhosis reduces risk of liver and non-liver complications. Gastroenterology.152(1):142–56. e2. https://doi.org/10.1053/j.gastro.2016.09.009.

Oyake, N., Shimada, T., Murakami, Y., Ishibashi, Y., Satoh, H., Suzuki, K., Matsumory, A., Oda, T., 2008. Hepatitis C virus infection as a risk factor for increased aortic stiffness and cardiovascular events in dialysis patients. J. Nephrol. 21 (3), 345–353.

Panel, A.I.H.G., Chung, R.T., Davis, G.L., Jensen, D.M., Masur, H., Saag, M.S., Thomas, D. L., Aronsohn, A.I., Charlton, M.R., Feld, J.J., Fontana, R.J., Ghany, M.G., Godofsky, E. W., Graham, C.S., Kim, A.Y., Kiser, J.J., Kottilil, S., Marks, K.M., Martin, P., Mitruka, K., Morgan, T.R., Naggie, S., Raymond, D., Reau, N.S., Schooley, R.T., Sherman, K. E., Sulkowski, M.S., Vargas, H.E., Ward, J.W., Wyles, D.L., 2015. Hepatitis C guidance: AASLD-IDSA recommendations for testing, managing, and treating adults infected with hepatitis C virus. Hepatology. 62 (3), 932–954. https://doi.org/10.1002/hep.27950.

Papatheodoridis, G., Papakonstantinou, E., Andrioti, E., Cholongitas, E., Petraki, K., Kontopoulou, I., Hadziyannis, S.J., 2003. Thrombotic risk factors and extent of liver fibrosis in chronic viral hepatitis. Gut. 52 (3), 404–409. https://doi.org/10.1136/gut.52.3.404.

Petta, S., Adinolfi, L.E., Fracanzani, A.L., Rini, F., Caldarella, R., Calvaruso, V., Cammà, C., Ciaccio, M., Marco, V., Grimaudo, S., Licata, A., Marrone, A., Nevola, R., Pipitone, R., Pinto, A., Rinaldi, L., Torres, D., Tuttolomondo, A., Valenti, L., Fargion, S., Craxì, A., 2018. Hepatitis C virus eradication by direct-acting antiviral agents

improves carotid atherosclerosis in patients with severe liver fibrosis. J. Hepatol. 69 (1), 18–24. https://doi.org/10.1016/j.jhep.2018.02.015.

Petta S, Maida M, Macaluso FS, Barbara M, Licata A, Craxì A, Cammà C,2016. Hepatitis C virus infection is associated with increased cardiovascular mortality: a meta-analysis of observational studies. Gastroenterology.150 (1):145–55. e4. https://doi.org/10.1053/j.gastro.2015.09.007.

Poujol-Robert, A., Rosmorduc, O., Serfaty, L., Coulet, F., Poupon, R., Robert, A., 2004. Genetic and acquired thrombotic factors in chronic hepatitis C. Am. J. Gastroenterol. 99 (3), 527–531. https://doi.org/10.1111/j.1572-0241.2004.04092.x.

Rafieian-Kopaei, M., Setorki, M., Doudi, M., Baradaran, A., Nasri, H., 2014. Atherosclerosis: process, indicators, risk factors and new hopes. Int J Prev Med. 5 (8), 927.

Ridker, P.M., Brown, N.J., Vaughan, D.E., Harrison, D.G., Mehta, J.L., 2004. Established and emerging plasma biomarkers in the prediction of first atherothrombotic events. Circulation. 109 (25_suppl_1), IV-6-IV-19. https://doi.org/10.1161/01.CIR.0000133444.17867.56.

Roed T, Kristoffersen US, Knudsen A, Wiinberg N, Lebech A-M, Almdal T, Thomsen RW, Kjær A,Weis N, 2014. Increased prevalence of coronary artery disease risk markers in patients with chronic hepatitis C–a cross-sectional study.Vasc Health Risk Manag. 10:55–62. https://doi.org/10.2147/VHRM.S53557.

Roed, T., Lebech, A.M., Kjaer, A., Weis, N., 2012. Hepatitis C virus infection and risk of coronary artery disease: a systematic review of the literature.Clin Physiol Funct. Imaging. 32 (6), 421–430. https://doi.org/10.1111/j.1475-097X.2012.01152.x.

Romero-Gómez, M., Viloria, M.D.M., Andrade, R.J., Salmerón, J., Diago, M., Fernández-Rodríguez, C.M., Corpas, R., Cruz, M., Grande, L., Vázquez, L., Muñoz-De-Rueda, P., López-Serrano, P., Gila, A., Gutiérrez, M.L., Pérez, C., Ruiz-Extremera, A., Suárez, E., Castilloet, J., 2005. Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients. Gastroenterology. 128 (3), 636–641. https://doi.org/10.1053/j.gastro.2004.12.049.

Schmidt FP, Zimmermann T, Wenz T , Schnorbus B , Ostad A, Feist C, Grambihler A, Schattenberg JM, Sprinzl MF, Münzel T, 2018a. Interferon-and ribavirin-free therapy with new direct acting antivirals (DAA) for chronic hepatitis C improves vascular endothelial function. Int. J. Cardiol. 271:296-300. doi: 10.1016/j.ijcard.2018.04.058.

Sitia, S., Tomasoni, L., Atzeni, F., Ambrosio, G., Cordiano, C., Catapano, A., Tramontana, S., Perticone, F., Naccarato, P., Camici, P., Picano, E., Cortigiani, L., Bevilacqua, M., Milazzo, L., Cusi, D., Barlassina, C., Sarzi-Puttini, P., Turiel, M., 2010. From endothelial dysfunction to atherosclerosis. Autoimmunity Rev. Autoimmun Rev. 9 (12), 830–834. https://doi.org/10.1016/j.autrev.2010.07.016.

Sterling, R.K., Lissen, E., Clumeck, N., Sola, R., Correa, M.C., Montaner, J., Sulkowski, M.S., Torriani, F.J., Dieterich, D.T., Thomas, D.L., Messinger, D., Nelson, Mark, 2006. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology. 43 (6), 1317–1325. https://doi.org/10.1002/hep.v43:610.1002/hep.21178.

Tada, T., Kumada, T., Toyoda, H., Mizuno, K., Sone, Y., Kataoka, S., Hashinokuchi, S., 2017. Improvement of liver stiffness in patients with hepatitis C virus infection who received direct-acting antiviral therapy and achieved sustained virological response. J. Gastroenterol. Hepatol. 32 (12), 1982–1988. https://doi.org/10.1111/jgh.13788.

Urbaczek, A.C., Ribeiro, L.Cd.A., Ximenes, V.F., Afonso, A., Nogueira, C.T., Generoso, W.C., Alberice, J.V., Rudnicki, M., Ferrer, R., Fonseca, L.M.D., Costa, P.I.D., 2014. Inflammatory response of endothelial cells to hepatitis C virus recombinant envelope glycoprotein 2 protein exposure. Mem. Inst. Oswaldo Cruz. 109 (6), 748–756. https://doi.org/10.1590/0074-0276140090.

van der Meer, A.J., 2015. Association between antiviral treatment and extrahepatic outcomes in patients with hepatitis C virus infection. Gut. 64 (3), 364–366. https://doi.org/10.1136/gutjnl-2014-308745.

Xu X-Y, Song R-X, Zhai Y-H, Wu X-F, Ai W-S, Liu H-B, 2014.The effectiveness of noninvasive biomarkers to predict hepatitis B-related significant fibrosis and cirrhosis: a systematic review and meta-analysis of diagnostic test accuracy. PLoS One. 9(6): e100182. https://doi.org/10.1371/journal.pone.0100182.

Zaki MSE,2017. The effect of Hepatitis C Virus infection on cardiovascular complications in end stage kidney disease patients on regular hemodialysis. Electron Physician. 9(2):3857.doi: 10.19082/3857.