Insulin Resistance and Portal Hemodynamic Changes before and after Treatment with New Direct Antiviral Drugs in Chronic Hepatitis C

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Background and study aim: Insulin resistance is typically associated with chronic hepatitis C virus and its improvement after treatment of HCV with new direct antiviral drugs may change portal hemodynamics. This study aimed at assessing changes in HOMA-IR, APRI score and platelet count/spleen diameter ratio as fibrotic markers and changes of portal hemodynamics after treatment with direct- acting antiviral drugs.

Patients and Methods: The Study included 100 chronic HCV patients who were eligible for treatment with direct-acting antiviral drugs. HOMA-IR, APRI score and platelet count/ spleen diameter ratio and changes of portal hemodynamics were calculated.

Results: The noninvasive indices of liver fibrosis were assessed before and after HCV treatment. Insulin resistance improved significantly after treatment from (3.2±0.9) to (2.9±0.7). Doppler parameters of the studied group also showed marked improvement after treatment where the portal vein flow velocity and flow volume increased (15.8 ±1.7 vs 16.1±1.3) and (728.3±128.2 vs 1059.9±29.6) respectively. Hepatic artery resistive index and calculated congestive index of portal vein also, showed improvement (0.6±0.1 vs 0.49±0.1) (0.077 ±0.014 vs 0.051 ± 0.017) respectively.

Conclusion: Direct-acting antiviral drugs may reduce inflammatory and fibrotic sequelae of HCV infection. Also, portal hypertensive indices improved and HOMA-IR, APRI score and platelet count/splenic diameter ratio show improvement.

INTRODUCTION

Persistence of HCV viremia for more than six months is considered as a chronic infection **[1]**. The rise in portal pressure, increased intra-hepatic vascular opposition secondary to sinusoidal fibrosis, obstruction in the portal venules terminals, the decline in intra-hepatic nitric oxide and elevated levels of intra- hepatic vasoconstrictor substances leads to the occurrence of portal hypertension **[2]**. Eradication of HCV infection with DAAs leads to reduction of liver fibrosis stage and hence can reduce disease-related complications and portal hypertension **[3,4]**. Serum fibrosis predictors and Doppler ultrasound parameters are changed with worsening of the stage of liver fibrosis **[5,6]**. Insulin resistance which occurs in HCV infections is associated with hepatic fibrosis progression and is measured using the HOMA-IR score **[7,8]**. Insulin resistance (IR) can affect hepatic stellate cells through increasing the level of the connective tissue growth factor (CTGF) which increases the production of extracellular matrix **[9]**. HCV-induced IR occurs due to direct inhibition of hepatitis C virus on the insulin signaling pathway as insulin modifies the endothelial production of nitric oxide and endothelin and can increase the rate of hepatic fibrosis progression to cirrhosis **[10,11]**.

AIM OF THE WORK

The present work aimed at assessing changes in HOMA-IR, APRI score and platelet count/spleen diameter ratio as non-invasive fibrotic markers and at assessing changes of portal flow after treatment with direct-acting antiviral drugs.

PATIENTS AND METHODS

Study design:
This prospective cohort study was conducted at the Tropical Medicine Department, Faculty of Medicine, Zagazig University between July 2018 and March 2019.

Patients:
This study included 100 patients with chronic HCV induced liver disease who were eligible for treatment with direct- acting antiviral drugs.

Inclusion criteria
Patients with a confirmed diagnosis of chronic HCV through the positivity of PCR were included in the study.

Exclusion criteria
Exclusion of patients from our study if they had:

- Advanced stage of cirrhosis (child classes B and C).
- Other liver disease (hepatitis B virus, autoimmune liver disease, Wilson's disease, hemochromatosis, and α1antitrypsin deficiency).
- Patients with ascites or hepatic encephalopathy.
- Hepatocellular carcinoma.
- Portal vein thrombosis.
- Body mass index ≥ 30 kg/m^2.
- History of diabetes.
- Treatment with beta-blockers, diuretics, or other vasoactive drugs.
- Patients with SVR achievement failure.

Methods
Our patients received treatment according to the National protocol for the therapy of HCV by Sofosbuvir (400 mg tab) plus Daclatasvir (60 mg tab) + ribavirin (600-800 mg cap) daily for 12 weeks and were followed up after achieving SVR.

The following was done to all patients:
1- Detailed history and physical examination.
2- Body mass index (BMI) calculation
3-Routine laboratory investigations; Complete blood picture, Liver function tests: serum bilirubin (total and direct), serum albumin, liver enzymes (ALT and AST), Renal function tests (serum creatinine and blood urea), Coagulation profile (PT, INR and PC), Fasting plasma glucose in addition to theVirologic investigations: ‣ Viral markers: HCV Abs and Hbs Ag by ELISA by (STATFAX 3000, USA). ‣Quantitative HCV RNA PCR by (TAQMAN REAL TIME PCR). Fasting insulin (μU/mL) was assessed by immunoenzymetric method.

Insulin resistance was determined by the HOmeostasis Model Assessment for Insulin Resistance (HOMA-IR) through the following equation: (HOMA-IR) = [fasting insulin (μU/mL)X fasting glucose (mmol/L)]/22.5.

5- Pelvi-abdominal ultrasonography and colored Doppler study: ultrasonography was used to define cirrhosis and colored Doppler was performed before and 6 months after completion of treatment by DAAs for 12 weeks. All measurements were done by a single specialist using color Doppler ultrasonography with subjects in the supine or left lateral position. A Power Vision SSA-380A system (Esaotemylab device) with multi hertz (3- to 5-MHz) convex and sector pulsed probes was used. Sono-graphic examinations were carried out 8 hours after the last meal.

In this study, four parameters were measured by Doppler ultrasound:
- Portal vein velocity (cm/sec): was measured directly using color Doppler ultrasound.
- Hepatic artery resistive index: the RI is calculated as follows: RI = (peak systolic velocity - end diastolic velocity) / peak systolic velocity.
- Portal vein congestive index: the CI is calculated as follows: Portal vein congestive index (CI) (cm.sec) = cross-sectional surface area/mean velocity.
- Portal vein flow volume (PVFV) (mL/min) =mean velocity X cross-sectional surface area X 60.

6- Calculation of modified Child-Pugh score.

7- Special investigations: AST/platelet count ratio score (APRI): APRI = [(AST/upper limit normal) X 100]/platelet count. Calculation of platelet count (/mm3)/spleen bipolar diameter (mm).

Statistical Analysis:
Data were checked, entered and analyzed using SPSS version 19 EPI-INFO 6 for data processing and statistics. Data were expressed as numbers and percentages for qualitative variables and mean ± standard deviation for quantitative ones. The comparison was performed using the student "t" test for comparison of means of two independent groups. Correlations were analyzed using the Spearman correlation coefficient. The significance of any test was detected if P<0.05.

RESULTS

The age of patients ranged from 33 to 65 years and sex distribution among them was nearly equal. Rural residence predominates in the studied population. All patients had average BMI. (Table 1)

All laboratory results of the studied group showed significant improvement after treatment. (Table 2)

Fasting serum insulin significantly improved after treatment (18.98±11.9 vs 14.3±2.5 p=0.004), and fasting plasma glucose mean value changed signficantly from (87.4±13.5) to (94.4±18.3) p= 0.04. HOMA-IR mean value recorded significant improvement from (3.2±0.9) to (2.9±0.7), p= 0.021. APRI score also improved to(0.4 ± 0.1) as the mean value prior starting DAAs (0.5 ± 0.2), also there was Platlet count spleen diameter ratio improvement (1726.2±585.29) to (1863.4± 536.7). (Table 3)

Doppler parameters changed significantly as hepatic artery resistive index improved from (0.6 ±0.1) to (0.49 ± 0.1) p=0.001.velocity of the flow had improved from(15.8±1.7) to (16.1± 1.3) (Cm/Sec.) p=0.002 and the flow improved significantly from (728.3±128.2) to (1059.9± 29.6) p=<0.001. Congestive index as well showed significant improvement ,the mean value from (0.077±0.014) to (0.051±0.017) p=<0.001. (Table 4)

Apart from age, body mass index and APRI score, portal vein velocity correlated with HOMA score and platelet count / spleen diameter ratio.

There was a significant correlation between hepatic artery resistive index, Portal vein flow and Portal vein congestive index with parameters of fibrosis. But, there was no correlation between portal vein diameter change and parameters of fibrosis.(Table 5) and (Figures 1-3)

Table (1) : Demographic data of the studied population (N = 100)

		No.	Percent
Sex	**Female**	47	47%
	Male	53	53%
Residence	**R**	67	67%
	U	33	33%
		Mean±SD	**Median (range)**
Age, years		43.5 ± 6.6	45 (33-65)
BMI		23.2 ± 2.4	23 (19-29)

Table (2): Mean serum laboratory data for all patients at base line and 6 months after end of treatment

	Before treatment	After 6 month	t-test	p
WBCs ($\times 10^3$/uL)	5.6 ± 1.6	6.92±2.41	1.1	0.26
HB (gm/dL)	12.7 ± 1.4	13.0±1.24	6.39	<0.001
PLT count ($\times 10^3$/uL)	210 ±96.7	234.6±49.79	5.02	<0.001
Albumin (gm/dL)	3.87 ± 0.4	4.1±0.59	4.19	<0.001
Bilirubin (mg/dL)	1.95 ± 0.26	1.1±0.09	7.52	<0.001
AST (IU/L)	40 ± 34.3	28.3±13.8	4.3	<0.001
ALT (IU/L)	42±13	30±12.6	7.02	<0.001
Creatinine (mg/dL)	0.74 ± 0.15	0.87±0.19	0.04	0.96
Prothrombin time (sec)	11.6±2.21	11.0±1.02	2.9	<0.001
Prothrombin conc. (%)	70.3±10.8	75.1±10.2	4.63	<0.001

Table (3): Changes in non-invasive parameters after DAAS treatment

	DAA Therapy		T-test	P
	Before treatment	**After 6 month**		
Fasting Insulin (µU/mL)	18.98 ±11.9	14.3±2.5	2.6	0.004
Fasting plasma glucose (mg/dL)	87.4 ± 13.5	94.4 ± 18.3	1.99	0.04
HOMA-IR	3.2 ± 0.9	2.9 ± 0.7	2.3	0.021
Platelet count / SBD ratio	1726.2 ± 585.29	1863.4 ±536.7	-3.88	<0.001
APRI score	0.5 ± 0.2	0.4 ± 0.1	3.3	0.001

Table (4): Color doppler of studied population before and after HCV treatment

	DAA Therapy		Test	P
	Before	**After**		
Portal Vein Diameter (cm).	0.9 ± 0.2	0.9 ± 0.1	0.9	0.345
Hepatic Artery RI.	0.6 ± 0.1	0.49 ± 0.1	-3.4	0.001
PVV (cm/sec)	15.8 ± 1.7	16.1 ± 1.3	3.1	0.002
PVFV (mL/min)	728.3 ± 128.2	1059.9 ± 29.6	16.1	<0.001
CI (cm. s)	0.077±0.014	0.051±0.017	12.2	<0.001

PVV: Portal vein velocity, PVFV: portal vein flow volume ,RI: resistive index and CI: congestive index.

Table (5): Correlations of different doppler ultrasound parameters with certain studied parameters

	Portal vein diameter (cm)^2		**Hepatic artery resistive index.**		**Portal vein velocity (cm^2/sec.)**		**Portal vein flow**		**Congestive index**	
	r	**P**	**r**	**p**	**r**	**P**	**r**	**P**	**r**	**P**
Age	-0.012	0.905	-0.012	-0.04	0.072	0.479	0.072	0.695	0.158	0.116
BMI	0.042	0.677	-0.004	-0.034	-0.047	0.642	-0.047	0.737	-0.044	0.667
HOMA.	0.141	0.161	0.146	-0.12	-0.293	**0.003**	-0.228	0.235	0.002	0.982
APRI.	-0.042	0.68	0.022	0.019	-0.009	0.926	-0.102	0.855	0.209	**0.037**
PLT/SD ratio	0.124	0.219	-0.302	0.092	0.354	**<0.001**	0.252	0.36	0.153	0.128

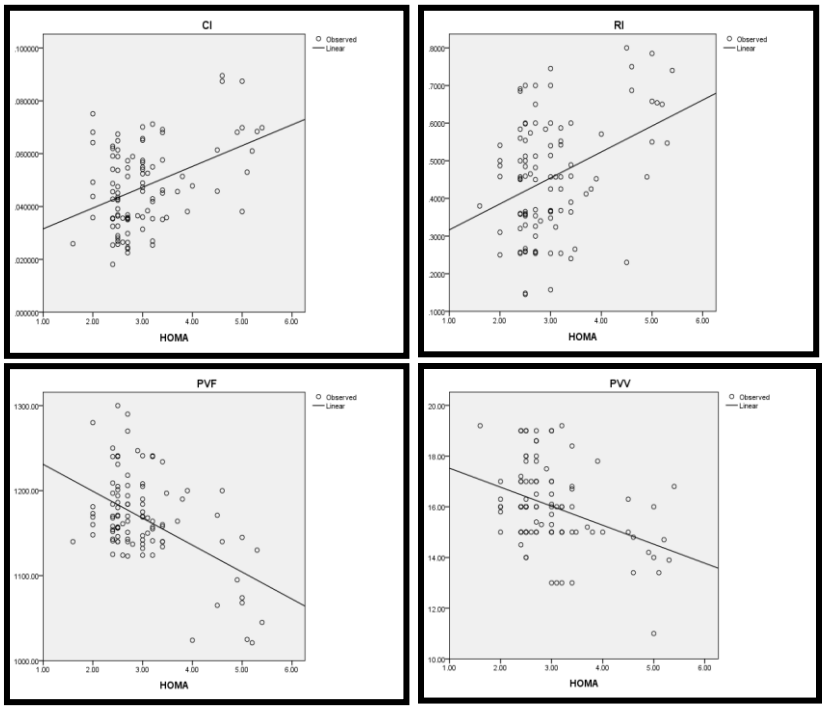

Figure (1): Graph presentation of correlation between HOMA –IR and certain parameters of Doppler ultrasound

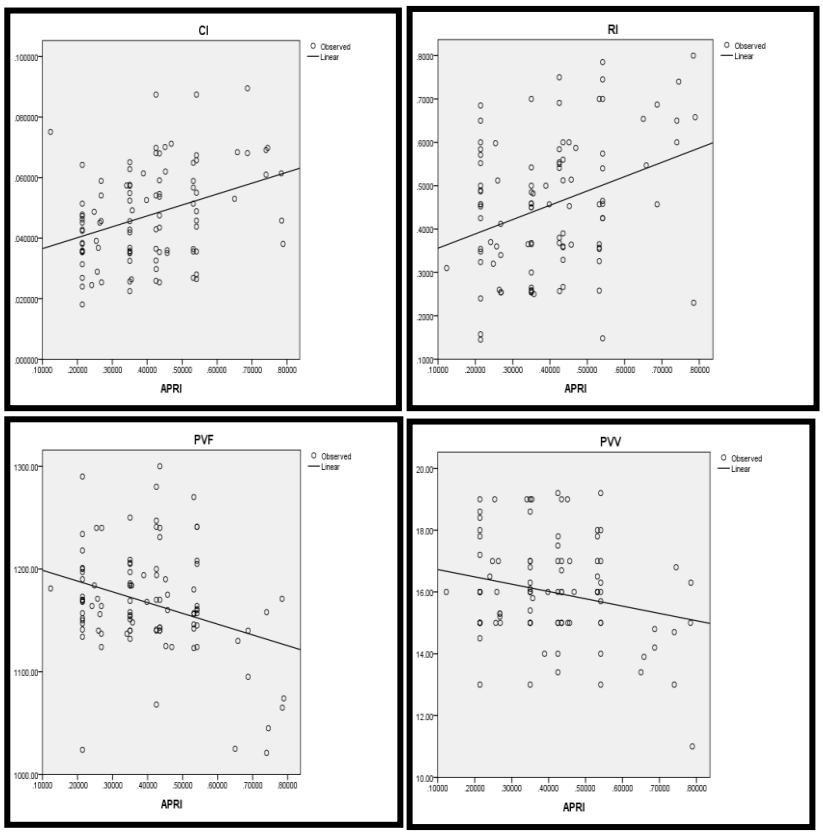

Figure (2): Graph presentation of correlation between APRI SCORE and certain parameters of Doppler ultrasound

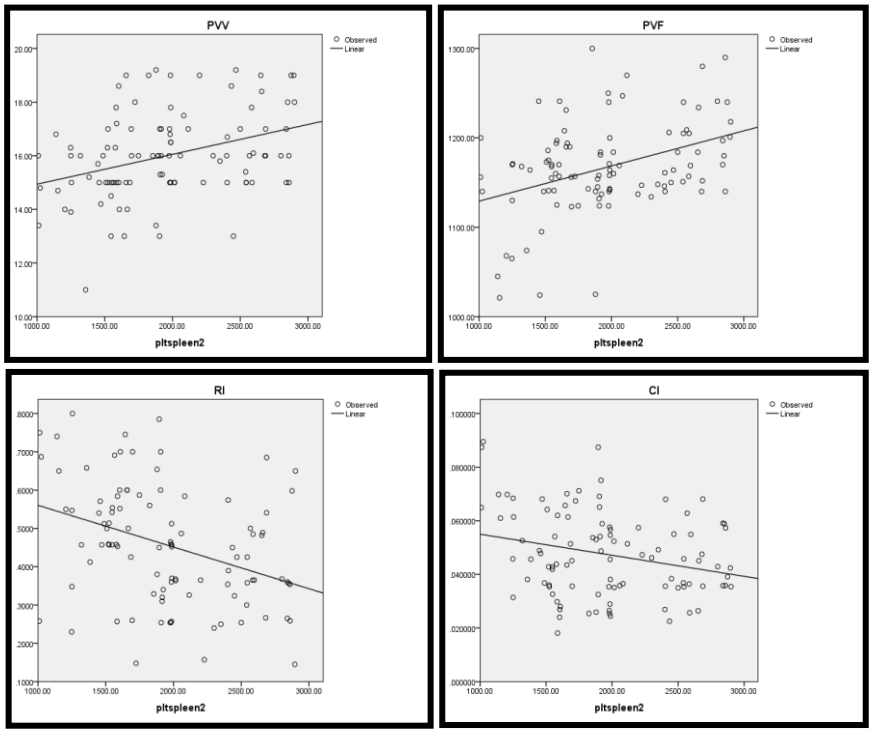

Figure (3): Graph presentation of correlation between platelets/spleen diameter ratio and certain parameters of Doppler ultrasound

DISCUSSION

Nearly 185 million people have been infected with HCV worldwide and 150 million people are chronically infected according to the WHO estimation **[12,13,14]**. About fifteen percent of the Egyptian population are positive for HCV antibodies and ten percent have an active infection with genotype 4 predominance **[15,16]**. Directly acting antiviral drugs improve hepatic inflammation, HCV induced insulin resistance, hepatic fibrosis and reduces liver disease complications **[17]**. In the present study, all laboratory parameters improved 3 months after the achievement of SVR and these results agree with Mahmoud et al. **[5]**.

In our study, there was a statistically significant improvement in the HOMA-IR score. This result agrees with results of Alzahaby et al. **[18]** who found significant decrease HOMA-IR after 12 weeks of achievement SVR in non-obese non-diabetic patients. Also, Moucari et al. **[19]** found a significant correlation between IR and high HCV RNA PCR values and HCV genotypes 1 and 4. Moreover, HCV clearance by DAAs and follow up for 12 weeks after successful SVR reverses or improves IR and stress on β-cells of islets of Langerhans and that this can prevent IR-related pathological sequelae such as progression of liver fibrosis, the occurrence of type 2 diabetes, metabolic syndrome and cardio-vascular affection **[20]**. This result was in line with that of Giannini et al. **[21]** who concluded that APRI decreased, while platelet count improved (from 143×106/mL [117-176] to 153 ×106/mL [139-186] with P= 0.003) and splenic bi-polar diameter decreased (from 120 mm [112-123] to 110 mm [102-116] with P= 0.0009) at the end of their follow-up for 60 weeks in patients with compensated chronic liver disease. Significant improvement in APRI scores at week 24 after completion of therapy regardless of the presence of cirrhosis in Chinese patients with HCV-Genotype 3 infection was reported by Tao et al. **[22]**. As regards Doppler parameters, there was marked improvement the portal vein velocity and flow volume, hepatic artery resistive index and the calculated congestive index of the portal vein. Only the portal vein diameter did not change significantly (0.9 ± 0.2 vs 0.9 ± 0.1).

There was a statistically significant negative correlation between portal vein velocity and HOMA-IR. There was a statistically significant positive correlation between portal vein velocity with APRI. There was no statistically significant correlation between portal vein

velocity with other parameters. Portal hypertension (PH) takes place due to fixed and functional revisable elements. The functional element of PH is related to reduced intra-hepatic nitric oxide with predominance in vasoconstrictor substances which can be improved with treatment. While the fixed element of portal hypertension is due to sinusoidal fibrosis, regeneration nodules pressure and the impediment in the terminal venules of the portal vein **[23]**. Bi-phasic change occurs in portal hypertension during HCV treatment. The first phase occurs rapidly during treatment and it is related to inflammation regression then the second phase which occurs late at six months to one year after treatment completion and it is related to fibrosis regression **[24]**. On this basis, we can verify the improvement of Doppler parameters associated with viral clearance and cessation of the hepatic inflammatory process. Also, Mahmoud et al. **[5]** concluded in a study on Egyptian patients that Doppler portal hypertensive parameters were enhanced with the improvement in fibroscan results after HCV therapy.

Finally, it can be concluded that treatment of chronic HCV with direct acting antiviral drugs may ameliorate inflammatory process and normalizes biochemical, parameters of portal hypertensive indices improve and liver fibrosis indirect markers namely HOMA-IR and APRI score improve adding to decrease long term sequ sequelae and complications of fibrosis and portal hypertension. It is recommended that follow-up intervals in noninvasive studies investigating regression of fibrosis and portal hypertension in patients with HCV-associated cirrhosis and SVR to DAA-based antiviral therapy should be expanded beyond 24 weeks after the end of treatment.

The limitations of the present study include: a relatively small number of patients and the need for histological follow up of fibrosis which may be extremely difficult.

Funding: NO
Conflict of interest: NO

Ethical consideration: After approval of ethical committees, Faculty of Medicine Zagazig University. Informed consents were taken from patients included in the study.

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