Impact of Sustained Virological Response on Metabolic Changes and Vitamin-D Status in Patients with Hepatitis C Viral Infection Treated by Direct Acting Antiviral drugs

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Background and aim: Hepatitis C virus (HCV) clearance is linked to better glycometabolic management in such individuals. The introduction of direct acting analogues (DAAs) in the previous decade has resulted in a significant improvement in the management of chronic hepatitis C infection. Until date, there has been a scarcity of research on the effects of these drugs on vitamin D (VD), lipid profile, and insulin resistance (IR).

Patients and Methods: In between April 2019 to February 2020, a cross sectional study was conducted on 100 patients with known chronic hepatitis infection and was eligible to therapy with DAAs. Lipid profile, vitamin D and insulin resistance was assessed at baseline and 3-months after therapy.

Results: Ninety percent of enrolled patients achieved sustained viral response (SVR). There were no significant changes in lipid profile after therapy with DAAs but at the same time IR was significantly decreased while VD was significantly increased.

Conclusion: Therapy with DAAs is effective in achievement of SVR and also, it could have beneficial effects on insulin resistance and vitamin D. Long term prospective studies on large scale are needed.

INTRODUCTION

HCV is a leading cause of chronic liver disease, with millions of individuals globally affected by its consequences. The recent discovery of a new class of direct-acting antivirals (DAA) has resulted in a cure rate of more than 90 percent. HCV eradication has been found to improve hepatic inflammation and functioning within a few months after cure, along with hepatic fibrosis **[1]**.

Vitamin D (VD) insufficiency is the most prevalent dietary deficiency, affecting millions of individuals throughout the world. Aside from a lack of sunshine exposure, chronic liver illness and genetic variations in the genes involved in VD metabolism are the leading causes of VD insufficiency. VD is largely engaged in calcium homeostasis, but it also plays key functions in the immune system, cell differentiation, and proliferation **[2]**.

VD deficiency is frequent in individuals with chronic hepatitis C (CHC), with a frequency close to 90%. Chronic liver disorders are one source of VD insufficiency, and the degree varies depending on the severity of the condition. Reduced liver metabolic functions as a result of hepatic damage and fibrosis are one potential explanation for this deficiency **[3]**.

The achievement of SVR decreases the risk of liver-related complications, liver transplantation, and mortality substantially. Despite earlier research indicating a decrease in cardio-vascular events in SVR patients,

new research suggests that HCV eradication may have an influence on certain comorbidities by raising total cholesterol serum levels and fractions. However, long-term evaluation of lipid profile and concomitant medication are unknown in patients with HCV cure after DAA **[4-6]**.

The current work was designed to evaluate change in lipid profile and IR in addition to level of VD in patients with CHC infection treated by direct acting antiviral agents.

Patients AND METHODS

Study setting and design

A cross sectional study was conducted at Outpatient Clinic of Tropical Medicine and Gastroenterology Department. It was conducted in the period from April 2019 to February 2020.

Inclusion& exclusion criteria

Any patient with chronic HCV infection and was eligible for treatment with DAAs was recruited in the study. The exclusion criteria included; diabetes mellitus, VD supplements within the last 3 months, decompensated liver cirrhosis, co-infection with hepatitis B virus (HBV) or human immunodeficiency virus, autoimmune diseases, history of steroid or immunosuppressive therapy, or patient's refusal

Methodology

The current study enrolled 100 patients with chronic HCV infection were diagnosed based on detectable HCV RNA with anti-HCV antibodies. All were subjected to complete history taking thorough clinical evaluation. Before initiation of therapy and three months after therapy; all patients were evaluated with abdominal ultrasound.

Also, the following laboratory data were ordered (at baseline and three months after therapy); complete blood count (CBC), liver function test, alpha fetoprotein, lipid profile, serum creatinine, random blood sugar (RBS). Also, insulin resistance determined via Homeostasis Model Assessment

(HOMA-IR) = fasting insulin (µu/ml) × fasting glucose (mg/dl)/ 405

An index value of > 2.5 was defined as IR. **[7]**.

Measurement of VD level

Level of VD was assessed by the Liaison 25(OH) VD total assay (DiaSorin, Saluggia, Italy), which was performed on the LIAISON® chemiluminescence analyzer following the manufacturer's instructions. Final concentrations are reported in ng/mL.

According to the Endocrine Society Practice Guidelines, the criteria for VD insufficiency/deficiency was defined as serum 25(OH) VD < 30 ng/mL. Serum 25(OH) VD concentrations < 20 ng/mL and between 20 and 29 ng/mL were defined as VD deficiency and VD insufficiency, respectively **[8]**.

Statistical analysis

Baseline characteristics are presented as the percentage or mean± standard deviation (SD). Categorical variables were analyzed using chi^2 test. Continuous variables with a normal distribution were compared within the same group (pre- and post-treatment) with paired t-test while comparison between different groups was done by Student t test. The normality of was assessed by Shapiro-Wilks test. All statistical analyses were performed in SPSS Version 22.0 (IBM Corp., Armonk, NY, USA). Differences were considered significant at $p<0.05$

RESULTS:

Baseline and follow up data of studied group (table 1):

Mean age of enrolled patients was 40.75 ± 11.86 years and majority (74%) of them was males. All patients received therapy for three months duration where majority of them (90%) received only sofosbuvir and daclatasvir while in 10 (10%) patients weight based ribavirin was added.

Nearly all patients showed normalization of aspartate transaminase and alanine transaminase. After therapy, there was significant reduction in HOMA (8.48 ± 2.98 vs. 5.06 ±1.35; $p< 0.001$). It was found that level of VD was significantly increased after DAAs therapy (27.56 ± 2.22 vs. 17.29 ± 4.56 (ng/ml); $p= 0.01$).

Change in lipid profile, VD and HOMA based on sustained virological response (table 2):

Out of the enrolled patients; 90 (90%) patients achieved 3-months SVR while 10 (10%) patients failed to achieve SVR. All baseline and 3-months

after therapy laboratory data showed no significant diffrences between both groups of patients with exception of significantly lower HOMA (4.56 ± 1.45 vs. 8.06 ± 2.22; *p*< 0.001) and higher VD (29.45 ± 3.09 vs. 19.50 ± 2.98 (ng/ml); *p*< 0.001) at 3-months after therapy among patients who achieved SVR.

It was found that 25 (27.8%) patients from those achieved SVR and 2 (20%) patients from those didn't achieve SVR had sufficient VD after therapy. Also, after therapy majority of both groups had insufficient VD.

In each separate group, there is no significant change in such parameters at 3rd month after therapy with exception of significant reduction in HOMA with significant increase in vitamin D in SVR group only.

Level of vitamin D in cirrhotic and non-cirrhotic patients (table 3):

Patients with liver cirrhosis had significantly higher HOMA at baseline (8.99 ± 2.11 vs. 6.56 ± 2.01; *p*=0.04) and at 3-months after therapy (7.89 ± 3.11 vs. 3.45 ± 1.10; *p*< 0.001) in comparison to those non-cirrhotic patients. Although baseline VD showed no significant difference between both groups, non-cirrhotic patients had significant higher VD at 3-months after therapy (26.11 ± 2.11 vs. 20.20 ± 3.09 (ng/ml); *p*< 0.001).

In each separate group, there is no significant change in HOMA and vitamin D at 3rd month after therapy with exception of significant reduction in HOMA with significant increase in vitamin D in non-cirrhotic group only.

Table (1): Baseline and follow up data of studied groups.

Item	Baseline data (n= 100)	3-months after therapy (n= 100)	*P* value
Age (years)	40.75 ± 11.86		
Male sex	74 (74%)		
BMI (kg/m^2)	27.59 ± 3.37		
Regimens of therapy SOF& Dacl SOF and Dacl with RIB	 90 (90%) 10 (10%)		
Leucocytes (10^3/ul)	6.23 ± 1.85	7.21± 1.09	0.22
Hemoglobin (mg/dl)	12.50 ± 1.44	11.51± 1.04	0.98
Platelets (10^3/ul)	215.96 ± 79.14	211.06 ± 60.45	0.20
Bilirubin (mg/dl)	0.81 ± 0.69	0.87 ± 0.19	0.45
AST (u/l)	45.55 ± 13.98	22.15 ± 6.78	**< 0.001**
ALT (u/l)	52.15 ± 11.43	29.23 ± 8.67	**< 0.001**
Serum albumin (g/dl)	4.11 ± 0.41	4.14 ± 0.21	0.34
INR	1.10 ± 0.13	1.09 ± 0.12	0.19
AFP (ng/dl)	9.98 ± 1.07	7.67 ± 2.11	0.37
Creatinine (mg/dl)	0.74 ± 0.16	0.78 ± 0.10	0.16
Cholesterol (mg/dl)	170.32 ± 30.50	169.12 ± 22.45	0.09
Triglyceride (mg/dl)	152.83 ± 32.6	160.22 ± 21.11	0.10
HDL (mg/dl)	38.32 ± 11.90	34.22 ± 9.11	0.22
LDL (mg/dl)	98.54 ± 13.35	101.11 ± 5.91	0.33
RBS (mg/dl)	109.78 ± 10.45	100.18 ± 9.09	0.06
HOMA	8.48 ± 2.98	5.06 ±1.35	**< 0.001**
Vitamin D (ng/ml)	17.29 ± 4.56	27.56 ± 2.22	**0.01**
Level of vitamin D Sufficient Insufficient Deficient	 17 (67.0%) 59 (29.0%) 24 (4.0%)	 27(27%) 60 (29.0%) 13 (4.0%)	**0.02**

Data was expressed as mean (SD), frequency (percentage). *P* value was significant if < 0.05. BMI: body mass index; AST: aspartate transaminase; ALT: alanine transaminase; INR: international randomized ratio; AFP: alpha feto-protein; LDL: low density lipoprotein; HDL: high density lipoprotein; RBS: fasting blood sugar; IR: insulin resistance; HOMA: homeostatic model assessment.

Table (2): Lipid profile, VD and HOMA based on sustained virological response.

Item	SVR group (n= 90)	No-SVR group (n= 10)	*P* value[2]
Cholesterol (mg/dl)			
Baseline	168.13 ± 24.56	171.23 ± 29.08	0.11
3-month after therapy	165.35 ± 21.11	170.12 ± 27.67	0.86
***P* value[2]**	0.44	0.50	
Triglyceride (mg/dl)			
Baseline	150.81 ± 45.67	155.67 ± 29.56	0.16
3-month after therapy	156.78 ± 22.34	161.20 ± 25.67	0.17
***P* value[2]**	0.09	0.07	
HDL (mg/dl)			
Baseline	40.11 ± 13.56	35.09 ± 12.34	0.92
3-month after therapy	33.40 ± 12.34	37.11 ± 7.89	0.15
***P* value[2]**	0.10	0.23	
LDL (mg/dl)			
Baseline	97.11 ± 10.34	100.45 ± 9.98	0.93
3-month after therapy	99.87 ± 8.89	105.10 ± 7.80	0.54
***P* value[2]**	0.20	0.64	
HOMA			
Baseline	8.11 ± 3.98	9.01 ± 2.01	0.33
3-month after therapy	4.56 ± 1.45	8.06 ±2.22	**< 0.001**
***P* value[2]**	**0.01**	0.07	
Vitamin D (ng/ml)			
Baseline	18.29 ± 2.56	16.05 ± 3.33	0.16
3-month after therapy	29.45 ± 3.09	19.50 ± 2.98	**< 0.001**
***P* value[2]**	**0.03**	0.19	
Level of vitamin D			
At baseline			0.23
Sufficient	15 (16.7%)	2 (20%)	
Insufficient	41 (45.6%)	4 (40%)	
Deficient	34 (37.7%)	4 (40%)	
3-month after therapy			**0.01**
Sufficient	25 (27.8%)	2 (20%)	
Insufficient	55 (61.1%)	5 (50%)	
Deficient	10 (11.1%)	3 (30%)	
***P* value[2]**	**0.04**	0.11	

Data was expressed as mean (SD), frequency (percentage). P value was significant if < 0.05. LDL: low density lipoprotein; HDL: high density lipoprotein; RBS: fasting blood sugar; HOMA: homeostatic model assessment. P value1compared between both groups at different times while P value2 compared between baseline and f3-month after therapy data in the same group

Table (3): Level of vitamin D in cirrhotic and non-cirrhotic patients .

	Non-cirrhotic (n= 90)	Cirrhotic (n= 10)	*P* value[1]
Vitamin D (ng/ml)			
Baseline	16.29 ± 2.98	18.11 ± 3.11	0.16
3-month after therapy	26.11 ± 2.11	20.20 ± 3.09	**< 0.001**
***P* value[2]**	**< 0.001**	0.33	
HOMA			
Baseline	6.56 ± 2.01	8.99 ± 2.11	**0.04**
3-month after therapy	3.45 ± 1.10	7.89 ± 3.11	**< 0.001**
***P* value[2]**	0.02	0.10	

Data was expressed as mean (SD. *P* value was significant if < 0.05. HOMA: homeostatic model assessment.

P value[1]compared between both groups at different times while *P* value[2] compared between baseline and f3-month after therapy data in the same group

DISCUSSION

DAA regimens are the current standard of therapy in patients with CHC infection, curing more than 95% of cases. Patients with CHC, particularly those with cirrhosis, have a high frequency of comorbidities, which necessitate the administration of several medicines in addition to antiviral therapy **[9]**.

The current study was conducted on 100 patients with chronic HCV infection who were eligible for therapy with DAAs. The study aimed to determine change in VD, lipid profile and IR as assessed by HOMA following DAAs and impact of these parameters among those patients.

In the current study SVR was achieved in 90%. In 2016, EASL issued guidelines for treatment of HCV genotype 4by including the combination of sofosbuvir and daclatasvir with or without ribavirin for 12 weeks **[10].** This result was consistent with many previous reported studies that reported efficacy of this combination in management of HCV infection **[11-13]**.

VD is a necessary hormone substance that plays a role in normal organ physiology as well as pathophysiology in a variety of illnesses. VD insufficiency and deficiency are common in individuals with chronic liver disorders, and they increase as hepatic function and cirrhosis severity worsen **[14]**.

In our study, level of VD was increased following DAAs. Also, we found that level of VD following DAAs was significantly higher among non-cirrhotic patients and also, in those who achieved SVR. Longer VD level monitoring is required to explore this hypothesis. Nevertheless, the most crucial result of the present study is that level VD may be increased following therapy with DAAs.

In consistent with our study, Patients with chronic hepatitis exhibited substantially greater levels of VD at baseline or throughout follow-up than those with liver cirrhosis. This study disagreed with the current study as regard significant reduction of VD following DAAs at 3- and 6- months after therapy **[15]**.

VD deficiency following DAA treatment, according to the authors, might be explained by other underlying variables rather than impaired metabolic liver function. To put it another way, decreased liver function may not be a substantial contributor to this nutritional deficiency in individuals with CHC infection **[15]**.

However, a study indicated a tendency toward a decreased incidence of VD deficiency 24 weeks after interferon therapy completion. This finding implies that restoring hepatic VD metabolism, particularly 25-alpha hydroxylation, may take time before showing therapeutic benefit **[16]**.

The impact of VD on chronic liver disorders, independent of etiology, has been fully documented in several researches. In individuals with chronic hepatitis B, serum VD levels were inversely linked to HBV viral load **[17]**. Low blood 25(OH) VD levels were linked with advanced illness and a predictor of responsiveness to ursodeoxycholic acid in individuals with primary biliary cholangitis **[18]**.

Low serum VD levels were also linked to severe histological characteristics and may have predicted negative treatment results in autoimmune hepatitis patients. VD insufficiency has numerous negative consequences in CHC patients, including deregulation of T-cell activities, which results in poorer response rates of HCV genotype 1 to pegylated interferon and RBV therapy **[19]**.

Our investigation found that the lipid profile of the subjects exhibited no significant changes three months following DAA treatment. However, we discovered that following treatment, insulin resistance as measured by HOMA improved considerably. In addition, patients who achieved SVR and those who did not have cirrhosis had considerably reduced HOMA following treatment.

Few researches have been conducted to investigate the effects of SVR on glucose and lipid metabolism, and the evidence that is available is both inadequate and inconsistent. Notably, a study proposed that DAA-induced fast suppression of HCV core proteins may result in a dysregulation of host lipid metabolism, shown as a reduction in lipid droplet formation in HCV-infected liver cells and a significant rebound of circulating HCV core proteins **[20]**.

Other study revealed irrespective of DAA protocol or fibrotic state, viral clearance caused a substantial reduction in insulin resistance as evaluated by HOMA, a rise in total cholesterol, low density lipoproteins (LDL), the LDL/high density lipoproteins (HDL) ratio, and non-HDL

cholesterol. Notably, the detrimental effect on the lipid profile may be offset in part by the reduction in insulin **[21]**.

To the best of our knowledge, this is the first research to look at all three indicators (VD, lipid profile, and IR) in a single prospective cohort of patients with CHC who were given DAAs. However, the current study has two major limitations: a small sample size and a short follow-up period.

In conclusion, DAA treatment was found to be safe and effective in patients with chronic hepatitis C infection, but the effect of those drugs on VD, lipid profile, and insulin resistance remains unknown and controversial. Future research on this topic with long-term follow-up are thus needed.

Ethical consideration: This work was conducted in accordance with Code of Good Practice and the guidelines of Declaration of Helsinki, 7th revision, 2013 and after being approved by the Medical Ethics Committee with No. 17200294. Also, a written informed consent was obtained from all participants before being enrolled in the study.

Abbreviations

DAAs: direct acting analogues, VD: vitamin D, SVR: sustained virological response, IR: insulin resistance, HOMA: homeostatic model assessment.

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HIGHLIGHTS

1. Low serum VD level was an independent predictor for insulin resistance among patients with HCV-induced patients.
2. VD favors the HCV response by improving the sensitivity to insulin. Insulin resistance (IR) is considered one of the factors in predicting HCV patients' response.
3. Vitamin D deficiency may be linked to a lack of response to anti-viral treatment, while vitamin D supplementation may potentiate SVR.

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