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Do Sofosbuvir and Daclatasvir Affect Vitamin D and Iron Status in Chronic Hepatitis C Virus Patients? Role of Hepcidin

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- Iron overload
- Sofosbuvir/daclatasvir
- Vitamin D

Abstract

Aim: This study was designed to assess the impact of sofosbuvir/daclatasvir on vitamin D and iron status in chronic HCV patients (CHC). **Methods:** Sixty-five CHC patients registered for sofosbuvir/daclatasvir based regimen were recruited. Serum vitamin D, total iron, total iron binding capacity, and serum hepcidin were measured prior the treatment and 12 weeks after the stoppage of the treatment. Transferrin saturation was calculated. **Results:** The present study showed that a great majority of CHC patients had vitamin D deficiency or insufficiency, high total serum iron, and high transferrin saturation. Vitamin D was negatively correlated ALT. 12 weeks after completion of treatment, patients who had vitamin D deficiency or insufficiency reduced and the median value of vitamin D significantly increased compared to the pretreatment value. Total serum iron, transferrin saturation tended to decrease, however, there were no significant differences. The number of patients who had concurrently vitamin D deficiency or insufficiency and high transferrin saturation was reduced. Serum hepcidin significantly increased 12 weeks after completion of the antiviral therapy. Despite there were no significant correlations between hepcidin and total serum iron, vitamin D, and transferrin saturation before treatment, a significant positive correlation between hepcidin and serum iron and a significant negative correlation between hepcidin and vitamin D were noted after treatment. **Conclusion:** CHC is associated with vitamin D deficiency and iron overload, which could be attributed to reduced hepcidin level. Treatment with sofosbuvir/daclatasvir increases hepcidin and thereby reduces iron level and its harmful effect on the liver, thus increases vitamin D.

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INTRODUCTION

Hepatitis C virus (HCV) is one of the global public health problems. World Health Organization (WHO) reported that more than 80 million people all over the world are infected with HCV. Approximately 700000 persons die every year from hepatitis C-related complications. Egypt is one of the highest prevalence rates of HCV, worldwide (1).

Several studies demonstrated that HCV is associated with vitamin D deficiency (2) and that vitamin D supplementation improved the sustained virologic response in patients treated with interferon (3, 4).

Additionally, iron overload was reported in HCV positive patients, which were linked to bad prognosis (5). Hepcidin, an iron regulatory peptide secreted by the hepatocytes, plays an important role in iron homeostasis (6). It reduces the expression of ferroportin, the only known exporter of intracellular iron to the plasma, and causes the internalization and degradation of ferroportin, consequently preventing iron efflux from macrophages and other professional iron-transporting cells, including hepatocytes, duodenal enterocytes and placental syncytiotrophoblast to the plasma; reducing serum iron concentration and increasing intracellular iron content (7). A reduced serum hepcidin has been reported in chronic HCV patients, which was correlated to the severity of liver histopathological changes and related to iron overload in these patients (8, 9).

There is an interaction between iron metabolism and vitamin D metabolism. Iron is essential for vitamin D activation (10) and vitamin D deficiency is associated with elevated hepcidin level (10). Vitamin D controls the hepcidin-ferroportin axis by decreasing hepcidin mRNA expression, while increasing ferroportin, thereby enhancing iron transport. Bacchetta et al. (11) showed that vitamin D (1,25(OH)2Vit D3) had a direct transcriptional suppression of the hepcidin gene promoter in both human monocytes and hepatocytes and on healthy volunteers, who received a single oral vitamin D2. Additionally, vitamin D reduces the release of proinflammatory cytokines and consequently reduces the expression of hepcidin (12).

The combination of sofosbuvir and daclatasvir, direct acting antivirals (DAAs), has achieved high rates of sustained virologic response; sofosbuvir is an inhibitor of NS5B polymerase while daclatasvir is an inhibitor of NS5A replication complex (13). Up to our knowledge, little is known about the association between vitamin D status and iron metabolism in chronic HCV patients, especially after receiving the DAAs. Thus, this study was designed to assess the impact of sofosbuvir/daclatasvir on vitamin D and iron status in chronic HCV patients (CHC).

Patients and methods

A total of sixty-five patients with chronic hepatitis C infection based on HCV antibody and HCV RNA were recruited from the National Center for Control of Viral Hepatitis, Assiut and from those registered for the treatment with a combination of Sofosbuvir 400 mg/day (Mylan laboratories, Maharashtra, India) and Daclatasvir (Global Napi Pharmaceuticals. Egypt) 60 mg/day, based regimen. The present study was designed using a quasi-experimental approach and conducted between November 2017 and July 2018. The study protocol was approved by the Ethical

Committee, Faculty of Medicine, Assiut University. The protocol was registered in clinicaltrials.gov (unique registration number #IRB0000871820032017#). Background about the study and its purpose were provided and all participants gave their informed consent before the inclusion in the study. None of the patients had any manifestations of liver cell failure, cirrhosis, or previously received treatment for HCV (naïve patients). Patients were excluded if aged less than 18 years old or more than 70 years old, had manifestations or history of manifestations of liver cell failure and cirrhosis, co-infected with the hepatitis B (HBV) or human immunodeficiency viruses (HIV), had hepatocellular carcinoma and/or other extra-hepatic carcinoma, renal disease, or received vitamin D, calcium therapy or iron supplementation in the last 3 months. Patients with a total serum bilirubin ≥ 3 mg/dL, a serum albumin < 2.8 g/dL, INR> 1.7, platelet count <50000/mm^3, a serum creatinine >2.5mg/L, pregnant or unable to use contraception were also excluded.

Full medical and nutritional histories were taken. Almost all patients eat daily dairy products, green vegetables and plants protein, twice weekly animal protein and poultry. Thorough clinical examination was done. Abdominal ultrasonography, ECG, and general laboratory investigations, including: HBs Ag, complete blood count, liver function tests (alanine aminotransferase (ALT), aspartate aminotransferase (AST), total and direct bilirubin, serum albumin, prothrombin time, prothrombin concentration and INR), alpha-fetoprotein (AFP), serum creatinine, random blood glucose and/or HbA1ca were carried out. Quantitative PCR for HCV RNA (IU/mL) was used to determine the HCV RNA concentration using Cobas Amplicor HCV Monitor version 2 (Roche Diagnostics, Branchburg, NJ) prior to the treatment and 12 weeks after the stoppage of the treatment to evaluate the sustained virologic response (SVR). SVR is defined as undetectable HCV RNA 12 weeks after the end of treatment. All included patients were assumed to have HCV genotype 4.

Venous blood samples were obtained from all patients and centrifuged for 15 min at 3000 RPM. Sera were collected from each patient before the start of the treatment and 12 weeks after the stoppage of the treatment and stored at −20°C until used. Serum vitamin D (25OH vitamin D) was measured using the commercially available enzyme linked immunoassay (ELISA) kit (Epitope Diagnostic, San Diego, USA). According to the Endocrine Society Clinical Practice Guideline, vitamin D deficiency is diagnosed if 25 OH vitamin D below 20 ng/mL, while vitamin D insufficiency is considered if 25 OH vitamin D of 21-29 ng/mL and sufficiency/optimal is diagnosed when 25 OH vitamin D ≥30 ng/mL (14). Serum total iron and total iron binding capacity (TIBC) were measured using commercially available kits (spectrum-Diagnostic, Cairo, Egypt). Transferrin saturation was calculated by the following formula: (serum iron/ TIBC)*100. Typically, transferrin is 20-50% saturated with iron (15). Transferrin saturations of less than 20% suggest iron deficiency, while those of more than 50% indicated iron overload (15). Additionally, serum hepcidin was measured using the commercially available ELISA kit (Glory Science Co., Hongkong, China). All biochemical analyses were conducted according to the manufacturer's instructions and were measured before the start of the treatment and 12 weeks after the stoppage of it. Fibrosis was evaluated by Ultrasonography.

Statistical analyses

and maximum values. Nominal data are expressed in the form of frequencies and percentage (%). The data had been investigated for normality using the Shapiro-Wilk test and the data were not normally distributed. Statistical differences between data before and 12 weeks after the treatment were analyzed using the Wilcoxon signed rank test. A value of P≤0.05 was considered statistically significant. Spearman's correlations were used. All statistical analyses were carried out with SPSS software version 20 (SPSS Inc., Chicago, IL, USA).

Continuous data are presented as median and their minimum

Results

This study included 65 CHV patients based on HCV antibody and HCV RNA by qPCR. The baseline patient demographic data and clinical characteristics are summarized in Table 1 & 2. The mean age of the patients was 51.5 ±11. 47 years old, 41.5% (n=27) were male, 29.2% (n=19) were smoking, 18.5% (n=12) were hypertensive, 9.2% (n=6) were diabetic, 18.5% (n=12) were overweight, and 33.8% (n=22) were obese (Table 1 & 2 and Figure 1). The mean viral load of HCV was 6.36 ± 13.74 million per mL (Table 2).

Table 1. Demographic data of the studied HCV patients (n=65).

	Frequency (n)	Percentage (%)
Sex		
Male	27	41.5
Female	38	58.5
Smoking		
Smoker	19	29.2
Non-smoker	46	70.8
Hypertension		
Hypertensive	12	18.5
Normo-tensive	53	81.5
Diabetes		
Diabetic	6	9.2
Non-diabetic	59	90.8
BMI		
Normal (below 24.9 Kg/m^2)	31	47.7
Overweight (25-29. 9 Kg/m^2)	12	18.5
Obese (≥30 Kg/m^2)	22	33.8

Table 2. Clinical data of the studied CHC patients.

Parameters	Median	Minimum-maximum
Age (year)	53	27-69
HCV (IU/mL)	1.5 x10^6	3390-84.9 x10^6
ALT (IU/L)	34	10-174
AST (IU/L)	32	14-346
AFP IU/L)	1.3	0-16
Albumin (mg/dL)	4.3	3.6-5.6
Total bilirubin (mg/dL)	0.6	0.2-1.1
WBCs (10^9/L)	6.3	3.3-10.6
HB (g/dL)	13.4	10.3-18.1
Platelet (10^9/L)	227	152-393
Prothrombin concentration (%)	100	81-100
INR	1	1-1.1
Creatinine (mg/dL)	0.8	0-1.3
Blood glucose (mg/dL)	95	65-200
Weight (Kg)	67	48-118
Height (meter)	1.59	1.43-1.89
BMI (Kg/m^2)	25.95	15.96-47.26

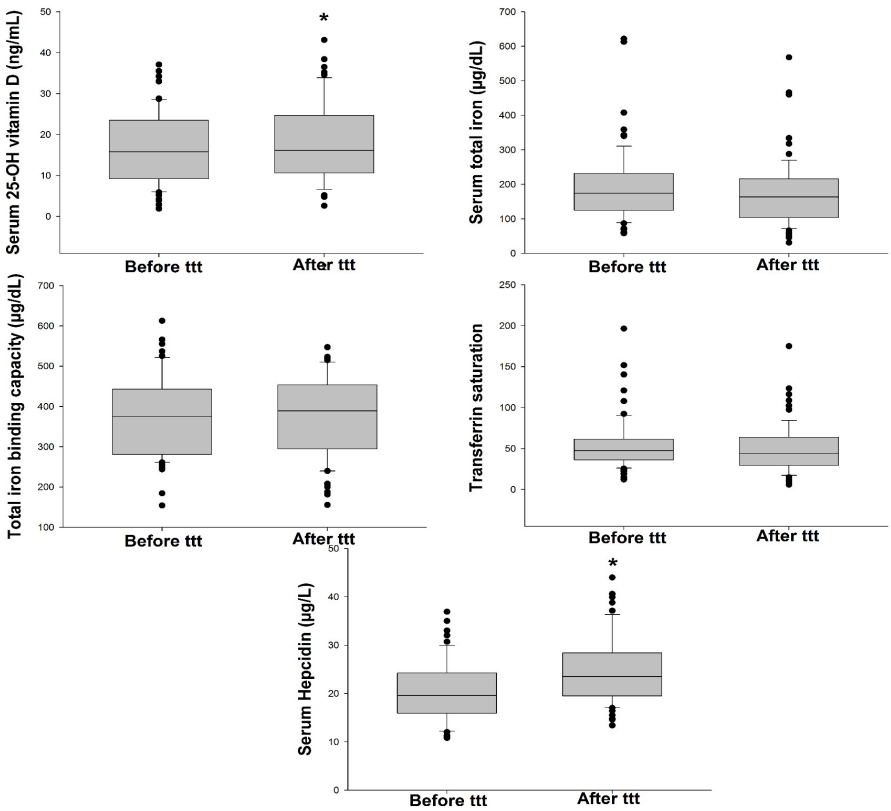

Figure 1. Serum levels of total 25-OH vitamin D (ng/mL), total iron (µg/dL), Total iron binding capacity (TIBC) (µg/dL), and Transferrin saturation (%) in the 65 HCV positive patients before and after treatment. Mean ±SD are shown. * significant s compared to mean values before treatment.

Overall, the majority of patients before receiving treatment were found to have a vitamin deficiency (66.2%, n=43), or insufficiency (27.2%, n=18). A greater proportion of patients had total serum iron above the normal range for each sex (60%, n=39) with transferrin saturation above 50% (43.1%, n=28). Patients who had concurrently vitamin D deficiency or insufficiency and high transferrin saturation were 38.5% (n=25) (Table 3).

12 weeks after completion of treatment, patients who found to have vitamin D deficiency or insufficiency were 63.1% (n=41) and 18.5% (n=12); respectively. 53.8% (n=35) of patients had total serum iron above the normal range for each sex. 44.6% (n=29) of patients had a transferrin saturation above 50%, suggesting iron overload (Table 3). Patients who had concurrently vitamin D deficiency or insufficiency and high transferrin saturation reduced from 38.5% (n=25) before treatment to 33.8% (n=22) after treatment (Table 3).

The median value of 25-OH vitamin D significantly increased after treatment compared to the pre-treatment median values (P=0.01). In contrast, total serum iron tended to decrease, however, there was no significant difference between the two values. Serum hepcidin significantly increased after treatment compared to the pre-treatment values. Additionally, there were no significant differences in the median values of total iron binding capacity and transferrin saturation pre-treatment and after treatment; however, transferrin saturation tended to decrease after treatment (Figure 1).

Table 3. Frequencies of vitamin D sufficiency, deficiency and insufficiency, iron status (within the normal range, above the upper limit of normal), and transferrin saturation (normal and iron overload) before and after treatment.

	Frequency	Percentage (%)
Age (year)		
<40 years	12	18.5
> 40years	53	81.
Vitamin D before treatment		
Deficiency (<20 ng/mL)	43 **(17 of them had combined deficiency and high transferrin saturation)**	66.2
Insufficiency (21-29 ng/mL)	18 **(8 of them had combined insufficiency and high transferrin saturation)**	27.2
Sufficiency (≥ 30 ng/mL)	4	6.2
Vitamin D after treatment		
Deficiency (<20 ng/mL)	41 **(18 of them had combined deficiency and high transferrin saturation)**	63.1
Insufficiency (21-29 ng/mL)	12 **(4 of them had combined insufficiency and high transferrin saturation)**	18.5
Sufficiency (≥ 30 ng/mL)	12	18.5
Total serum iron before treatment		
Normal	26	40
Above normal range	39	60
Total serum iron after treatment		
Normal	30	46.2
Above normal range	35	53.8
Transferrin saturation (%) before treatment		
Normal	37	56.9
Above 50%	28	43.1
Transferrin saturation (%) after treatment		
Normal	36	55.4
Above 50%	29	44.6

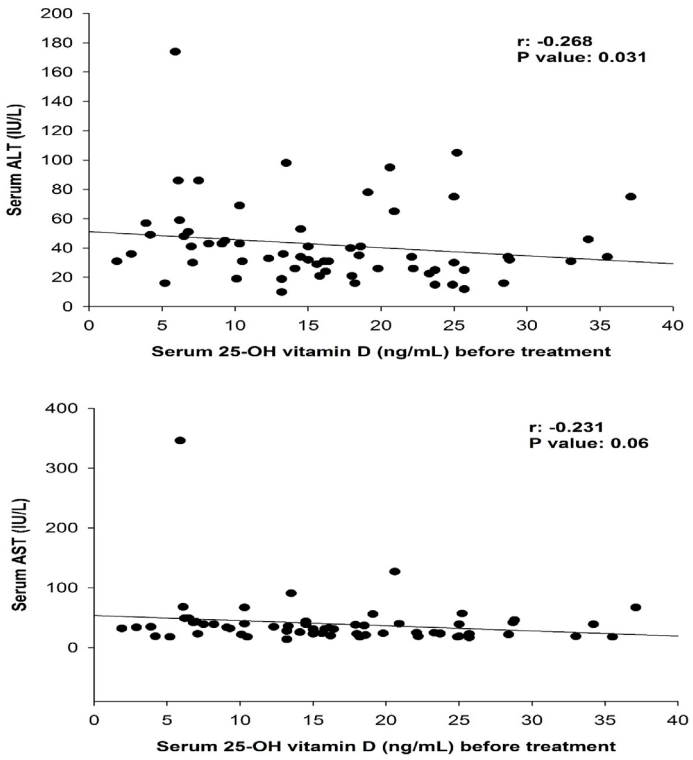

Figure 2. Spearman's Correlation between serum 25-OH vitamin D (dependent variable) and alanine aminotransferase (ALT), aspartate aminotransferase (AST) as independent variables.

Figure 2 shows that serum 25-OH vitamin D before treatment had a significant negative correlation with serum ALT (r=-0.268, P value= 0.031) but not with AST (r=-0.231, P value= 0.06).

Figure 3 shows no significant correlations between serum hepcidin before treatment and total serum iron (r=-0.084, P-value= 0.508), transferrin saturation (r=-0.136, P-value 0.281), and vitamin D (r=-0.231, P-value 0.064) before treatment. In contrast, after treatment, serum hepcidin correlated positively with total serum iron (r=0.274, P-value= 0.027) and negatively with transferrin saturation (r=-0.245, P-value= 0.04), and vitamin D (r=-0.277, P-value= 0.025) (Figure 4).

Discussion

The present study was designed to assess the levels of vitamin D, iron, transferrin saturation, and total iron binding capacity in patients infected with HCV before and after treatment with sofosbuvir/ Daclatasvir based regimen. Serum hepcidin level was determined as a link between vitamin D and iron metabolism. Up to our knowledge, the present study is the first to demonstrate the change in vitamin D, total serum iron, hepcidin, and transferrin saturation before and after receiving sofosbuvir/daclatasvir.

The current study demonstrated that most of CHC patients had either vitamin D deficiency or insufficiency (93.8%, n=61), which is in agreement of Eltayeb et al. (4), who demonstrated that nearly 76% of studied Egyptian children had either vitamin D deficiency or insufficiency. Villar et al. (16) reviewed eleven studies involving 1575 CHC patients through a meta-analysis and reported that nearly 71% of the population studied had low vitamin D levels.

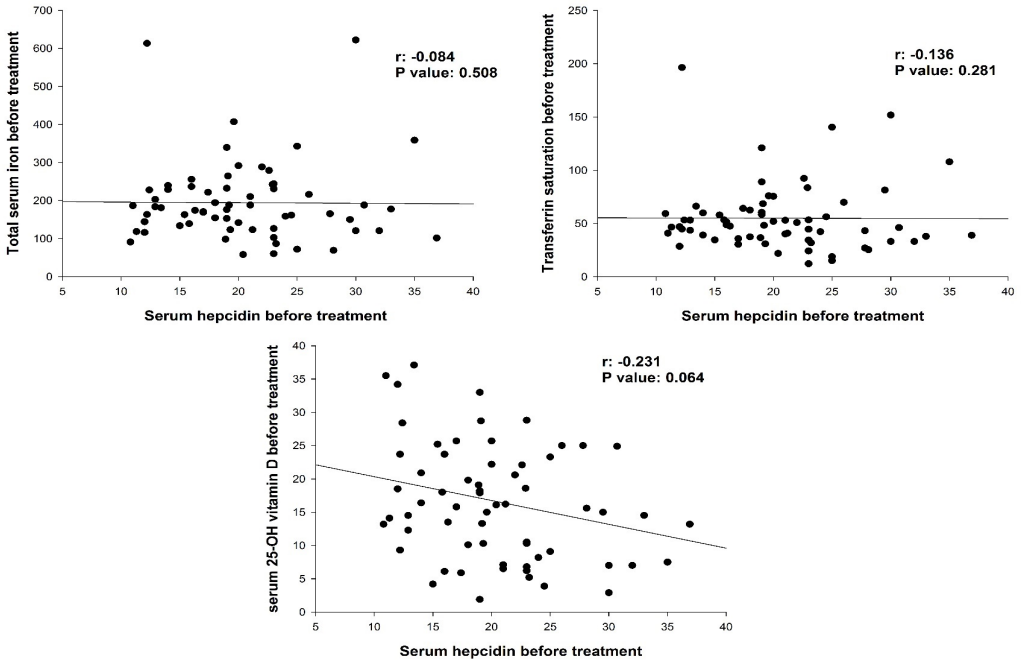

Figure 3. Spearman's Correlation between serum hepcidin before treatment (dependent variable) and total serum iron, transferrin saturation, and 25-OH vitamin D before treatment.

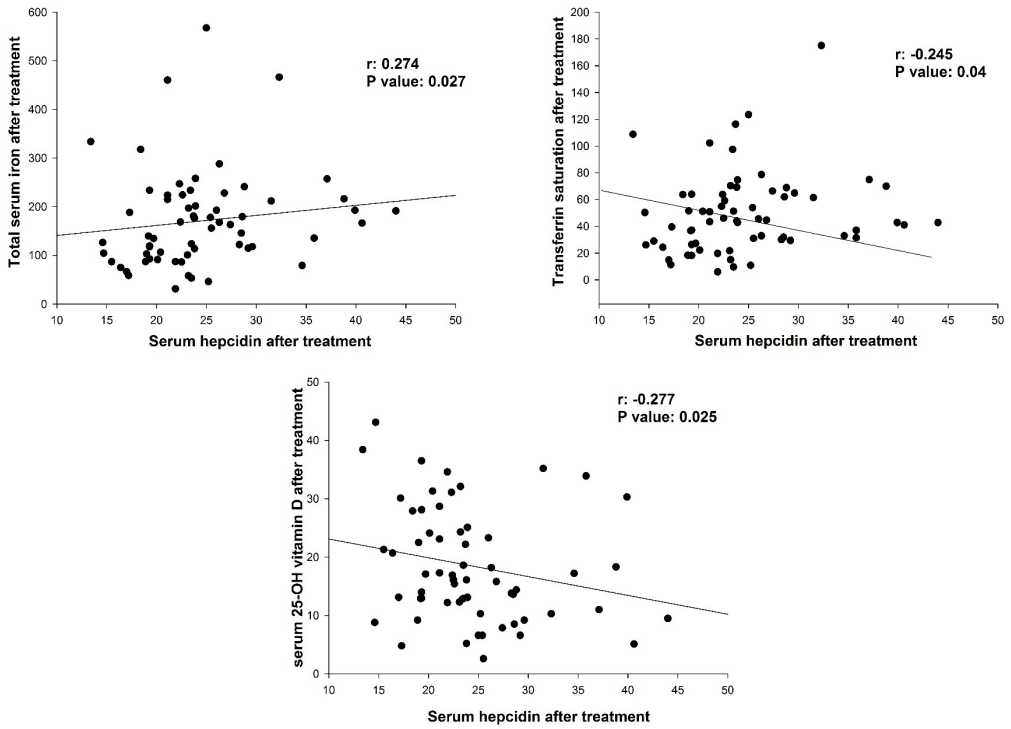

Figure 4. Spearman's Correlation between serum hepcidin after treatment (dependent variable) and total serum iron, transferrin saturation, and 25-OH vitamin D after treatment.

Since HCV is an inhibitor of hepcidin expression resulting in iron overload. The liver damage-induced by the viral infection or iron overload could be the cause of vitamin D deficiency. This could be true as a significant increase in vitamin D level was observed after antiviral treatment. Eradication of virus by antiviral therapy resulted in a significant increase in hepcidin levels and subsequently reduced iron overload, improving the status of liver cells and thus regain their capability to do the first hydroxylation of vitamin D. However, we can't rule out the direct inhibitory effect of the virus itself on vitamin D to evade vitamin D-induced innate immune responses and interferon production; Gal-Tanamy et al. (17) showed that vitamin D3 inhibited viral replication in cell culture through interferon production and enhancement of innate immunity. Additionally, HCV hinders patients lipid metabolism and consumes it in viral cellular entry, replication, and virion release (2) leading to hypolipidemia, increased hepatic steatosis and fibrosis and this could be also the cause of vitamin D deficiency (2).

12 weeks after completion of the antiviral therapy, vitamin D level significantly increased; however, its level is still subnormal. One explanation for this is the seasonal variation of vitamin D level, as we collected the second blood samples on May and June (spring). Previous studies reported that vitamin D levels increase in summer and autumn reduce in winter and spring (18, 19); this also means that our patients need to receive vitamin D supplementation. Our data suggests that vitamin D deficiency or insufficiency may not only caused by CHC virus, but many factors may contribute to this deficiency. For example, most of the studied groups had dark-skin

which may interfere with ultraviolet B radiation reaching to the skin (20). Furthermore, most of the studied patients (52.3%, n=34) are overweight or obese; obesity reduce the bioavailability of vitamin D due to fatty tissue uptake (21). Age could be also a contributing factor for vitamin D deficiency due to decreased skin capacity to synthesize vitamin D, poor socioeconomic status, poor dietary intake and lack of exposure to sunlight could also be the cause of vitamin D deficiency and insufficiency (22).

Previous studies showed that high-dose vitamin D therapy suppresses hepcidin expression both directly and indirectly via reducing pro-hepcidin inflammatory cytokines (11, 12). Bacchetta et al.(11) reported that vitamin D down-regulates hepcidin expression in human monocytes via binding to vitamin D response element on human hepcidin promoter. Furthermore, Zughaier et al. (12) reported that administration of high doses of oral vitamin D3 (cholecalciferol, 50,000 IU weekly for 12 weeks, followed by 50,000 IU every other week for 40 weeks) to patients with early stages CKD (stages 2/3) resulted in a significant reduction in serum hepcidin in a time-dependent manner and ameliorated anemia. Additionally, vitamin D resulted in reduced hepcidin expression in vitro studies using human monocytic THP-1 cells in the presence of an inflammatory stimulus (i.e. exposure to LPS) in a dose-dependent manner (12). In contrast, Moran-Lev et al. (23) reported no significant correlation between serum 25-OHD levels and circulating levels of hepcidin, which agrees with the results of the present study before treatment. Nevertheless, the absence of correlation between circulating hepcidin and 25-OH vitamin D can't rule out the important local intracrine mechanism regulating 1,25(OH) 2D3 and hepcidin expression (24); however, further research should be conducted to elucidate the molecular interaction.

On the contrary, a significant negative correlation between vitamin D and hepcidin was observed 12 weeks after completion of therapy which was not supported by further reduction of hepcidin after treatment. Yet unresolved question concerning vitamin D and hepcidin interaction is: why the increase in vitamin D level couldn't suppress hepcidin level? Our data suggest that inspite that vitamin D increased after treatment with antiviral therapy, its level still subnormal and this could provide a relatively weak or nearly negligible suppression on hepcidin the supressive effect of vitamin D on hepcidin was observed in vivo using a high dose of vitamin D (12). Besides, the stimulatory effect of serum iron, which is still high, could overcome the inhibitory effect of vitamin D.

Reduced hepcidin in CHC patients was in agreement with previous studies, which showed reduced serum and or hepatic hepcidin level in CHC patients (25-27). The relationship between reduced hepcidin and hepatic iron deposition in CHC patients is a matter of controversial. Fujita et al. (26) and Aoki et al. (25) reported a positive correlation between hepcidin and hepatic iron deposition and serum iron, while Sikorska et al. (27) and Eddowes et al. (28) demonstrated no association between hepcidin and hepatic iron deposition. Recently, Ismail et al. (29) reported a lower hepcidin level among the Egyptian CHC patients compared to the controls, which was correlated negatively with liver enzymes and serum iron and positively with viral load and

ferritin. Additionally, Abd Elmonen et al. (30) reported a negative correlation between hepcidin expression and serum ferritin and hepatic iron concentration, in Egyptian patients (30). This discrepancy in the association of serum hepcidin and viral load, liver enzymes, serum iron could be attributed to the different methods in assessing these parameters (27). The ethnic differences, which lead to functional changes of iron homeostasis regulatory genes or domination of other than HCV genotype 1, and different tissue studied, might also explain the above discrepancies. Disrupted BMP/SMAD signaling pathway and subsequently impaired hepcidin induction and iron overload might be the cause of the absence of correlation between hepcidin and serum iron before treatment (28). Hence, BMP has an antiviral activity via potentiating IFN activity and down-regulating USP18, an inhibitor of IFN signaling (28), disruption of BMP/SMAD signaling by HCV could be a way of virus to attenuate immune system and thus viral replication and spread.

In line with previous studies (26, 31), the current study demonstrated a significant increase in serum hepcidin 12 weeks after completion of antiviral therapy together with tendency of total iron and transferrin saturation to be decreased. Furthermore, a positive correlation between hepcidin and total serum iron. Fujita et al. (26) demonstrated an increase in serum hepcidin synthesis following successful interferon and ribavirin therapy in CHC patients; however no correlation between HCV viral load and serum hepcidin level was found. Moreover, Ryan et al. (31) showed a significant increase in serum hepcidin, concurrently with a significant reduction in serum iron, transferrin saturation, and viral load following the initiation of interferon (IFN-α) therapy to CHC patients; suggesting the role of hepcidin as an antiviral agent. Yet the unresolved question, why serum iron and transferrin saturation were still in high level in spite the increase in hepcidin levels. This could be due to that hepcidin level is still not high enough to normalize the iron status and thus, these patients need interventional therapy to reduce serum iron levels and transferrin saturation. Ware et al. (32) reported that resolution of iron overload was obtained after at least six months of serial phlebotomy (6 months); this means that resolution of iron overload need a long time and this could explain that serum iron and transferrin saturation is still high after treatment. This also may raise the point that these patients may need a treatment of iron overload after confirmation of iron overload with liver biopsy.

Taken together, in the present study, we found no significant correlations between hepcidin and total serum iron, vitamin D, and transferrin saturation before treatment and this could be attributed to impaired BMP6-HJV/SMAD pathway. After treatment this system may be recovered from the inhibitory effect of the virus and thus a positive feedback between hepcidin and serum iron was regained; in an attempt to reduce iron overload and this could be seen in the tendency of both total serum iron and transferrin saturation to decrease after treatment.

The novelty of this study was the assessment of the effect of HCV therapy (sofosbuvir/daclatasvir) on iron and vitamin D status indicating the efficiency of HCV therapy on restoring iron and vitamin D status; however, this effect was subtle may be due to the short duration

of follow up after stoppage of treatment or these cases need intervention al therapy to normalize iron and vitamin D status.

To conclude, HCV is associated with vitamin D deficiency and iron overload, which could be attributed to the reduced hepcidin level. Treatment with sofosbuvir/daclatasvir increases hepcidin and thereby reduces iron level and its harmful effect on the liver, thus increasing vitamin D. However, these effects are subtle and may need either a long duration of follow up or intervention by therapy.

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