25-Hydroxy-vitamin D_3 level is a predictor to insulin resistance in patients with hepatitis C virus-induced liver cirrhosis

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Background
There is an established relationship between liver disease and hepatogenous diabetes mellitus, and a growing evidence for the role of vitamin D deficiency in the pathogenesis of type 1 and type 2 diabetes mellitus. However, data on the impact of vitamin D serum level on insulin resistance among liver cirrhosis patients are lacking.
Objectives of the study
The primary objective of the current study was to investigate the relationship between vitamin D status and insulin resistance among hepatitis C virus (HCV)-induced liver cirrhosis patients using a homeostasis model for assessment of insulin resistance (HOMA-IR). The secondary objectives were to assess the association between deterioration of liver function on the one hand and insulin resistance and vitamin D deficiency on the other.
Participants and methods
Fifty patients with biopsy-proved HCV-induced liver cirrhosis were enrolled in this cross-sectional study. Routine clinical, laboratory, and imaging workout was performed to assess the degree of liver decompensation using the model of end-stage liver disease (MELD) score and the Child–Turcotte–Pugh Score (CTPS). Serum level of 25-hydroxy-vitamin D_3 [25(OH)D_3] was estimated. Fasting plasma glucose and fasting insulin were also measured to calculate HOMA-IR as an indicator of insulin resistance. Patients were subclassified according to serum 25(OH)D_3 levels into tertiles, according to the MELD score into three groups, and according to CTPS into Child A, B, and C.
Results
A significant inverse correlation was found between serum 25(OH)D_3 level and insulin resistance as assessed by HOMA-IR, whether using one-by-one correlation ($r=-0.976$, $P=0.000$) or using 25(OH)D_3 tertiles' correlation ($r=-0.830$, $P=0.000$). Linear multiple regression analysis determined low serum 25(OH)D_3 level as an independent predictor for increase in HOMA-IR among HCV-induced liver cirrhosis patients. No significant association was identified between low serum 25(OH)D_3 level and the severity of liver dysfunction as assessed by the MELD score or CTPS.
Conclusion
The present study showed that low serum 25(OH)D_3 level was an independent predictor for insulin resistance among patients with HCV-induced liver cirrhosis.

Keywords:
hepatitis C virus-induced liver cirrhosis, 25-hydroxy-vitamin D_3, insulin resistance, liver cell dysfunction

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Introduction

There is increasing evidence of high frequency of glucose intolerance (GI) and diabetes mellitus (DM) among patients with liver cirrhosis, termed 'hepatogenous diabetes' [1]. Although there are some data explaining the pathogenesis of GI and/or DM among those with chronic hepatitis C infection, the role of vitamin D deficiency in the pathogenesis of hepatogenous diabetes in patients with hepatitis C virus (HCV)-induced cirrhosis has not been addressed as yet.

The proposed pathophysiologic scenario of hepatogenous diabetes is an initial hyperinsulinemic state because of increased insulin resistance. Intrahepatic insulin resistance starts with HCV-induced tumor necrosis factor -α (TNF-α) overproduction, which phosphorylates the serine residues of the insulin receptor substrates 1 and 2 (IRS 1 and IRS 2), and stimulates overproduction of the suppressor of cytokines 3. These changes block transactivation of glucose transporter-4, with subsequent inhibition of cellular glucose uptake [2]. Extrahepatic insulin resistance occurs in the skeletal muscles and adipose tissue [3] similar

to that preceding type 2 DM. Impaired insulin extraction, considered to be unique for cirrhotic liver because of functional decompensation and/or portosystemic shunting [2], and more recently pancreatic islet cell hypertrophy [4], have been considered as additional causes for the hyperinsulinemic state in liver cirrhosis patients. A responsive increase in anti-insulin hormones, insulin-like growth factors, and cytokines has been considered as an additional factor that could aggravate insulin resistance [2]. Initially, hepatic insulin resistance has been suggested to cause fasting hyperglycemia. Finally, reduced insulin production by the pancreatic β-cells occurs with the onset of frank diabetes [5].

Genetic predisposition, etiology of liver cirrhosis, as well as unknown environmental factors were proposed as pathogenic factors for hepatogenous diabetes.

Vitamin D deficiency has been recognized recently to be associated with the degree of liver dysfunction and to predict hepatic decompensation in patients with chronic liver disease [6]. However, vitamin D deficiency has been suggested to be involved in the pathogenesis of DM. A relation between vitamin D deficiency early in life and development of type 1 DM later on has been reported extensively, mainly attributed to its immunomodulatory action [7]. Recently, circumstantial evidence of its role in the development of type 2 DM through suspected genetic and/or environmental modulatory effects has also been reported [8,9]. However, the role of vitamin D deficiency in the development of hepatogenous diabetes among patients with HCV-induced liver cirrhosis remains to be evaluated.

Objective of the study

In the current study, we aimed to evaluate the correlation between vitamin D level and the development of insulin resistance as assessed by the homeostasis model for assessment of insulin resistance (HOMA-IR) in HCV-induced liver cirrhosis patients. A secondary objective was to determine the correlation between the degree of hepatic decompensation, as assessed by the model of end-stage liver disease (MELD) score and the Child–Turcotte–Pugh Score (CTPS), and insulin resistance assessed by HOMA-IR, as well as vitamin D by measuring 25-hydroxy-vitamin D_3 [25(OH)D_3] serum levels.

Study design

In a cross-sectional hospital-based observational study, 50 previously biopsy-proved hepatitis C-induced liver cirrhosis patients, 33 men (66%) and 17 women (34%), were recruited from the inpatient unit of the Internal Medicine Department, Kasr El Aini Hospital, Cairo University, from January 2012 to November 2012. All the participants had established HCV infection, previously proved by PCR of HCV-RNA and liver cirrhosis previously confirmed by pathological examination of liver biopsy. Blood samples were withdrawn for routine laboratory investigations and abdominal ultrasound was performed. The degree of liver decompensation was evaluated using the MELD score and CTPS. Fasting plasma glucose and insulin were evaluated after an 8-h overnight fast to calculate HOMA-IR. The rest of the sample was stored according to the manufacturer's instruction to measure serum 25(OH)D_3 level. Patients with positive hepatitis B serological markers, obese patients, defined as BMI 30 kg/m^2 or more, alcoholics, those with hypercholesterolemia or hypertriglyceridemia, dialysis patients, patients with hepatocellular carcinoma, patients known to have type 1 or type 2 DM before liver disease, and those with first-degree relatives with type 2 DM were excluded from the current study. The study design was approved by the medical ethical committee of the Internal Medicine Department, and an informed written consent was obtained from each participating patient.

Calculation of model of the end-stage liver disease score

It was calculated according to the following equation [10]:

$$\text{MELD score} = 9.6 \times \log \text{creatinine (mg/dl)} + 3.8 \times \log \text{bilirubin (mg/dl)} + 11.20 \times \log \text{international normalized ratio} + 6.4$$

Child–Turcotte–Pugh Score

A numerical score was given for each of the variables signifying liver decompensation [11] as follows:

	Numerical score		
Variables	1	2	3
Ascites	None	Slight	Moderate/severe
Encephalopathy	None	Grade 1–2	Grade 3–4
Bilirubin (mg/dl)	<2	2–3	>3
Albumin (mg/dl)	>3.5	2.8–3.5	<2.8
Prothrombin time (extra seconds above control)	1–3	4–6	>6

Then, Child A was assigned for those with CTPS ranging between 5 and 6, Child B (7–9), and Child C (10–15) [12].

Calculation of a homeostasis model for assessment of insulin resistance [13]

$$\text{HOMA} - \text{IR} = \text{fasting glucose (mmol/l)} \times \text{fasting insulin (mU/l)}/22.5$$

Glucose was converted from mg/dl into mmol/l by dividing by 18.

(1) Fasting plasma glucose was measured using the oxidase–peroxidase method [14].
(2) Plasma insulin levels were analyzed using enzyme-linked immunosorbent assay (ELISA) (Dako, Carpinteria, California, USA) according to the manufacturer's instructions [15].

Estimation of 25-hydroxy-vitamin D_3

Blood samples were withdrawn and left to clot for 20 min, and then centrifuged at 12.000 rpm for 10 min; then, the separated serum was kept frozen at −80°C till analysis.

Serum samples were examined for the $25(OH)D_3$ level by an ELISA using the kit supplied by (Immunodiagnostic, New Jersey, USA). Monoclonal antibody identifying $25(OH)D_3$ was used in this assay. The samples were incubated with the detection antibody after the extraction step. Then, peroxidase-conjugated antibody was added to a microplate well, forming a complex of $25(OH)D_3$-detection antibody–peroxidase conjugate. Tetramethylbenzidine was used as a substrate, and the color density developed was proportional to the concentration of vitamin D. Finally, to terminate the reaction, stop solution was added and the microplate was read using an ELISA reader at 520 nm [16].

Results

Demographic data of the enrolled cirrhotic patients were represented as mean ± SD in (Table 1). Serum $25(OH)D_3$ level ranged from 10.1 to 23.5 ng/ml, with an average of 14.11 ± 3.36 ng/ml. Patients were subclassified into tertiles according to the serum $25(OH)D_3$ level as follows: 32 patients (64%) in the first tertile, 12 in the second (24%), and six in the third one (12%) (Table 2).

According to the MELD score, patients were classified into three groups as follows: 12 patients (24%) were included in the first group (score range 0–10), 29 (58%) in the second group (score range 11–20), and nine (18%) in the third group (score > 20) (Table 3).

Finally, according to the CTPS, participants were subclassified as follows: Child A including 11 patients (22%), Child B including 17 patients (34%), and Child C including 22 patients (44%) (Table 4).

HOMA-IR and fasting insulin levels showed a significant inverse correlation with serum vitamin D levels [$25(OH)D_3$] whether by one-by-one correlation or by group correlation using the three tertiles of the $25(OH)D_3$ level (Table 2). Figure 1 shows the inverse correlation between HOMA-IR and $25(OH)D_3$ serum level.

Linear multiple regression analysis showed that only serum vitamin D level was the independent predictor for insulin resistance represented by HOMA-IR in HCV-induced liver cirrhosis patients. Correlation of the vitamin D level with the MELD score or CTPS showed no significant values, whether by one-by-one correlation or by correlating groups (Table 5).

Table 1 Demographic data of 50 hepatitis C virus-induced liver cirrhosis patients

Variables	Mean ± SD
Age (years)	49.16 ± 9.37
Bilirubin (mg/dl)	2.59 ± 1.60
PT (s)	18.04 ± 4.27
PC (%)	57.41 ± 22.30
INR	1.66 ± 0.50
AST (IU/l)	60.60 ± 39.78
ALT (IU/l)	52.73 ± 46.73
GGT (IU/l)	50.36 ± 48.49
Albumin (g/dl)	2.75 ± 0.92
Creatinine (mg/dl)	0.93 ± 0.1929
Cholesterol (mg/dl)	127.60 ± 40.77
TGs (mg/dl)	93.96 ± 37.67
Hb (g/dl)	8.31 ± 2.33
RBCs ($\times 10^6$/ml)	3.27 ± 0.85
WBCs ($\times$ 1000/ml)	5.02 ± 1.84
Platelets ($\times$ 1000/ml)	112.67 ± 76.30
FPG (mg/dl)	98.50 ± 15.84
2 h PPG (mg/dl)	139.60 ± 30.61
F insulin (mU/l)	28.77 ± 7.64
HOMA-IR	6.94 ± 1.96
$25(OH)D_3$ (ng/ml)	14.11 ± 3.36

ALT, alanine aminotransferase; AST, aspartate transaminase; F insulin, fasting insulin; FPG, fasting plasma glucose; GGT, γ glutaryl transferase; Hb, hemoglobin; HOMA-IR, homeostasis model for assessment of insulin resistance; $25(OH)D_3$, 25-hydroxy-vitamin D_3; INR, international normalized ratio; PC, prothrombin concentration; 2 h PPG, 2 h postprandial glucose; PT, prothrombin time; RBCs, red blood corpuscles; TGs, triglycerides; WBCs, white blood cells.

Discussion

Recently, vitamin D deficiency has been recognized as a world health problem especially in developing countries [17] and in dark-skinned populations because of the inhibitory effect of skin melanin on dermal vitamin D synthesis [18]. Its role in the development of GI and type 2 DM has also been reported [8,9,19,20], with a beneficial effect of its supplementation on glycemic control among patients with type 2 DM in a recent short-term prospective study [21]. However, there are no sufficient data on the role of vitamin D deficiency in the setting of hepatogenous DM. The current study showed that low serum $25(OH)D_3$ level was an independent predictor for insulin resistance among HCV-induced liver cirrhosis patients.

The liver is responsible for activation of the inert vitamin D by 25-hydroxylation, forming $25(OH)D_3$. The latter is considered the main circulating vitamin D metabolite and is used for classification of vitamin D status [22] because of its slower clearance rate compared with the more active final product 1,25-dihydroxy-vitamin D [$1,25(OH)2D_3$], which is considered to be less reliable for assessment of the actual vitamin level, being more instantaneously affected by hyperparathyroidism [23].

There has been an established association between liver disease and insulin resistance, with subsequent GI found by previous researchers in about 60–80% of patients with chronic liver disease and frank DM observed in 20–60% of them [24–26]. Particularly, HCV as a cause of liver disease has been considered to increase the likelihood for development of diabetes [24], with suggested HCV core-protein-induced impairment in IRS signaling [27].

Although recently acknowledged to increase the probability of GI and/or type 2 DM in many population-based prospective studies [8,9,28], vitamin D deficiency was suggested to be associated directly with parameters of metabolic syndrome including obesity, increased waist circumference [29], and dyslipidemia [30]. However, in the present study, obese individuals with liver cirrhosis, and/or dyslipidemic patients fulfilling criteria of the metabolic syndrome, were excluded. Waist circumference, although

Table 2 Classification of patients according to 25-hydroxy-vitamin D_3 levels into tertiles

	25-Hydroxy-vitamin D_3 tertiles (mean ± SD)			
Variables	Group 1 ($n=32$)	Group 2 ($n=12$)	Group 3 ($n=6$)	*P*-value
Age (years)	49.81 ± 8.495	50.33 ± 9.689	43.33 ± 12.612	0.269
Bilirubin (mg/dl)	2.447 ± 1.493	2.508 ± 1.448	3.533 ± 2.358	0.313
PT (s)	18.57 ± 4.617	16.95 ± 3.891	17.40 ± 2.840	0.503
PC (%)	55.97 ± 23.296	62.52 ± 24.001	54.83 ± 12.529	0.665
INR	1.730 ± 0.556	1.505 ± 0.405	1.572 ± 0.256	0.379
AST (IU/l)	62.97 ± 30.961	64.25 ± 62.924	40.67 ± 17.489	0.432
ALT (IU/l)	51.04 ± 44.903	61.50 ± 60.617	44.17 ± 22.973	0.725
GGT (IU/l)	48.41 ± 40.869	50.33 ± 55.249	60.83 ± 76.256	0.852
Albumin (g/dl)	2.703 ± 1.035	3.000 ± 0.701	2.533 ± 0.579	0.531
Creatinine (mg/dl)	0.929 ± 0.202	0.983 ± 0.153	0.820 ± 0.198	0.242
Cholesterol (mg/dl)	124.34 ± 40.313	128.67 ± 40.302	142.83 ± 47.839	0.601
TGs (mg/dl)	89.72 ± 29.607	86.00 ± 39.453	132.50 ± 54.749	0.024
Hb (g/dl)	8.159 ± 2.630	8.683 ± 1.951	8.383 ± 1.278	0.807
RBCs ($\times 10^6$/ml)	3.230 ± 0.953	3.276 ± 0.642	3.483 ± 0.618	0.803
WBCs ($\times$ 1000/ml)	4.813 ± 1.625	5.900 ± 2.388	4.317 ± 1.222	0.133
Platelets ($\times$ 1000/ml)	133.49 ± 79.845	95.92 ± 44.375	141.83 ± 107.200	0.492
FPG (mg/dl)	102.16 ± 14.411	92.17 ± 18.542	91.67 ± 13.337	0.092
2 h PPG (mg/dl)	144.97 ± 29.411	129.92 ± 35.171	130.33 ± 24.213	0.259
F insulin (mU/l)	32.351 ± 4.476	26.456 ± 6.336	14.335 ± 3.747	0.000
HOMA-IR	8.078 ± 1.099	5.783 ± 0.706	3.183 ± 0.677	0.000
25(OH)D_3 (ng/ml)	11.998 ± 1.141	16.342 ± 1.304	20.933 ± 1.573	0.000
MELD	15.03 ± 5.492	14.00 ± 4.411	15.50 ± 3.728	0.790
CTPS	9.97 ± 3.763	8.58 ± 3.315	10.50 ± 3.146	0.449

ALT, alanine aminotransferase; AST, aspartate transaminase; CTPS, Child–Turcotte–Pugh Score; F insulin, fasting insulin; FPG, fasting plasma glucose; GGT, γ glutaryl transferase; Hb, hemoglobin; HOMA-IR, homeostasis model for assessment of insulin resistance; 25(OH)D_3, 25-hydroxy-vitamin D_3; INR, international normalized ratio; MELD, model of end-stage liver disease; PC, prothrombin concentration; 2 h PPG, 2 h postprandial glucose; PT, prothrombin time; RBCs, red blood corpuscles; TGs, triglycerides; WBCs, white blood cells.

Table 3 Classification of patients according to the model of end-stage liver disease score

	MELD score (mean ± SD)			
Variables	0–10 ($n=12$)	11–20 ($n=29$)	>20 ($n=9$)	*P*-value
Age (years)	43.08 ± 5.648	50.55 ± 10.602	52.78 ± 4.790	0.026
Bilirubin (mg/dl)	1.120 ± 0.168	2.568 ± 1.338	4.633 ± 1.229	0.000
PT (s)	13.39 ± 1.619	17.92 ± 2.727	24.63 ± 0.812	0.000
PC (%)	90.08 ± 12.325	51.42 ± 11.130	33.11 ± 2.759	0.000
INR	1.114 ± 0.136	1.649 ± 0.318	2.407 ± 0.265	0.000
AST (IU/l)	87.33 ± 60.268	55.21 ± 28.646	42.33 ± 16.470	0.017
ALT (IU/l)	91.11 ± 67.284	45.24 ± 33.353	25.68 ± 4.765	0.001
GGT (IU/l)	80.67 ± 68.007	45.72 ± 40.807	24.89 ± 5.326	0.021
Albumin (g/dl)	3.875 ± 0.796	2.541 ± 0.629	1.944 ± 0.364	0.000
Creatinine (mg/dl)	0.882 ± 0.239	0.935 ± 0.197	0.973 ± 0.091	0.551
Cholesterol (mg/dl)	148.33 ± 38.770	119.62 ± 41.100	125.67 ± 36.339	0.120
TGs (mg/dl)	110.75 ± 29.280	83.03 ± 37.326	106.78 ± 39.949	0.050
Hb (g/dl)	9.517 ± 1.900	8.224 ± 2.513	6.989 ± 1.458	0.043
RBCs ($\times 10^6$/ml)	4.084 ± 0.801	3.092 ± 0.717	2.766 ± 0.541	0.000
WBCs ($\times$ 1000/ml)	4.242 ± 0.872	5.622 ± 2.090	4.089 ± 1.086	0.019
Platelets ($\times$ 1000/ml)	188.75 ± 74.301	99.61 ± 64.059	53.32 ± 20.962	0.000
FPG (mg/dl)	102.67 ± 12.070	99.79 ± 16.923	88.78 ± 14.007	0.109
2 h PPG (mg/dl)	145.42 ± 31.572	143.00 ± 30.488	120.89 ± 25.082	0.125
F insulin (mU/l)	30.474 ± 6.966	27.500 ± 8.135	30.614 ± 6.695	0.390
HOMA-IR	7.667 ± 1.833	6.703 ± 2.056	6.733 ± 1.719	0.343
25(OH)D_3 (ng/ml)	13.051 ± 2.660	14.631 ± 3.721	13.856 ± 2.872	0.387
CTPS	5.42 ± 1.165	10.03 ± 2.639	14.33 ± 0.707	0.000

ALT, alanine aminotransferase; AST, aspartate transaminase; CTPS, Child–Turcotte–Pugh Score; F insulin, fasting insulin; FPG, fasting plasma glucose; GGT, γ glutaryl transferase; Hb, hemoglobin; HOMA-IR, homeostasis model for assessment of insulin resistance; 25(OH)D_3, 25-hydroxy-vitamin D_3; INR, international normalized ratio; MELD, model of end-stage liver disease; PC, prothrombin concentration; 2 h PPG, 2 h postprandial glucose; PT, prothrombin time; RBCs, red blood corpuscles; TGs, triglycerides; WBCs, white blood cells.

considered a cornerstone criterion of metabolic syndrome and found in previous studies to be inversely correlated with the 25(OH)D_3 level and positively correlated with insulin resistance [29], waist circumference was not a valid item to assess in the participating patients with cirrhosis, being biased by the intra-abdominal organomegaly and/or ascites. Accordingly, a direct effect of low vitamin D levels might be suggested to explain the development of insulin resistance among patients with HCV-induced cirrhosis in the current research. One of these direct influencing factors of vitamin D could be its anti-inflammatory property.

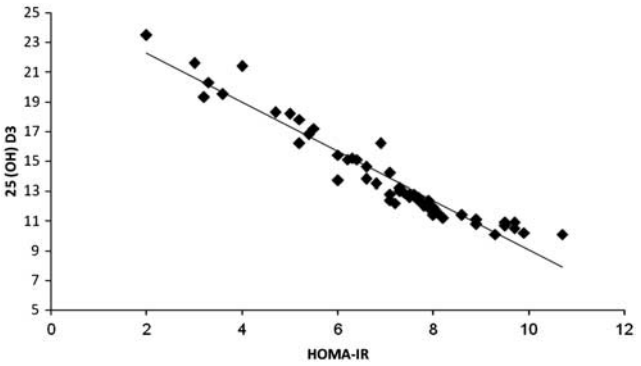
Inflammation participates in the defense mechanisms against infections as HCV, but may be deleterious, being a cornerstone for the development of fibrosis, which is

Table 4 Classification of patients according to the Child–Turcotte–Pugh Score

	CTPS (mean ± SD)			
Variables	Child A ($n=11$)	Child B ($n=17$)	Child C ($n=22$)	*P*-value
Age (years)	42.45 ± 5.466	50.53 ± 10.875	51.45 ± 8.354	0.022
Bilirubin (mg/dl)	1.113 ± 0.174	1.900 ± 0.584	3.866 ± 1.578	0.000
PT (s)	13.09 ± 1.300	16.78 ± 1.787	21.50 ± 3.559	0.000
PC (%)	91.91 ± 11.095	57.01 ± 10.240	40.46 ± 9.855	0.000
INR	1.097 ± 0.129	1.481 ± 0.145	2.073 ± 0.432	0.000
AST (IU/l)	89.09 ± 62.886	58.76 ± 26.426	47.77 ± 26.002	0.015
ALT (IU/l)	97.73 ± 66.345	47.66 ± 22.575	34.14 ± 34.181	0.000
GGT (IU/l)	86.73 ± 67.843	46.88 ± 47.555	34.86 ± 24.847	0.011
Albumin (g/dl)	4.045 ± 0.559	2.829 ± 0.621	2.050 ± 0.362	0.000
Creatinine (mg/dl)	0.907 ± 0.233	0.901 ± 0.243	0.962 ± 0.118	0.578
Cholesterol (mg/dl)	155.36 ± 31.639	121.41 ± 46.912	118.50 ± 34.682	0.034
TGs (mg/dl)	117.36 ± 19.122	89.06 ± 44.978	86.05 ± 34.980	0.061
Hb (g/dl)	10.000 ± 0.941	7.906 ± 2.714	7.782 ± 2.180	0.021
RBCs ($\times 10^6$/ml)	4.282 ± 0.436	3.126 ± 0.864	2.878 ± 0.539	0.000
WBCs ($\times$ 1000/ml)	4.464 ± 0.432	6.131 ± 2.465	4.427 ± 1.281	0.006
Platelets ($\times$ 1000/ml)	184.73 ± 76.544	115.45 ± 82.767	74.50 ± 36.346	0.000
FPG (mg/dl)	102.55 ± 12.652	95.94 ± 17.869	98.45 ± 15.883	0.569
2 h PPG (mg/dl)	144.55 ± 32.962	133.41 ± 26.192	141.91 ± 33.122	0.584
F insulin (mU/l)	29.952 ± 7.055	27.785 ± 9.370	28.950 ± 6.627	0.764
HOMA-IR	7.518 ± 1.845	6.541 ± 2.469	6.959 ± 1.533	0.442
25(OH)D_3 (ng/ml)	13.319 ± 2.614	15.101 ± 4.156	13.745 ± 2.946	0.316
MELD	8.00 ± 1.612	13.47 ± 1.875	19.32 ± 2.767	0.000

ALT, alanine aminotransferase; AST, aspartate transaminase; CTPS, Child-Turcotte-Pugh Score, F insulin, fasting insulin; FPG, fasting plasma glucose; GGT, γ glutaryl transferase; Hb, hemoglobin; HOMA-IR, homeostasis model for assessment of insulin resistance; 25(OH)D_3, 25-hydroxy-vitamin D_3; INR, international normalized ratio; MELD, model of end-stage liver disease; PC, prothrombin concentration; 2 h PPG, 2 h postprandial glucose; PT, prothrombin time; RBCs, red blood corpuscles; TGs, triglycerides; WBCs, white blood cells.

Figure 1



Correlation between HOMA-IR and 25(OH)D3 among hepatitis C virus-induced liver cirrhosis patients. 25(OH)D3, 25-hydroxy-vitamin D3; HOMA-IR, homeostasis model for assessment of insulin resistance.

Table 5 Correlation between homeostasis model for assessment of insulin resistance and other variables

Variables	Spearman's correlation	*P*-value
MELD score	−0.142	0.325
MELD as groups	−0.152	0.293
25(OH)D_3	−0.976	0.000
25(OH)D_3 in tertiles	−0.830	0.000
CTPS	−0.118	0.414
Child class	−0.040	0.781

25(OH)D_3, 25-hydroxy-vitamin D_3; CTPS, Child–Turcotte–Pugh Score; MELD, model of end-stage liver disease.

a sine qua non of liver cirrhosis. The cytokine TNF-α was suggested to play a pivotal role in the pathogenesis of chronic hepatitis C, especially that progressing to liver cirrhosis [31,32], and was also supposed to induce intrahepatic insulin resistance and hence hepatogenous diabetes by phosphorylating IRS 1 and IRS 2 [2]. Macrophages produce excessive amounts of TNF-α in response to nuclear factor κB, which activates gene transcription of the former in response to inflammation and stress [33]. In animal models, 1,25(OH)2D_3 was shown to upregulate the nuclear factor κB-α inhibitor by increasing its messenger RNA stability and decreasing its phosphorylation, emphasizing the anti-inflammatory role of 1,25(OH)2D_3 [34]. Moreover, vitamin D receptors, which are nuclear steroid receptors for the active form 1,25(OH)2D_3, have been found recently to be distributed in many tissues including the liver [35]. Through these receptors, 1,25(OH)2D_3 might be considered to accelerate hepatic insulin resistance, with the consequent development of GI and finally diabetes.

Moreover, vitamin D in its fully active form 1,25(OH)2D_3 has been considered to increase intracellular calcium, suggested to enhance insulin secretion through phosphoinositide/protein kinase-C [36] and/or the cyclic-AMP pathway [37]. Conversion of proinsulin into insulin was another potential mechanism for the promotive effect of vitamin D on insulin [38]. Recent detection of extrarenal 1-α-hydroxylase in skeletal muscles, adipocytes [39], and pancreatic islet cells [40] was considered to induce local activation of 25(OH)D_3 in these tissues, with subsequent enhancement of insulin action. In addition, at the genetic level, there is a vitamin D response element sequence in the insulin receptor gene promoter [41], which has been detected in cellular tests to increase transcription and protein expression of these insulin receptors [42].

There is a disagreement on the cutoff level for assessment of vitamin D status; however, the average serum 25(OH)D_3 level recorded previously in South African Moroccan women was less than 75 nmol/l [43], and was less than 37.5 nmol/l in 48% of a cohort of Tunisian women [44]. An Egyptian Turkish study reported values below 37.5 nmol/l in 71% of rachitic children, as well as in 48% of the those in an apparently healthy control group [45]. The present study found a serum level of 25(OH)D_3 ranging from 10.1 to 23.5 ng/ml equivalent to 25.21–58.66 nmol/l, which seemed to begin below the suggested cutoff level in the last two studies carried out in the same geographic regions.

Deficiency of 25(OH)2D_3 in cirrhotic patients has been attributed recently to synthetic decompensation of the cirrhotic liver [6] with reduced activity of 25-α-hydroxylase, which is one of the cytochrome P450-dependent steroid hydroxylases synthesized in the liver [7] and responsible for 25-α-hydroxylation of the inactive vitamin D. In contrast to other researches [46–49], which showed that 25(OH)D_3 deficiency in cirrhotic patients was associated directly with liver dysfunction as assessed by both the MELD score and/or CTPS, and was even a predictor of future liver decompensation and overall mortality in cirrhotic patients [6], the current study drew no similar conclusions. This could be attributed to the difference in the etiology of liver cirrhosis, being HCV posthepatitic cirrhosis in the present study, with the exclusion of alcoholic or nonalcoholic fatty liver disease [2]. In addition, differences in the ethnic group studied and different study designs, being cross-sectional in the current one compared with the prospective study method used by Putz-Bankuti *et al.* [6], might explain the discrepancy in the conclusions. Moreover, the different viral genotype, such as HCV genotype 4, which is the most prevalent among Egyptian patients [50] compared with genotype 1 evaluated in other researches [48], could reflect a genotype-specific effect on vitamin D hydroxylation in the liver. However, 25(OH)D_3 deficiency in cirrhotic patients might be explained by other mechanisms such as malnutrition because of dietary restrictions [51] and/or reduced appetite [52] in cirrhotic patients, malabsorption attributed to impaired biliary-facilitated absorption of fat-soluble vitamins such as vitamin D [53] as well as gastrointestinal congestion [54], and reduced exposure to sunlight by cirrhotic patients [46], who usually prefer staying indoors because of limited movement by organomegaly and/or ascites.

The current results are not in agreement with those of previous investigators, who found that hepatogenous diabetes was an indicator of advanced liver damage [25,55]. Hepatogenous diabetes was considered as an unfavorable prognostic factor for cirrhosis patients with suggested considerable influence on the short-term [25] and/or the long-term prognosis [56], attributed to deterioration of liver cell function [25,56]. This disagreement may be because of the difficulty in, or even impossibility of, differentiating between hepatogenous diabetes and type 2 DM. Therefore, in the present study, those with first-degree relatives with type 2 DM were excluded, being more susceptible to develop type 2 DM as well [57]. Moreover, patients with a history of diabetes, antedating that of the known liver disease, were excluded. In addition, 50% of the participating patients had normal fasting and 2 h postprandial plasma glucose readings recorded within 6 months before being enrolled in the study. Thus, the insulin resistance detected was relatively recent while carrying out the study, following established liver cirrhosis. Both HOMA-IR and oral glucose tolerance test are known to be sensitive methods for assessment of insulin resistance [26,58], with the former being more easily performed in this particular group of patients, with gastric upsets and abdominal distension, which might interfere with performing oral glucose tolerance test properly. Moreover, HOMA-IR and fasting insulin levels approximated the high values recorded in hepatogenous diabetes in previous studies [59].

Conclusion

To our knowledge, the current study was the first in the English language to investigate vitamin D status as a link between HCV-induced liver cirrhosis and development of insulin resistance. An obvious association was found between low 25(OH)D_3 level and insulin resistance in these patients, unrelated to metabolic syndrome criteria. This suggested a specific role of vitamin D in hepatogenous diabetes, warranting further molecular studies for more accurate delineation of the related pathophysiologic mechanism. Moreover, prospective interventional studies to evaluate the effect of vitamin D replacement among liver cirrhosis patients to prevent GI, improve the clinical course of cirrhosis, and reduce their mortality are recommended.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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