

Original Article

Association of vitamin D binding protein polymorphisms with response to therapy in Egyptian chronic hepatitis C patients

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Abstract

Introduction: Vitamin D binding protein (VDBP) is a potential modulator of immune response and is associated with clinical progression of many diseases. Our aim was to assess influence of baseline 25-hydroxyvitamin D levels and VDBP single nucleotide polymorphisms (SNPs), rs4588 (C > A) and rs7041 (G > T), on baseline clinical parameters and response to interferon based therapy in chronic hepatitis C patients in Egypt.

Methodology: Genotyping was performed by RFLP (Restriction Fragment Length Polymorphism) in 112 treatment naïve hepatitis C patients and 50 healthy controls. Vitamin D levels were assessed by ELISA. HCV RNA quantification was performed by PCR to assess therapy outcome.

Results: Patients with VDBP WT+ diplotype (3 or 4 VDBP major alleles) had higher viral response rates at weeks 12, 48, and 72 ($p = 0.046$, 0.034 and 0.029 , respectively) and lower base line viral load ($p = 0.016$). Multivariate logistic regression identified VDBP WT+ diplotype as an independent predictor of sustained viral response (SVR; $p = 0.014$, RR = 4.716, 95% CI = 1.371 – 16.609). Interestingly, WT- diplotype (less than 3 VDBP major alleles) was associated with significant liver fibrosis ($p = 0.045$).

Conclusions: VDBP WT+ diplotype is associated with lower baseline viral load and better therapy outcome in HCV treatment naïve patients. The rs4588 genotype is associated with SVR in the Egyptian population.

Key words: rs7041; rs4588; fibrosis; viral load; Egypt; SVR.

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Introduction

Hepatitis C is a global pandemic that affects nearly 150 million individuals worldwide [1]. Prevalence of hepatitis C virus (HCV) antibodies is estimated to be around 1.6% worldwide with HCV prevalence of about 1.1% [2]. Chronic hepatitis C causes cirrhosis in 10 – 20% of infected patients with 1-5 % annual risk of hepatocellular carcinoma [3]. Egypt has one of the highest prevalences of HCV infection worldwide with 14.7% of adults seropositive for HCV and 10% with HCV viraemia. Genotype 4 accounts for more than 90% of HCV infections in Egypt [2]. Pegylated interferon and ribavirin have been the standard of care for treatment of chronic HCV with a response rate of about 55% [4]. Recently, direct acting antivirals with response rates reaching 90% have been used in patients with genotype 4 [5]. Due to the high expense of these drugs, patients may still be treated with pegylated interferon and ribavirin in resource limited countries.

25-hydroxyvitamin D plays an important role as an immunomodulator and has been shown to inhibit HCV

replication *in vitro* [6]. Some studies showed that baseline vitamin D levels are associated with better therapy outcome [7,8] while others showed no such association [9,10].

Polymorphisms of the vitamin D pathway have been shown to affect hepatitis C treatment outcome in patients infected with genotypes 1, 2, and 3 [11–13]. Vitamin D binding protein (VDBP) polymorphisms were associated with sustained viral response (SVR) in hepatitis C patients in only one previous study [7]. VDBP is a polymorphic multifunctional protein that consists of 3 main domains and has 2 important binding sites: one for vitamin D metabolites between residues 35–49, and one for G actin. It transports 25-hydroxyvitamin D and 1, 25-hydroxyvitamin D in serum to different tissues as 88% of 25-hydroxyvitamin D and 85% of 1, 25-hydroxyvitamin D are bound to VDBP. It plays an important role in the inflammatory cascade as it binds G actin produced from tissue injury to prevent its polymerization into F actin, which can cause intravascular coagulation and thromboembolism

[14]. It is involved in chemotaxis, complement mediated recruitment of neutrophils to inflammatory sites and it enhances neutrophil chemotactic activity of complement activated peptide C5a [15]. It is also transformed by deglycosylation into a potent macrophage activating factor (MAF) which has an important role in phagocytosis [16].

Our aim was to assess the effect of two VDBP polymorphisms (rs4588 and rs7041) and 25-hydroxyvitamin D levels on different baseline clinical parameters and the outcome of interferon-based therapy in Egyptian patients with chronic hepatitis C.

Methodology

Study Design and Patients

A prospective observational study was conducted at Cairo-Fatemic Hospital, Cairo, Egypt. Patients were enrolled from September 2013 to July 2014. Inclusion criteria were: age ≥ 18 years and ≤ 60 years, fasting blood sugar < 115 mg/dl or 20% upper normal limit (140 mg/dl), HBA1C $\leq 7.5\%$ in diabetic patients, normal serum creatinine, normal thyroid stimulating hormone level, negative hepatitis B surface antigen, hemoglobin ≥ 12 g/dl for male or ≥ 11 g/dl for female, total leukocytes $\geq 3,500/\text{mm}^3$, ANC $\geq 1500/\text{mm}^3$, platelets $\geq 100,000/\text{mm}^3$, presence of HCV RNA by PCR and elevated liver enzymes within the preceding six months for patients with F1 stage fibrosis.

Exclusion criteria were: hypersensitivity to interferon or ribavirin, decompensated liver cirrhosis (presence of ascites, esophageal varices or hepatic encephalopathy), hepatocellular carcinoma, body mass index > 35 Kg/m² and vitamin D supplementation.

This study was performed according to the declaration of Helsinki. Ethics committee approval was obtained from the institutional review board at the National Committee for Combating Viral Hepatitis and Faculty of Pharmacy, Cairo University. Signed informed consent was obtained from all patients.

Patients received either peg-interferon alpha-2a (180 mcg/Week) or peg-interferon alpha-2b (1.5 mcg/kg/week) plus weight based oral ribavirin (11-15 mg/kg/day) for 48 weeks. Vitamin D levels and frequencies of VDBP polymorphisms were compared with healthy controls of matched age and gender.

Laboratory investigations and 25-hydroxyvitamin D Assay

Baseline blood samples were obtained from patients after eight hours of fasting to determine complete blood count, fasting blood glucose and liver biochemical profile. A liver biopsy was also obtained to assess

inflammatory activity and fibrosis stage. Quantitative HCV PCR was performed at baseline, weeks 12, 48 and 72 to determine outcome of therapy. Early viral response (EVR), end of treatment response (EoTR), sustained viral response (SVR) were defined as previously described [17].

The 25-hydroxyvitamin D level was quantified using enzyme linked immunosorbent assay (Catalog no. EQ 6411-9601, Euroimmun, Luebeck, Germany) according to manufacturer's instructions. Cutoff values of 10, 20, and 30 ng/ml were used to define 25-hydroxyvitamin D severe deficiency, deficiency and insufficiency, respectively [18]. The METAVIR score was used to assess degree of inflammatory activity and stage of liver fibrosis [19].

Genotyping

DNA was extracted from fresh EDTA whole blood samples using the Wizard Genomic DNA purification Kit (Promega, Madison, USA) according to manufacturer's instructions. Genotyping was performed by polymerase chain reaction followed by restriction fragment length chain polymorphism (PCR-RFLP) as previously described [20]. In brief, PCR amplification of the VDBP encoding gene (483 bp fragment) was performed in a 25 μl reaction containing 150 ng genomic DNA, 2.5 μM MgCl₂, 1X Taq colorless buffer, 0.5 μM of each primer, and 1.25 units of DNA Taq polymerase (Promega, Madison, USA). Thermal cycling conditions were as follows: initial denaturation for 2 minutes at 95 °C, 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 40 seconds, extension at 72 °C for 45 seconds and final extension at 72 °C for 7 minutes. The PCR product was digested with restriction enzymes HaeIII and StyI (Ipswich, Massachusetts, USA) for rs7041 and rs4588, respectively. One μg of PCR product was incubated with 1 unit of the restriction enzyme in a 25 μl total reaction volume at 37 °C for 1 hour. Digestion products were loaded on 2.5% agarose gel and subjected to electrophoresis for 30 minutes then visualized under UV light. Digestion with StyI yielded a 483 bp fragment for the CC genotype, 305+178 bp fragments for the AA genotype and 483+305+178 bp fragments for the CA genotype while digestion with HaeIII yielded a 483 bp fragment for the TT genotype, 297+183 bp fragments for the GG genotype and 483+297+183 bp fragments for the GT genotype. Haplotype frequencies were estimated using Phase software (Stephens lab, University of Chicago, USA).

A major allele model was implemented for both loci studied (G for rs7041 and C for rs4588), where patients

with 3 or 4 major alleles were classified as the wild diplotype [WT+] patients (GC/GC, GC/GA, GC/TC) and those with less than 3 major alleles (TA/TA, GA/TA, TC-TA, GA/GA, TC/TC) were classified as the non-wild type [WT-] patients.

Sample size and statistical analysis

A sample size calculation was performed a priori by G power software. For 25-hydroxyvitamin D levels, a total sample size of 92 (46 in each arm) was required to detect an effect size of 0.6 with an 80% power at a significance value of 0.05 (two tailed). A sample size of 116 (58 in each arm) was required to detect a viral response rate difference of 25% between VDBP WT+ and WT- groups with 80% power at a 0.05 significance level (two tailed). Continuous data was analyzed using Student's t-test for normally distributed data and the Mann-Whitney test for non-normal data. Nominal data was analyzed by the Pearson Chi-Square test and Chi square test for linear trend when appropriate. The Chi-Square G test was used to examine if the population studied is in Hardy-Weinberg Equilibrium. Stepwise logistic regression with a forward approach was performed to determine independent predictors of SVR.

Results

Viral response

One hundred and twelve patients completed treatment with interferon/ribavirin. Interferon alpha 2a was used in 60 (53.6%) patients and interferon alpha 2b was used in 52 (46.4%) patients. Ninety nine (88.4%) patients achieved EVR and seventy seven (68.8%) patients achieved EoTR. SVR data was available for

only 84 patients due to loss to follow up. Of those, 44 (52.4%) patients achieved SVR. Baseline demographics and clinical data for chronic HCV patients included in this study are shown in Table 1.

25-hydroxyvitamin D levels and VDBP polymorphisms in HCV patients and healthy controls

Vitamin D levels and VDBP genotype frequencies were compared with 50 healthy controls of matched age and gender. Mean vitamin D levels were significantly lower in patients than controls (21.01 ± 7.92 vs. 28.66 ± 16.06 , $p = 0.014$). The proportion of patients with sufficient vitamin D (> 30 ng/ml) was significantly lower than controls (13/94 [13.8%] vs. 18/50 [36%], respectively, $p = 0.002$).

VDBP genotype frequencies for both loci did not differ significantly from Hardy-Weinberg equilibrium ($p > 0.05$). VDBP polymorphism frequencies did not differ significantly among patients and controls (Table 2).

Baseline 25-hydroxyvitamin D levels, VDBP polymorphisms and viral response rates

Comparison of baseline demographics and clinical data according to SVR is shown in Table 3. Baseline 25-hydroxyvitamin D serum samples were available in 94 patients. Peg-interferon alpha 2b was associated with a reduced likelihood of achieving SVR ($p = 0.001$) and an association with EVR and EoTR approached significance, although a larger sample size would be needed for verification ($p = 0.082$ and 0.052 respectively).

Table 1. Baseline demographics and clinical data for HCV patients included in the study.

Age (years)		37.86 ± 11.74
Gender (Male)		73 (54.1%)
Interferon alpha 2a		60 (53.6%)
BMI (Kg/m ²)		27.04 ± 3.56
ALT (IU/L)		58.69 ± 43.79
AST (IU/L)		46.67 ± 30
Glucose (mg/dL)		91.34 ± 16.42
ALP (IU/L)		88.4 ± 54.89
HCV log		5.44 ± 0.98
Albumin (g/dL)		4.31 ± 0.4
Vitamin D (ng/mL) ^a		21.01 ± 7.92
Degree of inflammatory activity ^b	A1	87 (85.3%)
	A2	13 (12.7%)
	A3	2 (2%)
	F1	70 (68.6%)
Stage of Fibrosis ^c	F2	17 (16.7%)
	F3	13 (12.7%)
	F4	2 (2%)

Data is presented as mean ± standard deviation for continuous data and count (percentage) for non-continuous data; ^a samples were available in 94/112 patients; ^{b,c} biopsy results were available for 102/112 Patients; BMI: body mass index, ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, HCV: hepatitis C virus.

Table 2. Frequencies of VDBP polymorphisms among HCV patients and healthy controls.

		Patients	Controls	p value
rs4588	CC genotype	65 (58%)	33 (66%)	0.212
	CA genotype	41 (36.6%)	17 (34%)	
	AA genotype	6 (5.4%)	0 (0%)	
	C Allele	0.76	0.83	0.422
	A allele	0.24	0.17	
rs7041	GG genotype	35 (31.3%)	19 (38%)	0.446
	GT genotype	47 (41.9%)	22 (44%)	
	TT genotype	30 (26.8%)	9 (18%)	
	G Allele	0.52	0.6	0.387
	T Allele	0.48	0.4	
Haplotype	G-C	117 (52.2%)	59 (59%)	0.233
	T-C	54 (24.2%)	23 (23%)	
	T-A	53 (23.6%)	17 (17%)	
	G-A	0 (0%)	1 (1%)	

Data is presented as count (percentage).

Table 3. Baseline demographics and clinical data for included patients classified according to sustained viral response.

	SVR+ (n = 44)	SVR- (n = 40)	p-value
Age (years)	39.89 ± 13.3	47 ± 11.45	0.081
Gender (Male)	22 (50%)	22 (55%)	0.647
BMI (Kg/m ²)	26.87 ± 3.78	27.66 ± 3.41	0.32
ALT (IU/L)	54.41 ± 34.13	53.83 ± 31.65	0.911
AST (IU/L)	39.55 ± 17.34	48.78 ± 23.73	0.059
HCV log	5.33 ± 1.01	5.56 ± 0.96	0.292
Glucose (mg/dL)	92.17 ± 16.74	91.56 ± 15.37	0.962
Interferon alpha 2b	13 (29.5%)	26 (65%)	0.001*
ALP (IU/L)	89.8 ± 55.71	84.07 ± 52.72	0.654
Albumin (IU/L)	4.29 ± 0.4	4.29 ± 0.45	0.971
Vitamin D (ng/mL) ^a	20.68 ± 7.11	22.03 ± 7.69	0.46

Data is shown as mean ± standard deviation for continuous data and as count (percentage) for non-continuous data; ^a baseline samples were available for 70/84 patients with SVR data; BMI: body mass index, ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, HCV: hepatitis C virus; * Result is statistically significant at the 0.05 significance level.

Table 4. Effect of VDBP polymorphisms on baseline clinical parameters and viral response to pegylated Interferon/ribavirin.

	VDBP diplotype			rs4588 genotype			rs7041 genotype		
	WT- n = 57	WT+ n = 55	p	CC n = 65	CA, AA n = 47	p	GG n = 35	GT, TT n = 77	p
Vitamin D (ng/mL)	19.18 ± 7.07	22.62 ± 8.35	0.035*	22.2 ± 8.44	19.09 ± 6.69	0.065	23.3 ± 8.6	20 ± 7.45	0.06
Baseline ALT (IU/mL)	54.95 ± 48.05	62.56 ± 38.95	0.1	61.2 ± 37.44	55.25 ± 51.53	0.237	62.3 ± 36	57 ± 47.1	0.213
Baseline HCV (log)	5.67 ± 0.92	5.21 ± 1	0.01*	5.29 ± 0.97	5.65 ± 0.96	0.054	5.3 ± 0.95	5.5 ± 1	0.314
Baseline ALP (IU/mL)	92.84 ± 57.7	83.92 ± 52	0.333	87.57 ± 56	89.6 ± 53	0.328	86.2 ± 55	89.4 ± 55	0.634
EVR	47/57 (82.5%)	52/55 (94.5%)	0.046*	60/65 (92.3%)	39/47 (83%)	0.128	32/35 (91.4%)	67/77 (87%)	0.55
EoTR	34/57 (59.6%)	43/55 (78.2%)	0.034*	50/65 (76.9%)	27/47 (57.4%)	0.028*	25/35 (71.4%)	52/77 (67.5%)	0.68
SVR ^a	17/42 (40.5%)	27/42 (64.3%)	0.029*	31/49 (63.3%)	13/35 (37.1%)	0.018*	16/27 (59.3%)	28/57 (49.1%)	0.385
Significant fibrosis (F2, F3, F4) ^b	21/52 (40.4%)	11/50 (22%)	0.045*	15/60 (25%)	17/42 (40.5%)	0.097	7/32 (21.9%)	25/70 (35.7%)	0.162

Data is shown as mean ± standard deviation for continuous data and as count (percentage) for non-continuous data; ^a Data was available for 84 patients; ^b Biopsy was available in 102 patients; * Result is statistically significant at the 0.05 significance level.

An association between baseline AST levels and SVR also approached significance ($p = 0.059$). Baseline 25-hydroxyvitamin D levels were not associated with viral response at any stage of treatment ($p = 0.61, 0.5,$ and 0.46 for EVR, EoTR, and SVR respectively). Moreover, 25-hydroxyvitamin D levels did not affect either baseline viral load or stage of liver fibrosis ($p = 0.73$ and 0.931 respectively).

Effect of VDBP genes polymorphisms and VDBP diplotype on baseline criteria and viral response is shown in Table 4. Vitamin D levels were significantly higher among WT+ patients ($P= 0.035$). At baseline, significant liver fibrosis (F2, F3 and F4) was more prevalent in patients with WT- diplotype (21/52 vs. 11/50, $P=0.043$). Moreover, VDBP WT+ diplotype was associated with lower HCV viral load at baseline ($P= 0.01$).

The rs7041 genotype showed no association with baseline viral load, stage of fibrosis or viral response rates. Regarding rs4588, our results show that the CC genotype was not associated with stage of liver fibrosis but it was significantly associated with better viral response at weeks 48 and 72 ($p = 0.028$ and 0.018 , respectively). When the combined effect of both VDBP loci was assessed, viral response rates were significantly higher among patients with WT+ diplotype at all stages of treatment (Table 4). In patients with the WT+ diplotype twenty-seven out of forty-two achieved SVR but only seventeen out of forty-two WT-diplotype patients achieved SVR ($p = 0.029$, Figure 1).

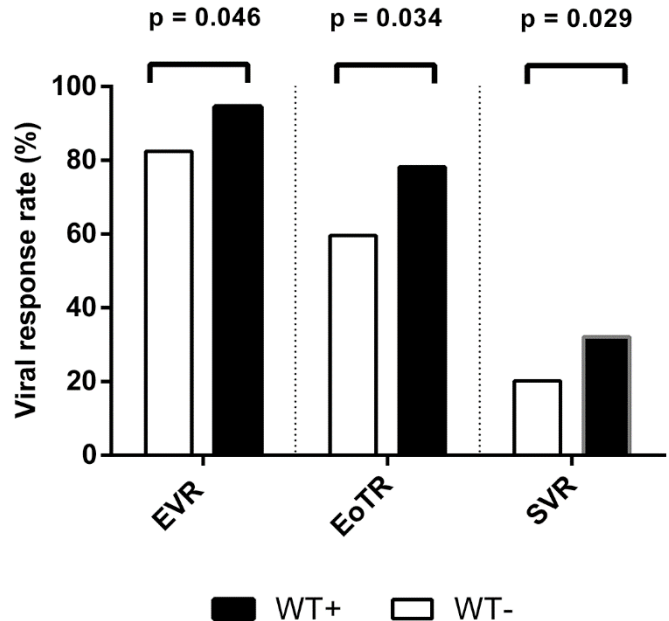
Interestingly, there was a trend towards an increase in SVR with increasing number of major alleles (0/4 [0%] vs. 4/11 [36.4%] vs. 13/27 [48.1%] vs. 27/42 [64.3%], $p = 0.015$). Those with 3 and 4 major alleles were included in one group as they are associated with the production of the GC1 isoform (Figure 2).

Finally, baseline demographics and clinical parameters that were significantly associated with SVR (Table 3) were included in multivariate logistic regression analysis to develop a model to identify independent predictors of SVR. Interferon type, baseline AST levels and VDBP WT+ diplotype were independent predictors of SVR (Table 5). Patients with the WT+ diplotype had a four times greater chance of achieving SVR ($p = 0.014$, $RR= 4.716$, $95\% CI= 1.371 - 16.609$).

Discussion

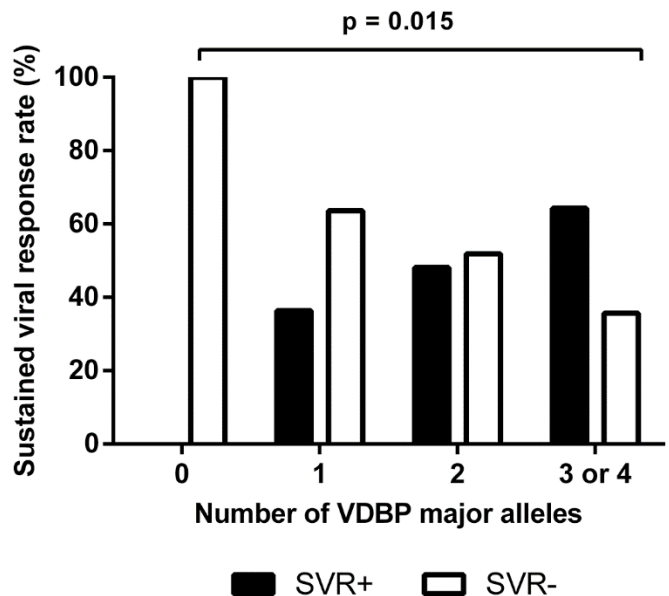
Viral response rates in the current study were 88.4% for EVR, 68.8% for EoTR and 52.4% for SVR. Response rates are similar to those reported in Egyptian HCV patients treated with interferon/ribavirin

Figure 1. Relationship between number of major alleles in VDBP loci (rs7041 G > T and rs4588 C > A) and viral response rates in HCV patients. Patients were classified as either WT+ (GC/GC, GC/GA, and GC/TC) or WT- (TA/TA, GA/TA, TC-TA, GA/GA, TC/TC). Proportions (%) of patients achieving EVR, EoTR, and SVR are shown for both groups. Statistical analysis was performed by the Pearson chi square test.



WT+: Wild-type diplotype carrier, WT-: Non wild-type diplotype carrier.

Figure 2. Relationship between number of VDBP major alleles and sustained viral response rate. SVR rate increases with increasing number of VDBP major alleles from zero to 3 or 4 major alleles ($p = 0.015$).



SVR+: Negative HCV RNA at week 72; SVR-: Positive HCV RNA at week 72.

Table 5. Predictors of SVR using multivariate logistic regression.

	Univariate analysis	Multivariate logistic regression	
	p-value	p-value	RR (95% C.I)
Degree of inflammatory activity (A1 vs. A2, A3)	0.550		
Stage of fibrosis (F1 vs. F2, F3 and F4)	0.778		
Baseline AST \geq 40 (IU/L)	0.03*	0.02*	0.231 (0.067 – 0.776)
Baseline blood glucose \geq 100(mg/dL)	0.452		
Interferon alpha 2b	0.001*	0.004*	0.157 (0.058 – 0.575)
WT+ diplotype	0.03*	0.014*	4.716 (1.371 – 16.609)
HCV RNA \geq 300,000 IU/mL	0.516		
Platelets \geq 250 \times 10 ⁹ /L	0.13		
BMI \geq 25 Kg/m ²	0.158		
Gender	0.695		

* Result is statistically significant at the 0.05 significance level.

combination [4]. Vitamin D levels in hepatitis C patients were significantly lower than controls and 25-hydroxyvitamin D insufficiency was more common in HCV patients. These findings confirm that patients with chronic liver disease have impaired 25-hydroxyvitamin D status compared to healthy controls [21].

Regarding VDBP loci rs4588 and rs7041, our results show that genotype frequencies of both loci studied are similar to those reported in Caucasian populations. However, they differ significantly from frequencies in African American populations where the T allele is more common than the G allele [22].

Patients with the VDBP WT+ diplotype had significantly higher levels of 25-hydroxyvitamin D than those who did not. Our findings are similar to results of previous studies [23] and are attributed to the different affinities of VDBP isoforms to vitamin D metabolites. VDBP polymorphisms, rs7041 (G > T) and rs4588 (C > A), produce 3 different isoforms: GS-1f (T-C), GC-1s (G-C), and GC-2 (T-A) [24]. These isoforms exhibit different affinities to vitamin D metabolites with GC-1f expressing the highest affinity and GC-2 expressing the least. Higher total concentrations of 25-hydroxyvitamin D were associated with isoforms GC-1f and GS-1s as they are more slowly metabolized than the GC2 isoform [25].

Although rs4588 and rs7041 genotypes were not associated with baseline viral load, HCV patients with the WT- diplotype had higher baseline viral burden than patients with the WT+ diplotype. This supports the finding that 25-hydroxyvitamin D levels affect HCV replication in vitro [6] and can be explained by the fact that the VDBP WT+ diplotype codes for GC-1 isoform of VDBP which is associated with higher circulating concentrations of 25-hydroxyvitamin D, as previously discussed.

Regarding baseline 25-hydroxyvitamin D and stage of liver fibrosis, contradictory results have been

reported in the literature. In the current study, 25-hydroxyvitamin D levels were not associated with stage of liver fibrosis, which is similar to previous results [26,27]. However, other studies have shown an association between baseline 25-hydroxyvitamin D levels and stage of liver fibrosis [28,29].

Interestingly, significant liver fibrosis (stages F2, F3 and F4 according to the METAVIR score) was more common in patients with the WT- diplotype compared to those with the WT+ diplotype. To our knowledge, this is the first study to report such an association. A study conducted in genotype 1 HCV patients found no association between VDBP rs7041 genotype and stage of liver fibrosis, but the authors did not assess the effect of rs4588 polymorphisms [30]. In the current study, we found no significant association between either rs7041 or rs4588 genotypes and stage of liver fibrosis. However, when both loci were assessed together, there was a significant association between VDBP diplotype and stage of liver fibrosis.

The VDBP WT+ diplotype was significantly associated with response to therapy at all stages of treatment (Table 4) where 27/42 patients with the WT+ diplotype achieved SVR and only 17/42 with WT- diplotype did. This was only reported in one previous study where VDBP WT+ was associated with non-response and SVR in HCV patients receiving interferon/ribavirin [7]. However, only 15 genotype 4 patients were included in that study which was not enough to draw valid conclusions in this cohort of patients. Although we did not assess HCV genotype, our study was conducted in Egypt where 90% of HCV infections are due to HCV genotype 4 [2]. Another study conducted in an African American population did not reach the same findings; this was attributed to the small number of WT+ diplotype African American patients in the study (25/95) where the T allele is dominant (T = 0.853). The authors also suggested that

the number of Caucasian patients included was not sufficient for the study to detect such differences. They speculated that VDBP diplotype may affect response to hepatitis C treatment in different populations [31].

VDBP polymorphisms have also been related to many adverse health outcomes and have been associated with many clinical aspects such as osteoporosis, diabetes, COPD and tuberculosis [32]. Several VDBP polymorphisms have been independently related to the incidence and prognosis of many diseases [33]. Non GC-2 isoforms have also been associated with higher macrophage activity *in vitro* [16]. VDBP polymorphisms may affect immunity either directly through affecting chemotaxis and macrophage activation as previously mentioned, or indirectly by affecting transportation of vitamin D metabolites, which play an important role in immune homeostasis [34]. In the current study, we also observed an increase in SVR with an increase in number of VDBP major alleles. This may be attributed to the lower total concentrations of 25-hydroxyvitamin D associated with the GC-2 isoform which is produced in the presence of 2 or fewer VDBP major alleles. This isoform is also associated with lower macrophage activity.

Our findings showed that baseline 25-hydroxyvitamin D levels were not associated with SVR. Our results are similar to those of a recent meta-analysis in which authors concluded that baseline vitamin D levels were not associated with HCV therapy outcomes [9]. On the contrary, other studies have shown a significant association between baseline vitamin D levels and therapeutic response to peg-interferon/ribavirin [35]. These contradictory results may be attributed to multiple factors that affect vitamin D levels such as gender, age, seasonal variation, ethnicity and genetic factors [36].

When multivariate logistic regression analysis was performed, interferon alpha-2b was associated with a reduced chance of achieving SVR. This is in agreement with the results of a study conducted in a large cohort of HCV infected Egyptian patients [37]. VDBP WT+ was also identified as an independent predictor of SVR. Polymorphisms of the vitamin D pathway affect biochemical and clinical function of 25-hydroxyvitamin D in the body and they can affect immune response directly [38]. A study suggested that free rather than total 25-hydroxyvitamin D levels should be evaluated [39] due to high ethnic variability in genotype distribution of GC globulin isoforms and their different binding affinities to vitamin D metabolites [36]. Perhaps this may explain the

contradictory results of studies assessing impact of vitamin D supplementation on viral response rates where some trials showed a significant increase in SVR with supplementation [40–42] while others did not [43]. These trials only considered total vitamin D levels after supplementation and did not take free levels or VDBP genotype into consideration.

Vitamin D binding protein polymorphisms were associated with hepatocellular carcinoma in hepatitis B virus (HBV) patients but to date, there are no studies to assess its role in predicting response to HBV therapy, therefore further research is encouraged in that area [44]. Similarly, vitamin D binding protein polymorphisms have been associated with insulin resistance in chronic hepatitis C (CHC) patients [45].

In the current study, we showed that VDBP diplotype may offer a suitable predictor of response to INF/RBV in CHC patients. Although interferon-free regimens are now recommended by the American Association for the Study of Liver Disease (AASLD) for treatment of HCV genotypes 1-6 [46], the European Association for the Study of the Liver (EASL) still suggests interferon/ribavirin as an acceptable therapy in the setting where none of the direct acting antivirals are available, which is the case in many resource limited countries [47]. Therefore, therapy can be optimized by selecting patients who are more likely to benefit from therapy. VDBP diplotype can aid in this selection process alongside other predictors such as IL28 genotype [48].

Moreover, peg-interferon/ribavirin combined with either simeprevir or sofosbuvir is currently recommended by EASL for treatment of HCV genotypes 1 and 4 in countries which cannot afford the high costs of dual-acting antiviral combinations [47]. The response rate with either of the aforementioned combinations is nearly 80% in genotypes 1 and 4 treatment naïve patients, respectively, which is much higher than that seen with conventional interferon/ribavirin therapy. Response rates are as low as 60% and 40% in prior partial responders and non-responders genotype 4 patients, respectively [49,50]. Therefore, these rates could be further improved by offering these combinations to those who are more likely to benefit from them. Our findings, which suggest an association between VDBP diplotype and HCV therapy outcome in patients treated with peg-interferon/ribavirin, encourage further research in that field. The role of VDBP polymorphisms in predicting response to interferon/ribavirin/direct acting antiviral combination therapy needs to be investigated through future research to assess whether it can offer a suitable

predictor of response to avoid wasting the already limited resources of such countries.

Conclusion

Our findings confirm that the VDBP WT+ diplotype can be used to predict sustained viral response in chronic Hepatitis C patients receiving interferon/ribavirin. The rs4588 CC genotype was significantly associated with response to HCV therapy in the studied Egyptian population. In areas where direct acting antivirals can not be afforded, VDBP diplotype can act, alongside other factors, as a predictor of response to increase response rates in patients receiving peg-interferon/ribavirin. Further research is needed to investigate its suitability as a predictor of response in patients receiving interferon/ribavirin/direct acting antiviral combination therapy which is still recommended by EASL for the treatment of HCV when dual direct acting antiviral therapy is not available.

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