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Influence of Vitamin D Receptor Gene Polymorphisms on Response to Pegylated Interferon in Chronic Hepatitis B Egyptian Patients

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Abstract

Background: We explored the effect of vitamin D receptor gene (*VDR*) polymorphisms in response to PEG-IFN treatment in Egyptian chronic hepatitis B (CHB) patients.
Methods: Two hundred hepatitis B virus (HBV) patients (42.3±10.7 years) on PEG-IFN α-2a (180 µg /kg for 48 weeks) and one hundred control subjects (37.3 ±12 years) were enrolled in the study. Vitamin D levels and hepatitis B surface antigen (HBsAg) expression were assessed by ELISA. VDR polymorphisms FokI T>C (rs 10735810), BsmI A>G (rs 1544410), ApaI (rs7975253), and TaqI C>T (rs 731236), were genotyped using real-time PCR.
Results: Hepatitis B virus patients expressed significantly greater AST (p=< 0.00001) and ALT (P=< 0.00001), and significantly less vitamin D (P=0.01), than control subjects. Patients with Ff or ff alleles of the FokI single-nucleotide polymorphism (SNP), bb alleles of BsmI SNP, or TT alleles of the Taq1 single nucleotide polymorphisms (SNP) showed greater response to PEG-IFN therapy than those with the FF (P=0.02 and P=0.0002), Bb (P=0.023), or Tt/tt alleles (P=0.01 and P=0.004 respectively). Logistic stepwise regression showed that HBV DNA (r: 0.910, P< .00001), FokI SNP polymorphism (r: 0.919, (P=0.037) and bAt haplotype (r: .926, (P=0.043) are independent factors that determine PEG-IFN treatment response in the HBV-infected patients.
Conclusions: *VDR* gene polymorphisms may be used as treatment response predictors in HBV patients receiving PEG-IFN. FokI SNP and bAt haplotype are independent factors that that can be used to determine PEG-IFN treatment responses in HBV-infected patients.

Keywords: Egypt, Hepatitis B virus, PEGylated interferon, Vitamin D receptor polymorphism.

Introduction

Hepatitis B virus (HBV) infection is a worldwide problem, with nearly 350 million people being chronically infected (1) and around one million deaths per year (2). Hepatitis B virus infection-associated complications such as cirrhosis, hepatic cell failure, and hepatic cell carcinoma (HCC) affect approximately15-40% of HBV patients; these complications could be ameliorated by long-term anti-HBV therapy (1). Pegylated interferons (PEG-IFNs) or conventional interferon and nucleos (t) ide analogues including lamivudine, telbivudine, adefovir, entecavir, and tenofovir are antiviral drugs approved for chronic hepatitis B (CHB) patient treatment. Interferon is more effective than nucleotide analogues on hepatitis B surface antigen (HBeAg) clearance, HBeAg seroconversion (3), and HCC prevention among CHB patients (4). Although PEG-IFNα has been suggested as a first-line therapy for CHB patients (1), its use was restricted as it is costly, caused several reported adverse reactions, and was inconvenient to inject. Its failure rate

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among CHB may reach 60-70% (5). Many factors may affect the PEG-IFN effect, such as baseline HBV DNA level, HBV genotype, alanine amino transferase (ALT) level, sex, age, and patient genetic factors (6); however, the exact molecular mechanism is not well known.

Vitamin D 1,25(OH)2D3 is an immunomodulator hormone that modulates the transcription of target genes in response to its stimulation (7). The vitamin D receptor (*VDR*) is present on many immune system cells including monocytes, stimulated macrophages, dendritic cells, natural killer cells, and activated B and T cells (8). Some *VDRs* have an immunoregulatory action independent of their endocrine function pathways (8, 9). Recent studies reported associations of *VDR* polymorphisms with immune-mediated diseases including rheumatoid arthritis (10), autoimmune liver disease (11), tuberculosis (12), and Grave's disease (13). These associations support its role in immune modulation. The aim of the present work is to examine the effect of *VDR* gene polymorphisms FokI T>C (rs 10735810), BsmI A>G (rs 1544410), ApaI (rs7975253), and TaqI C>T (rs 731236) in response to PEG-IFN treatment in Egyptian HBV patients.

Materials and Methods

Hepatitis B-infected patients were referred to the outpatient clinic affiliated with the Endemic Medicine Department of Cairo University. Two hundred patients and 100 control subjects enrolled in the study. Hepatitis B virus was diagnosed by ELISA for HBsAg. Informed consent was given by each study participant.

The protocol of this study was approved by the Ethical Committee, Faculty of Medicine at Cairo University in accordance with the tenets of the Declaration of Helsinki.

Patient characteristics

Inclusion criteria:

- Male and female patients aged from 18 to 60 years.
- Patients who have been HBsAg-positive for more than six months whether they have normal or abnormal ALT levels.

Exclusion criteria:

- Co-infection with chronic hepatitis C virus (HCV).
- HDV positive and Bilharzial liver disease.

Medical histories were obtained from each patient including risk factors for acquiring their HBV infection, history of HBV vaccination, and full clinical and abdominal ultrasound examinations. Complete blood count, AST, ALT, bilirubin, albumin, alkaline phosphatase (ALKP), prothrombin time (PT), protein C (PC), and INR were determined using commercial kits. Body mass index (BMI) was determined from patient weight and height.

All patients received a PEG-IFN α-2a therapy regimen (F. Hoffmann-LaRoche, Basel, Switzerland) of 180 μg weekly for 48 weeks. The HBV loads were tested at the end of the PEG-IFN therapy by PCR for HBV.

HBV serological assessment

The HBsAg was measured using a commercial enzyme linked immunoassay (ELISA) kit (DiaSorin, USA). Hepatitis B virus DNA was extracted using the QIAamp MinElute Virus Spin protocol. The DNA was amplified using a quantitative real-time PCR StepOne kit (Applied Biosystems, Foster City, USA) according to the manufacturer's instructions.

Vitamin D assay

A vitamin D Assay A Liaison automatic analyzer (Thermo Scientific, Cairo, Egypt) was used to determine the participants' 25-hydroxy vitamin D (25-[OH]D) levels.

Vitamin D receptor polymorphism (VDR) genotyping

Whole blood DNA was purified using a Quick-gDNA™ MiniPrep kit (Zymoresearch, USA, CA, Catalogue No. D3024) according to the manufacturer' specifications. The DNA concentration was determined at 260 nm using a Nanodrop ND-1000*spectrophotometer. The *VDR* FokI T>C (rs 10735810), BsmI A>G (rs 1544410), ApaI (rs7975253), and TaqI C>T (rs 731236) single nucleotide polymorphisms (SNPs) were genotyped using specific primers and TaqMan FAM and VIC probes (TaqMan SNP genotyping assays, Applied Biosystems, Foster City, CA). Negative and positive controls were included to ensure accuracy of the genotyping. The reactions were carried out in a total volume of 20 μL

containing 50 ng of DNA. The PCR mix per well consisted of 10 μL of 2x MOD™ PCR master mix solution (Intron Biotechnology. Korea, Catalog No. 25341), 2 μL of primers and TaqManprobe, 1 μL of template DNA, and 7 μL of nuclease-free water. The thermal cycler conditions consisted of an initial hold for 10 min at 95 °C, followed by 40 cycles of 15 s at 92 °C, and 1 min at 60 °C each. The genotyping was analyzed with StepOne Applied Biosystems version 2.1 software.

Statistical analysis

Numerical variables between the study groups were compared using the Mann-Whitney *U* test for independent samples when comparing two groups and the Kruskal-Wallis test with Mann-Whitney *U* test for independent samples as post hoc multiple two-group comparisons when comparing more than two groups. To compare categorical data, the Chi square ($\chi 2$) test was used. The Fisher Exact test was used when the expected frequency was less than five. P values less than 0.05 were considered statistically significant. To detect the relative independent risk factors that could affect the treatment outcome, the logistic stepwise regression test was used. All statistical calculations were performed using computer programs Microsoft Excel 2007 (Microsoft Corporation, NY, USA) and SPSS (Statistical Package for the Social Sciences; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

Results

Clinical demographic characteristics of all subjects

The study included 200 patients (125 male & 75 female) aged 42.3±10.7 years and 100 control subject (50 male & 50 female) aged 37.3±12 years. The patients have significant greater AST and ALT values and significant lower Vitamin D level than the controls subjects (Table 1).

Table 1. Basic Characteristics of HBV Patients and Control Subjects

		Control	HBV Patients	P value
Sex	Male	50	125	0.04
	Female	50	75	
Age (Year)		37.3 ±12	42.3±10.7	< 0.00001
AST(IU/L)		26.04 ±6.03	47.9 ±32.3	< 0.00001
ALT (IU/L)		27.9±6.3	50.3±36.2	< 0.00001
Albumin (mg/dl)		4.1±0.5	4.1±0.43	0.3
Vit D level (ng/mL)		25.3±9.8	16.9±8.3	0.01

VDR polymorphism

The genotype allele distributions of VDR SNPs FokI T>C (rs 10735810) BsmI A>G (rs 1544410), ApaI (rs7975253), and TaqI C>T (rs 731236) are shown in Table 2. The genotype frequencies of these SNPs were not significantly different between patients and controls (P=0.07) (Table 2 and Figure 1 (Fig. 1)).

Table 2. Genotypic frequenciesof VDR Gene among HBV patients and control subjects

	Allele	HBV patients (N)	Control (N)	P
FOKI (rs 10735810)	FF	131	64	.89
	Ff	56	28	
	ff	13	8	
BSMI (rs 1544410)	BB	40	26	.16
	Bb	127	52	
	Bb	33	22	
API (rs7975253)	AA	180	82	.144
	Aa	7	6	
	aa	13	12	
TAqI (rs 731236)	TT	62	44	.076
	Tt	84	32	
	tt	54	24	

N= number of patients

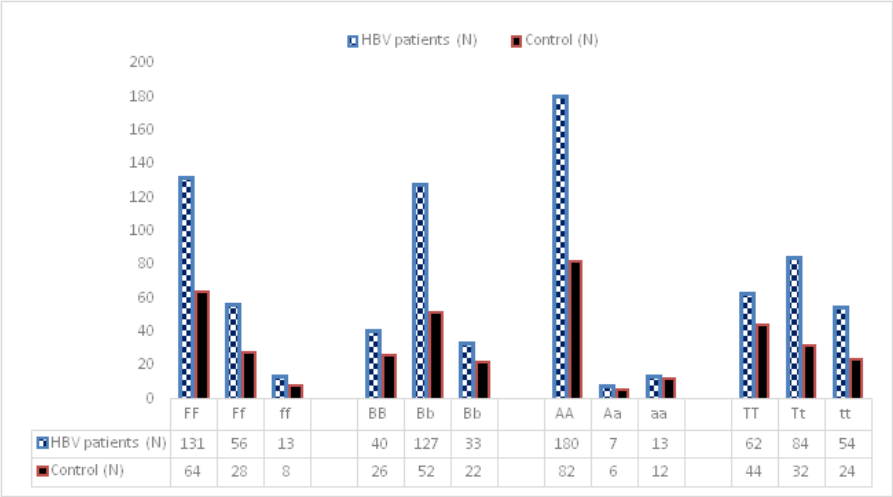

Fig. 1. Vitamin D Receptor gene alleles distribution among HBV patients and control subjects

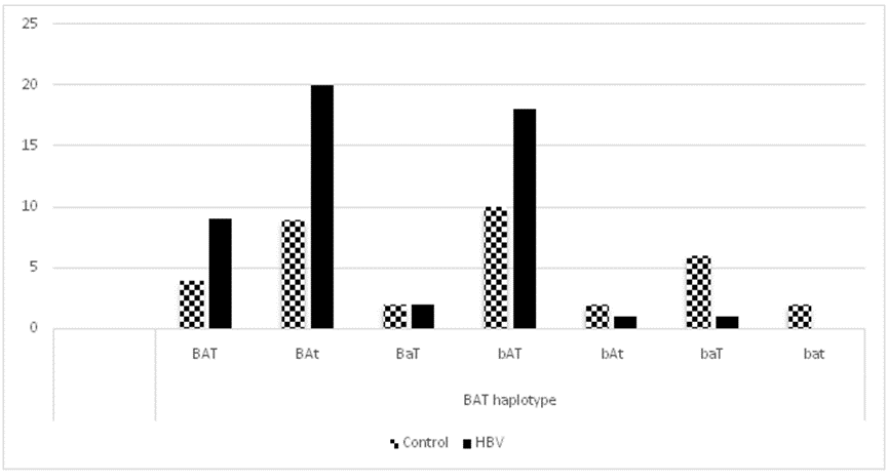

Fig. 2. bAt haplotype distribution among Hepatitis B Viral infected patients and control subjects

In the Fok1 SNP; patients with FF allele showed lower AST and ALT than in Ff patients. Patients with FF allele showed greater BMI but lower AST compared to ff patients. Patients with Ff allele showed greater BMI compared to ff patients (Table 3).

Table 3. Effect of VDR alleles on different clinical parameters among HBV patients

	Allele (N)	BMI (Kg/m^2)	AST (IU/L)	ALT (IU/L)	Bilirubin (mg/dl)	Albumin (g/dl)	Alkaline Phosphatase (IU/L)
FOKI (rs 10735810)	FF (131)	27.6±5.1	46.2±17.4 *	47.9±17.9 *	.9±1.2	4.1±.42	138.7±59.5
	Ff (56)	28.9±5.1 ***	52.7±18.5	55.4±18.8	.8±.3	4.2±.43	126.03±65.8
	ff (13)	24.6±2.5 **	62.1±22.3 **	58.9±20.6	.7±.3	4.3±.42	133.9±70.1
BSMI (rs 1544410)	BB (40)	26.5±4.82	51 ±16.9	52.4±17.2	.76±0.27	4.3±.42	125.9±61.4
	Bb (127)	28.1±4.84 #	48.02±17.9	49.9±18.3	.9±0.2	4.1±0.42 ###	133.6±59.8
	Bb (33)	27.9±5.8	50.7±22.2	51.9±21.9	.7±0.22	3.9±0.43 ##	150.6±69.7
API (rs7975253)	AA (180)	27.9±5.1	49.6±18.5	51.2±18.8	0.8±0.26	4.1±0.4	135.7±62.1
	Aa (7)	25.9±3.9	44.6±25.6	45.1±23.7	0.9±0.5	3.9±0.6	128.9±48.9
	aa (13)	26.5±4.4	44.3±13.64	46.5±13.4	0.8±0.28	4.2±0.3	126.2±70.1
TAqI (rs 731236)	TT (62)	28.1±4.9	54.9±21.03	56.5±22.4	1.01±1.7	4.1±.4	122.5±65.6
	Tt (84)	27.9±5.3	45.9±16.4 ¥	47.8±16.8¥	.8±.3	4.1±.5	136.9±56.2¥
	tt (54)	27.2±4.7	47.2±17.2	48.6±15.3	.77±.23	4.2±.4	145.7±64.8¥¥

Mann-Whitney test– N= Number of patients
*Significant FF Vs Ff **Significant FF Vs ff. ***Significant Ff Vs ff.
#Significant BB Vs Bb ##Significant BB Vs bb ###Significant Bb Vs bb.
¥Significant TT Vs Tt. ¥¥Significant TT Vs tt

In the Bsm1 SNP, BMI was significantly greater in patient with Bb allele than patient with BB allele. Albumin was significantly greater in patient with either BB or Bb allele than patients with bb allele (Table 3)

In the TaqI SNP; patient with TT allele showed significant greater AST and ALT compared to patients with Tt alleles, while alkaline phosphatase was significantly lower in patients with TT alleles compared to patients with either tt or Tt alleles (Table 3).

VDR alleles vs baseline HBV DNA and, Vitamin D levels

At baseline, for Fok1, significantly more HBV DNA was amplified from FF than either ff or Ff patients. For ApaI1, significantly more HBV DNA was amplified from Aa than from AA or aa patients. For ApaI, aa patients expressed significantly more vitamin D than AA patients. After treatment, for Fok1, significantly more HBV was amplified from FF than from Ff or ff patients. For Bsm1, significantly more HBV DNA was amplified from Bb than from bb patients. For Taq1, significantly more HBV DNA was amplified from TT than from Tt patients, and significantly more HBV DNA was amplified from tt than from TT or Tt patients (Table 5).

For the TaqI SNP, significantly less HBVDNA was amplified from the TT patients than from the Tt or tt patients (Table 5).

Table 4. Effect of VDR gene alleles on different clinical parameters among HBV patients

	Alleles	PC %	PT	INR	TSH	Hb (g/dl)	TLC (cells/µl)	Platelet ×103/mm3
FOKI (rs 10735810)	FF (131)	100.04±46.2	12.8±.8	1.1±.09	1.5±.9	13.9±1.5	6442.7±3799.6	205±65
	Ff (56)	115.6±66.4	12.6±1.7	1.1±.09	1.7±1.2	13.8±1.4	6051.6±1629.7	196±59
	ff (13)	125±74.04	12.6±.6	1.1±.1	1.5±.8	13.8±1.2	6553.8±2085.1	201±43
BSMI (rs 1544410)	BB (40)	107.9 ±58.7	12.7±.7	1.08±.08	1.68±1.03	13.9±1.5	6338.8±2100.1	195 ±47
	Bb (127)	105.5±52.6	12.7±1.2	1.09±.09	1.51±.9	13.9±1.5	6341.9±3751.1	204±64
	bb (33)	105.7±60.3	13.04±1.12	1.11±.08	1.56±1.09	13.9±1.3	6336.4±2067.3	203±72
API (rs7975253)	AA (180)	107.93±56.96	12.7±1.07	1.093±.09	1.56±.97	13.86±1.49	6350.3±3374.3	204±59
	Aa (7)	92.57±39.2	13.4±2.3	1.097±.07	1.41±.4	14.58±1.19	5714.3±1188.04	210±111
	aa (13)	86.62±16.07	12.7±.84	1.13±.11	1.58±1.1	13.96±1.4	6540.8±1628.06	173±67
TAqI (rs 731236)	TT (62)	106.6±58.7	12.8±.95	1.096±.09	1.4±.8	13.7±1.3	6634.2±2280.5	193±59
	Tt (84)	106.3±52.9	12.6±1.4	1.096±.097	1.7±1.09	13.9±1.5	6252.5±2280.5	205±65
	tt (54)	104.9±54.5	12.8±.64	1.094±.08	1.6±.88	14.1±1.6	6139.8±1761.5	209±62

Table 5. Effect of VDR alleles on baseline HBV DNA, Vitamin D level, and HBV DNA after 48 weeks of treatment

	Gene allele	Baseline HBV DNA (IU/ml)	Vitamin D levels (ng/mL)	HBV DNA after 48 weeks (IU/ml)
FOKI (rs 10735810)	FF (131)	2494693.3±1.119E7	17.6 ±8.9	1655837.9±9938934.1
	Ff (56)	944027.4 ±1239825.8 *	15.9±7.2	672929.9±1205241 ***
	ff (13)	492396.6 ±544967.7	15.1±4.4	315322.4±497442.7*
BSMI (rs 1544410)	BB (40)	587525.35±933034.973	16.3±8.7	352970.6±837498.4
	Bb (127)	2181878.4±1.006E7	16.97±7.9	1904171.8±1.009E7
	bb (33)	2590058.8±1.065E7	17.76±9.3	83315.06±191736.7 ###
API (rs7975253)	AA (180)	1888533.5±9562933.4	16.65±8.3	1327288.9±8494711.2
	Aa (7)	3739000 ±3421870.4 §	18.07±8.03	926848.6±1539322.2
	aa (13)	1535575.4±1776676.3	20.83±7.5 §§	1022931.2±1888636.8
TAqI (rs 731236)	TT (62)	1313169.9±2196733.6	16.1±6.8	915751.1±1790196.6
	Tt (84)	2278191.1±1.089E7	17.1±8.3	228233.2±8773497.4 ¥
	tt (54)	2097906.1±1.092E7	17.7±9.8	1828701.6±1.096E7¥¥

- Mann-Whitney test. * Significant FF Vs Ff. - ** Significant FF Vs ff. - ### Significance Bb Vs bb - §significance AA Vs Aa.

-§§ significance AA Vs aa- ¥ Significant TT Vs Tt. -¥¥ Significant TT Vs t

BAT Haplotype effect

Linkage disequilibrium was found between bAt haplotypes: seven haplotypes were identified among the patients included in our study; these were: BAT, BAt, BaT, bAT, bAt, baT, and bat. No significant differences were found between the frequencies of the bAt haplotyes (Table 6).

Table 6. BAT Haplotype Distribution*

		BAT haplotype							Total
		BAT	BAt	BaT	bAT	bAt	baT	bat	
Control	Count	4	9	2	10	2	6	2	35
	% of Total	4.7%	10.5%	2.3%	11.6%	2.3%	7.0%	2.3%	40.7%
HBV	Count	9	20	2	18	1	1	0	51
	% of Total	10.5%	23.3%	2.3%	20.9%	1.2%	1.2%	.0%	59.3%

Chi square test *P=0.07 Chi Square test

Hemoglobin was significantly greater in the BaT than in the BAT or Bat patients (Table 7). No significance differences in clinical parameters were found between any patients with other BAT haplotypes alleles (Tables 7 and 8).

Table 7. Effect of BAT haplotypes on different clinical parameters

Allele (number)	BMI (Kg/m^2)	AST (IU/L)	ALT (IU/L)	Bilirubin (mg/dl)	Albumin (g/dl)	Alkaline Phosphatase (IU/L)	BMI (Kg/m^2)	PT	INR	TSH	Hb (g/dl)	TLC (cells/µl)	Platelet ×10^3/mm^3
BAT (9)	27.1±2.09	62.3±5.3	60.3±8.1	.7±0.7	4.3±0.13	115.1±17.9	114±18.1	12.97±0.2	1.07±0.02	1.36±0.16	13.9±0.43	6800±889.8	190±16
BAt (20)	26.08±.8	50.7±3.5	52.7±3.4	.74±0.6	4.3±0.08	134.1±15.9	110±15.6	12.7±0.16	1.09±0.16	1.56±0.17	13.8±0.34	5982.5±390.8	190±8
BaT (2)	24.7±1.9	44.5±10.5	43.5±8.5	.75±0.5	4.3±0.1	151±38	94±6	12.5±0.5	1.06±0.6	1.83±0.6	16.6±0.5 *	6950±2650	234±19
bAT (18)	28.09±1.2	53.2±5.61	58.9±5.43	.72±0.51	4.01±0.09	136.4±16.7	106.5±12.5	12.7±0.16	1.1±0.025	1.33±0.22	13.7±0.3	7105.6±544.7	201±15

Mann-Whitney test. bAt and bat alleles were excluded as they include only one case each.
* significant BAT compared to BaT (p=0.02)
Chi square test - *P=0.07 Chi Square test

Table 8. BAT haplotype effects on Vitamin D levels and HBV DNA level after treatment

Allele (Number of patients)	HBV DNA after 48 Weeks (IU/ml)	Vitamin D levels (ng/mL)	Baseline DNA (IU/ml)
BAT (9)	250458.8±116819.569	15.02±1.962	277907.7±112672.1
Bat (20)	496487.9±254105.4	15.8 ±2.158	684582.6±252921.3
BaT (2)	2019 ±21	18.5 ±0.8	239000±59000
bAT (18)	87223.2±49580.568	14.2 ±1.04	268474±86582.7

Mann-Whitney test. bAt and bat alleles were excluded as they included only one case each.

Stepwise Logistic Regression

Stepwise logistic regression was tested to identify potential independent risk factors that could affect patient responses to PEG-IFN treatment as measured by HBV DNA. Of all the factors studied, only baseline HBV DNA, FOKI SNPs, and BAT haplotypes were significantly related and categorized as independent risk factors (Table 9).

Table 9. Logistic Stepwise Regression Test

	R	R^2	Adjusted R^2	Coefficient	SE	Standardized coefficient	P value
Baseline HBV DNA	.910	.829	.825	.854	.057	.910	<.00001
FOKI	.919	.844	.837	145648.777	66691.254	.123	.037
BAT haplotype	.926	.857	.848	-67177.978	32356.042	-.115	.043

Dependent variable: HBV DNA after 48 Weeks, R: correlation coefficient; SE: standard error; P: significance probability.

Discussion

The role of *VDR* polymorphisms as a predictor of PEG-IFN response among HCV-infected patients has been studied elsewhere (14-18). To our knowledge the effect of *VDR* polymorphisms as a predictor of PEG-IFN response in HBV patients had not been previously reported. Our study aimed to provide information in this field.

In our study, patients with FOKI Ff or ff alleles responded more strongly to PEG-IFN treatment than those with the FF allele as evidenced by significantly lower HBV viral load as determined by PCR.

Our results support the results of previous studies that demonstrated the role of FOKI C>T polymorphisms in response to PEg-IFN therapy in HCV-infected patients (14, 16, 19). The C>T polymorphism could be considered as a molecular marker to predict the risk and progress of HCC in HBV patients (20).

The FokI polymorphism is a non-synonymous SNP in *VDR* that seems to affect *VDR* protein structure and activity (21); it results in a threonine-methionine change and addition of three amino acids, which makes the protein less functionally active than its wild-type counterpart (22). Moreover, FOKI polymorphisms may contribute to increased susceptibility to HBV-related HCC in a Chinese population (23).

The predication of the HBV response to PEG-IFN may allow clinicians to minimize HBV infection complications and utilize effective selection antiviral drugs at the initiation of treatment, minimizing drug-related side effects and decreasing overall cost.

FokI C>T (rs2228570, exon 2), BsmI G>A (rs1544410, intron 8), ApaI C>A (rs7975232, intron 8), and TaqI T>C (rs731236, exon 9) are the most commonly genotyped SNPs of *VDR* (24).

In our study, patients with the bb allele in the *VDR* BSMI SNP responded more to PEG-IFN treatment than those with Bb alleles as evident by significant lower HBV DNA at the end of treatment. While in the TaqI SNP, patients with the TT allele expressed significantly less HBV than the patients with the Tt or tt alleles (Table 5). No significant differences were found between ApaI patients.

Our results are in agreement with previous studies that found that TaqI may be considered as a predictor for the response of HCV patients treated with PEG-IFN (14). Similarly, Li et al. reported that TaqI SNP polymorphism was significantly associated with primary biliary cirrhosis (25).

Also Wang et al. reported an association between BsmI allele polymorphisms and PEG-IFN plus ribavirin therapeutic responses in chronic HCV patients (26).

Moreover, the BB and Bb alleles were shown to be markers of inflammation and antioxidant activities in vitamin D-deficient elderly patients who received mega doses of Vitamin D, while the bb allele was not (27).

On the other hand, another recently published study showed TaqI rs731236, BsmI rs1544410, and ApaI rs7975232 polymorphisms had no effect on responses to PEG-IFN plus ribavirin therapy in Asian chronic HCV patients. No role was found for TaqI rs731236 A in HCC predication among HCV patients (28) and no relationship was identified between *VDR* TaqI and chronic HBV infection susceptibility (29). Our study found no significant association between ApaI polymorphisms and patient responses to PEG-IFN.

The role of the *VDR* ApaI polymorphism in the development of HCC among chronic hepatitis C patients is proved (28), also it might contribute to a decreased susceptibility to HCV infection in a high-risk Chinese population (30).

Three common alleles combinations of the *VDR* "bAt-haplotype" consist of BsmI, ApaI and TaqI (22) in strong linkage disequilibrium and hence patients were categorized as carriers and non-carriers (15, 16).

In the present study, baseline HBV DNA expression, FOKI allele polymorphisms, and bAt haplotype are independent factors determining the HBV patient responses to PEG-IFN treatment.

The previous results in this point in HCV infected patient are contradictory where the influence of bAt [CCA]- haplotype *VDR* polymorphisms on antiviral response to Peg-IFN plus ribavirin therapy (15, 16). Whereas recently published data showed no relationship (26, 28).

We found no relationship between Vitamin D level and treatment response, however the vitamin D3 level was significantly lower in HBV patients than in controls.

Our result supported by others that showed the risk of hepatitis C viral (HCV) infection and chronicity in subjects with low vitamin D levels (30, 31), moreover vitamin D supplemented subjects with higher serum

vitamin D levels exhibited high sustained virological responses (better treatment response) among HCV individuals (32).

In the present study, although the Vitamin D levels were lower in HBV patients than in healthy controls, vitamin D wasn't an independent factor that determined treatment response; this has also been reported for chronic HCV patients (33).

Such results disagree with previous studies that reported serum vitamin 25(OH) D3 level was an independent factor that significantly contributed to sustained virological response (34). Such conflicting results could be explained as the 25(OH) D3 serum level cannot be considered as an established predictor of treatment outcome (18). This may be justified as *VDR* variants modulate biological effects of vitamin D without influencing serum vitamin D levels (35), and vitamin D serum levels fluctuate according to season and age (36). Also, according to genome-wide association analysis, none of the *VDR* genes studied here are related to the vitamin D blood levels (35), but may be related to its action.

Vitamin D binds to *VDRs* on the surfaces of monocytes and lymphocytes, activating the innate immunity systems and enhancing immune responses by inhibiting Th1 cell functions and activating Th2 cell responses (37, 38).

The inhibitory role of vitamin D in viral replication is still unclear; it may directly inhibit viral replication through up-regulation of IFN-β expression (39), or by inhibiting a viral assembly step (40). More studies are needed to explore its exact molecular effect on viral replication and infection susceptibility.

In our study BMI wasn't an independent factor of PEG-IFN response, and no significant differences were found between BMI and different *VDR* alleles. Our result is in contrast with studies that reported that BMI affects IFN-β treatment responses (41, 42).

Obesity and metabolic syndrome are known to affect serum inflammatory markers; these are associated with a low-grade inflammatory state and release of cytokines that initiate immune responses (41) and worsen the disease course of several autoimmune diseases (43). Obesity may affect vitamin D action by decreasing its circulating form (44-46) or by modulating its response (47, 48). We acknowledge some limitations in the present study such as multiple vitamin D assessments to avoid biological variations, vitamin D intake status, HBV genotype, and insulin resistance, which were not assessed but we believe should be explored in the further studies.

Patients with Ff and ff alleles of the FOKI polymorphism responded more to PEG-IFN therapy than those with FF alleles, Patients with bb alleles of the BSMI polymorphism responded more to PEG-IFN than those with Bb alleles, patients with TT alleles of the TaqI polymorphism responded more to PEG-IFN than those with Tt or tt alleles as evident by having significant lower HBV DNA level. Baseline HBV DNA, FOKI polymorphism, and the bAt haplotype are independent factors that may determine PEG-IFN treatment response in the HBV infected patients in our study.

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