The Egyptian Journal of Hospital Medicine (Jul. 2016) Vol. 64, Page 287- 303

The Role of Vitamin D during Therapy in Chronic Hepatitis C Virus Infection and Its Relation to CYP 27 B1-1260 Promoter Polymorphism

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ABSTRACT

Objective: vitamin D is a potent immunomodulator. A number of genetic polymorphisms in the vitamin D pathway have been shown to affect vitamin D signaling, and stratification according to such polymorphisms has already being implemented in randomized controlled clinical intervention studies.

Aim of the work: the study was attempted to examine whether vitamin D improved viral response and predicted treatment outcome in patients with chronic hepatitis C virus (CHCV) infection.

Patients and methods: ninety two patients with CHCV, whose age ranged between 20 and 56 years, were selected from the National Hepatology and Tropical Medicine Research Institute were included in this study, before and after the treatment with pegylated interferon (PEG-IFN), ribavirin (RBV) and vitamin D supplementation drops; 2000 IU/day, 10 drops/day, six patients whom received identical therapy without vitamin D were included to serve as controls. All the patients had body mass index (BMI) ≤ 30, were subjected to the following: history, clinical examination, abdominal ultrasonography and collection of blood samples for routine laboratory investigations. CBCs and analysis of the expression of CYP 27 B1-1260 gene, vitamin D receptor (VDR), and the levels of serum 25-hydroxyvitamin D before and after chronic hepatitis C virus treatment.

Results: the treatment group with vitamin D had BMI ≤ 30 and high viral load 90900004.00 IU/ML, (P= 0.098). Sixty three percent of treated patients were HCV RNA negative at 48 weeks after treatment (SVR). Baseline serum vitamin D level was 8.5 minimum, mean level (32.9 ± 27 ng/mL). It increased after 48 wk vit D treatment, to a mean level of (54.9 ± 38 ng/mL). VDR show highly significant difference between patients and controls as regarding Ff=55.4% for patients (P=0.01), and 66.7% ff for the controls (P=0.006) alleles. CYP27B1 show non significant relation between patients and controls, with CYP27B1 genotype frequencies of the promoter polymorphism CC = 51.1% for patients, 66.7% for the control group, C allele frequency 69% for the patients, 83.3% for the controls, AC 35.9% for the patients, 33.3% for the controls. The majority of cases had A1F1 and A1F2 biopsy results.

Conclusion: our study suggests a role of vitamin D in the response to treatment of chronic HCV patients. However, serum concentration is not a suitable predictor of treatment outcome. VDR had a predictive positive treatment outcome. CYP27B1-1260 was found to be an independent predictor of sustained virologic response (SVR).

Recommendations: The level of recommended supplementation of vitamin D depends on the patient's individual deficiency, although 2000 IU daily is a common dose. Patients taking vitamin D supplements should have serum measurements made after starting therapy to determine whether they are reaching target levels.

Key words: Hepatitis C virus, vitamin D, genotype 4, VDR, SVR, CYP27B1, fibrosis.

INTRODUCTION

Vitamin D plays a role on the degree of liver damage in patients with chronic hepatitis C (CHC): low vitamin D levels have been associated with high hepatic necroinflammatory activity and progression of liver fibrosis. Vitamin D is known to have pleiotropic functions, dealing with both innate and adaptive immunity.

Calcitriol mediates its biological effects by binding to the vitamin D receptor (VDR), which is expressed not only by intestine, bone and kidney but also on cell membranes of T lymphocytes, B lymphocytes, dendritic cells and

Received: 01/07/2016
Accepted: 10/07/2016
DOI : 10.12816/0029021

macrophages[1]. Vitamin D is a group of fat-soluble secosteroides. In humans, vitamin D is unique because it can be ingested as cholecalciferol (vitamin D3) or ergocalciferol (vitamin D2) and because the body can also synthesize it from cholesterol when sun exposure is adequate hence its nickname, "sunshine vitamin"[2].

Historically, sun exposure was the main source of vitamin D, but food and supplements are now important sources, especially among urban populations and people who work indoors. During its conversion from a precursor to an active hormone, vitamin D is first modified in the liver by microsomal vitamin D 25-hydroxylases, which form 25-hydroxyvitamin D [25(OH)D], a stable metabolite that is the best single indicator of vitamin D states[3]. A second hydroxylation step, mediated by the mitochondrial cytochrome p450 oxidase, CYP27B1, products the most biologically active metabolite, 1,25-dihydroxyvitamin D [1,25(OH)2D]. In individuals with adequate renal function, most of the circulating [$1,25(OH)_2D$] is produced by the kidney; whoever, CYP27B1 activity occurs in many extra-renal tissues, including innate immune cells, such as macrophages and dendritic cells. The local metabolism of 25(OH)D by these cells is likely to be an important factor in generating the high local concentrations of $1,25(OH)_2D$ needed for its paracrine and autocrine activities[3].

Upon binding $1,25(OH)_2D$ the VDR is phosphorylated and forms a heterodimer with its preferred binding partner, the retinoid X receptor (RXR), forming a nuclear transcription factor. This VDR/RXR heterodimer bind vitamin D response elements (VDREs) in DNA and recruits co-regulatory protein complexes to modulate the expression of hundreds of genes. In addition to acting as a ligand-activated transcription factor, the VDR is also thought to activate cell signaling pathways independent of its genomic effects[4].

The vitamin D receptor belongs to the nuclear receptor superfamily of steroid / thyroid hormone receptors, and VDRs are expressed by cells in most organs, including the brain, heart, skin, gonads, prostate and breast. VDR activation in the intestine, bone, kidney and parathyroid gland cells leads to the maintenance of calcium and phosphorus levels in the blood (with the assistance of parathyroid hormone and calcitonin) and maintenance of bone content[5]. Given the prevalence of bone disease, inflammation, and fibrosis in HCV-positive patients, both classical and newly-discovered effects of vitamin D may be relevant to disease management[3].

The multiple steps in vitamin D bioactivation are controlled by intricate regulatory pathways. CYP27B1 expression in the renal proximal tubule is stimulated by the parathyroid hormone (PTH), which is regulated by free serum calcium levels $1,25(OH)_2D$ itself can directly and indirectly inhibit CYP27B1 expression, thereby providing a tight negative feedback loop. CYP27B1 expression in keratinocytes is stimulated by both PTH and inflammatory cytokines such as TNFα and IFNγ. $1,25(OH)_2D$ negatively regulates its own activity in these cells by inducing the expression of the $1,25(OH)_2D$ catabolic enzyme, CYP24A1. The functional expression of CYP27B1 and intracellular synthesis of $1,25(OH)_2D$ in macrophages are induced by both inflammatory cytokines, such as IFNγ, and toll-like receptor (TLR) ligands, such as lipopolysaccharide, because vitamin D metabolism is controlled by multiple factors, the amount of vitamin D consumed in the diet is only one of many variable that determine the local activity of the vitamin D system[3]. The study was attempted to examine whether vitamin D improve viral response and predicted treatment outcome in patients with chronic hepatitis C virus (CHCV) infection.

PATIENTS AND METHODS

Ninety two patients with chronic hepatitis C virus (HCV) infection, whose age ranged between 20 and 56 years, were selected from the National Hepatology and Tropical Medicine Research Institute were included in this study, before and after chronic hepatitis C therapy with vitamin D supplementation, and six patients whom received identical therapy without vitamin D. All the patients had BMI ≤ 30, were subjected to the following: history, clinical examination, abdominal ultrasonography and collection of blood samples for routine

laboratory investigations. CBCs and analysis of the expression of CYP 27 B1-1260 gene, vitamin D receptor (VDR), and the levels of serum 25-hydroxyvitamin D at the onset and after the treatment.

Procedure of preparation of 25-OH Vitamin D (total) ELISA (EIA-5396): Lot No: 80k094, 80k024. "According to the manufacturer instructions" DRG Instruments GMBH, Germany Division of DRG International, Inc Frauenbergstr. 18, D-35039 Marburg.
1- Dispense 25 ul of each standard, control and sample with new disposable tips into the vials. 2- Dispense 50 ul Denaturation Buffer into each vial. 3- Seal vials and incubate for 30 minutes at 37 °C. 4- Add 200 ul of Neutralization Buffer to each vial. 5- Add 50 ul of Enzyme Conjugate to each vial. 6- Add 50 ul of enzyme complex to each vial. 7- Thoroughly mix for 10 seconds. It is important to have a complete mixing of the solution in this step. 8-Use 200 ul of this mixed solution for the ELISA. 9- Secure the desired number of Microtiter Wells in the frame holder. 10- Transfer 200 ul of the mixed solution of each Standard, Control and sample with new disposable tips into the appropriate wells. 11- Seal wells carefully and incubate for 60 minutes at 37°C. 12- Briskly shake out the contents of the wells. Rinse the wells 4 times with diluted Wash Solution (300 ul per well). Strike the wells sharply on absorbent paper to remove residual droplets. 13- Add 200 ul of Substrate Solution to each well. 14- Incubate for 15 minutes at room temperature. 15- Stop the enzymatic reaction by adding 100 ul of stop solution to each well. 16- Determine the absorbance (OD) of each well at 450 ± 10 with a microtiter plate reader. It is recommended that the wells be read within 10 minutes after adding the stop solution.

Molecular analysis:

For VDR and CYP27B1-1260 polymorphisms, genomic DNA was isolated from whole blood using the genomic DNA purification Kit (Qiagene, cat no 95430, USA) according to the manufacturer's instructions. For the detection of the VDR polymorphisms, the polymerase chain reaction (PCR) technique was applied and followed by restriction fragment length polymorphism assays. The PCR amplifications were carried out in a total volume of 25 μL using PCR master mix (Applied Biosystem, cat no 245169, USA). The sequences of primers used for FokI were (f) 5-TGCAGCCTTCACAGGTCATA-3 (r) 5-GGCCTGCTTGCTGTTCTTAC- 3 .The cycling conditions for all the VDR polymorphisms were set as 40 cycles at 95°C for 30 s, 61°C for 30 s and 72°C for 1 min. In a total volume of 20 μL, amplified DNA (10 μL) was digested with 2 U of restriction endonucleases using the buffers and temperatures recommended by the manufacturers. The presence of restriction sites for the FokI (Ff) polymorphism was analyzed by digestion PCR product with FastDigest FokI (Thermoscientific, cat no 14211, Germany). All PCR products were sized by electrophoresis on a 4% agarose gel stained with ethidium bromide, for FF genotyping the PCR products was 265 bp, for ff genotyping the PCR products was 169 pb and for Ff genotyping the PCR products was 265, 169& 96 pb [(6)]. CYP27B1 rs10877012 promoter polymorphism was genotyped in an ABI 7300 real-time PCR system using the primers 5-GGGAGTAAGGAGCAGAGAGGTAAA-3 and 50-AACAGAGAG AGGGCCTGTCT-3(Applied Biosystem, cat no 10298, USA) as well the labeled primers 5-TGTGGGAGATTCTTTTA-3 and 5-CTGTGGGAGATTATTTTA-3 with VIC and FAM for the alleles C and A, respectively[(7)].

Statistical analysis: Analysis of data of all patients, were coded and entered using the statistical package SPSS (Statistical Package for the Social Science) version 22. Data was summarized using mean, standard deviation, median, minimum and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the non-parametric Kruskal-Wallis and Mann-Whitney tests[(8)]. Comparison between value measure before and after treatment was done using the non parametric Wilcoxon signed rank test. For comparing categorical data, Chi square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5[(9)]. Correlation between variables was done using Spearman

correlation coefficient[10]. Genotype and allele frequencies were compared between the disease and the control groups and between responders and non-responders using chi-square tests. Odds ratio (OR) with 95% confidence intervals was calculated. P-values less than 0.05 were considered as statistically significant.

Ethical consideration: Informed consent was obtained from each patient at the time of drawing blood samples. The Research Ethical Committee of the General Organization for Teaching Hospitals and Institutes approved the protocol.

RESULTS

The study included 92 patients with CHCV infection, they received the treatment associated with vitamin D supplementation and 6 control patients had received the same treatment without vitamin D supplementation.

We found that there's highly significant difference between patients and controls as regarding VDR alleles Ff with 55.4% for the patients group and ff alleles=66.7% for the controls. F allele=59.2% for the patients and f allele=66.7% for the controls.

No significant difference as regarding CYP27B1 polymorphism in the comparison between the both groups, with the increase in alleles % of CC =51.1% for the patients and 66.7% for the control group, C allele=69% for the patients and 83.3% in the control group table (1).

Figure (1) illustrates the variants polymorphism alleles in CHCV patients and controls as regarding CYP27B1.

Figure (2) illustrates the variants of VDR alleles in CHCV patients and controls.

Table (2) shows that there's increase in serum vitamin D levels with significant difference in the responder than that in non responder to treatment of CHCV with vitamin D supplementation after 48 weeks with the comparison with the same patients before treatment.

Figure (3) illustrates that serum vitamin D levels higher in the responder than that in the non responder to treatment of chronic HCV associated with vitamin D supplementation.

Table (3) Show the difference in mean serum levels of vitamin D before and after the treatment associated with vitamin D supplementation for 48 weeks in chronic HCV patients. There's significant increase after treatment.

Figure (4) illustrates the increased level of serum vitamin D after treatment of chronic HCV associated with vitamin D supplementation than before treatment.

Table (4) show the comparison between the chronic HCV patient and controls as regarding serum level of vitamin D after treatment associated with vitamin D for 48 weeks. The level increased in patients than controls with non significant difference.

Figure (5) illustrates the increased level of vitamin D in patients than that in the controls after treatment.

Table (5) shows that there's non significant difference in the comparison between the responder to treatment of CHCV associated with vitamin D supplementation after 48 weeks and the non responder to treatment as regarding the viral load before treatment.

Table (6a) shows the relationship between the viral load and serum levels of vitamin D in CHCV patients.

Table (6b) show that there's no correlation coefficient between the viral load before treatment and the serum levels of vitamin D after treatment in CHCV patients.

Table (7) shows that the CHCV patients responder to treatment with vitamin D supplementation with 63% and that the biopsy results A1F1= 59.8%, 23.9% for the A1F2.

Table (8) shows highly significant difference in the comparison between responder and non responder to treatment of CHCV patients with vitamin D supplementation as regarding vitamin D receptor alleles ff, the F and f allele, the Ff alleles =61.8% for the non responders and 51.7% for the responder.

No significant difference as regarding CYP27B1 polymorphism with increased levels of the alleles CC=53.4% in the responders, 69.8% for the allele C in the responder and 67.6% in the non responder.

Figure (6) illustrates the variants polymorphism in the responder and non responder to treatment of CHCV patients with vitamin D supplementation as regarding CYP27B1 alleles.

Figure (7) illustrates the variants of VDR alleles in CHCV patients' responder to treatment with vitamin D supplementation after 48 weeks and non responder.

Table (9) shows that there's non significant difference between the serum vitamin D levels in CHCV patients between before and after treatment as regarding CYP27B1 polymorphism.

Figure (8) illustrates the levels of CYP27B1 alleles as regarding serum vitamin D levels before treatment in CHCV patients.

Figure (9) illustrates the levels of CYP27B1 alleles as regarding serum vitamin D levels after treatment in CHCV patients.

Figure (10) illustrates the genotype CYP27B1 (CC).

Figure (11) illustrates the genotype CYP27B1 (CA).

Figure (12) illustrates the genotype CYP27B1 (AA).

Figure (13) illustrates an agroase gel electrophoresis show VDR genotyping (Ff, ff and FF).

DISCUSSION

Vitamin D has benefits in chronic HCV infection [(11)]. Abu Mouch *et al.* [(12)] observed the benefit of adding vitamin D to conventional antiviral therapy in patients with chronic hepatitis C. The results of the present genetic validation study suggest an association between the CYP27B1-1260 promoter SNPs and the treatment of chronic hepatitis C although CYP27B1 was not significantly associated with sustained virologic response. Cooper *et al.* [(13)] postulated that an association between the CYP27B1-1260 promoter SNP rs 10877012 and sustained virological response (SVR) to treatment of chronic hepatitis C, with PEG-IFN-α and ribavirin. The association was found only in patients with a poor-response IL28B genetic background, whereas CYP27B1-1260 rs 10877012 was not significantly associated with SVR in patients with good-response IL28B genotype. CYP27B1-1260 rs10877012 is a functional polymorphism in the promoter of the 1α-hydroxylase, the enzyme required for the bioactivation of $25(OH)D_3$ to $1,25(OH)_2D_3$ (calcitriol). The CC genotype of CYP27B1 is associated with poor response to interferon-α-based treatment of chronic hepatitis C[(14)]. 1α-hydroxylase is expressed not only in the kidney but also in inflamed tissue and even in immune cells, were it serves as a local, inducible producer of calcitriol[(15)]. Bioactive vitamin D is an important immune modulator, as for example T cells and macrophages crucially depend on calcitriol in various conditions[(16-18)]. Vitamin D deficiency has been reported in HCV-infected patients without advanced fibrosis and with a functional CYP27B1-1260 polymorphism associated with diminished active $1,25(OH)_2$ vitamin D_3 concentrations, resulting in a poor response to INF-based therapy[(14)]. Moreover, this polymorphism (rs10877012) was associated with response to IFN-based therapy [(19)].

In the present study, we found that there's significant difference between responder and non responder to treatment as regarding serum levels of vitamin D; SVR equal 63%. Analyzing the impact of functionally relevant genetic polymorphisms in vitamin D cascade on SVR may provide stronger evidence on an intrinsic role of vitamin D metabolism in the pathogenesis and treatment of chronic hepatitis C than analyzing exclusively vitamin D serum levels, which are affected by various parameters including season, sunlight exposure, nutrition, and the metabolic syndrome [(20,21)]. Gal-Tanamy *et al.* [(22)] observed that vitamin D supplementation was reported to improve the probability of achieving a sustained virologic response when combined with antiviral treatment against hepatitis C, and suggesting that vitamin D has a role as a natural antiviral mediator.

In our study, we observed that the serum levels of vitamin D was higher in the patients after the treatment associated with vitamin D supplementation than the controls but the difference insignificant in between, and that the level of serum vitamin D increased after treatment than before treatment the increase with significant difference. Yokoyama *et al.* [(23)] found that the baseline vitamin D concentration for patients was lower in the patients than in the control group, whereas their SVR rates did not differ. Increased production of 1,25-dihydroxy vitamin D_3 results in the synthesis of cathelicidine, a peptide capable of destroying many viral infectious agents, low serum levels of 25-hydroxyvitain D (<20 ng/mL) prevent

macrophages from initiating the innate immune response, which may explain why African Americans, who are often vitamin D deficient, are more prone to contracting viral infections[16]. A panel from the endocrine society concluded that 32 ng/ml should be used as the threshold of 25(OH)D sufficiency in patients with various disease conditions[24]**.** Nimer and Mouch [25] found that the normal serum level of vitamin D > 32ng/mL. The 73rd Annual scientific meeting of the American College of Gastroenterology, researchers from the University of Tennessee in Memphis measured the vitamin D levels in people with chronic liver disease. After dividing every vitamin D deficiency into three categories (mild, moderate and severe), the investigators found that 92.4% of those had some degree of vitamin D deficiency, at least 33% of participants were severely deficient , severe deficiency was more common among those with cirrhosis[26].

In the present work, we found that VDR genetic polymorphisms showed a highly significant difference between the patient and the control group, and also there's highly significant difference between the responder and non responder. Holick [3] postulated that the levels of vitamin D binding protein and VDR are additional variables that strongly influence the magnitude of biological effects of vitamin D. VDR activation by $1,25(OH)_2D$ has long been known to increase intestinal calcium and phosphate absorption, fostering healthy bones. Lead researcher Dr. Satheesh P. Nair commented, "Since deficiency is common among these patients, vitamin D replacement may hopefully prevent osteoporosis and other bone complications related to end stage liver disease" [27]. A study of VDR genetic polymorphisms has found that the bAt (CCA) haplotype is significantly associated with fibrosis progression rate as compared to the bat (CAA) and BaT (TAG) haplotypes [28]. A growing number of studies reveal pleiotropic roles of $1,25(OH)_2D$ beyond bone and calcium metabolism, including the induction of antimicrobial genes and the reduction of inflammation and fibrogenesis. Persistent HCV infection modulates the balance between immunostimulatory and inhibitory cytokines that can prolong inflammation and lead to fibrosis and chronic liver disease[29] . Gutierrez *et al.* [30] have shown that vitamin D_3 increase VDR protein expression and inhibits viral replication in cell culture.

The study suggest that there's no correlation coefficient between the viral load and the serum levels of vitamin D in chronic hepatitis C, and showed that the majority of patients were A1F1 59.8%, Garcia-Alvarz [31] added that the risks for advanced liver fibrosis and odds for achieving sustained virologic response were affected by vitamin D levels in patients with hepatitis C, virus infection. Researchers conducted a meta-analysis of 14 included studies published from 2011 to 2014 after a literature search of PubMed, Scopus, Liacs and Cochrance library databases. Seven studies focused on vitamin D and advanced liver fibrosis, and have found that low vitamin D levels increased risk for advanced liver fibrosis, decreased SVR rate among patients with HCV.

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Table (1): Comparison between CHCV patients and controls as regarding CYP27B1 polymorphism and vitamin D receptor.

Variables		Patients (n=92)		Control (n=6)		P value	OR (95%CI)
		Count	%	Count	%		
CYP27B1	AA	*12*	*13.0%*	*0*	*.0%*	*1*	*----------)*
	AC	*33*	*35.9%*	*2*	*33.3%*	*1*	*1.119 (0.194-6.438)*
	CC	*47*	*51.1%*	*4*	*66.7%*	*0.679 NS**	*0.522 (0.091-2.993)*
	Allele A	*57*	*31%*	*2*	*16.7%*	*0.516 NS**	*2.244 (0.476-10.573)*
	Allele C	*127*	*69%*	*10*	*83.3%*		
VDR	FF	*29*	*31.5%*	*2*	*33.3%*	*1*	*0.921 (0.159-5.316)*
	Ff	*51*	*55.4%*	*0*	*.0%*	*0.010 S**	*------*
	ff	*12*	*13.0%*	*4*	*66.7%*	*0.006 S**	*0.075 (0.012-0.455)*
	Allele F	*109*	*59.2%*	*4*	*33.3%*	*0.078 NS**	*2.907 (0.845-10.001)*
	Allele f	*75*	*40.8%*	*8*	*66.7%*		

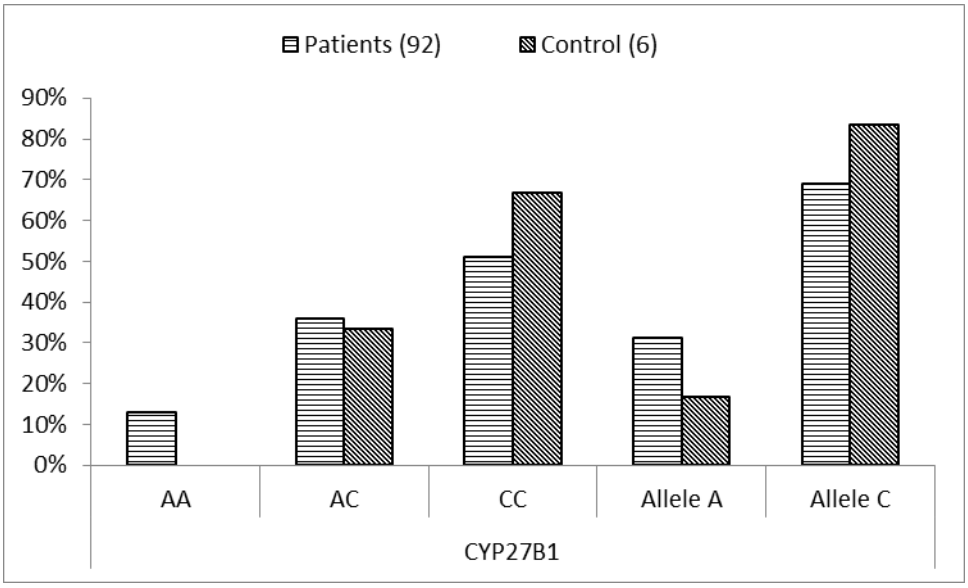

Figure (1): Illustrates the variants polymorphism alleles in CHCV patients and controls as regarding CYP27B1.

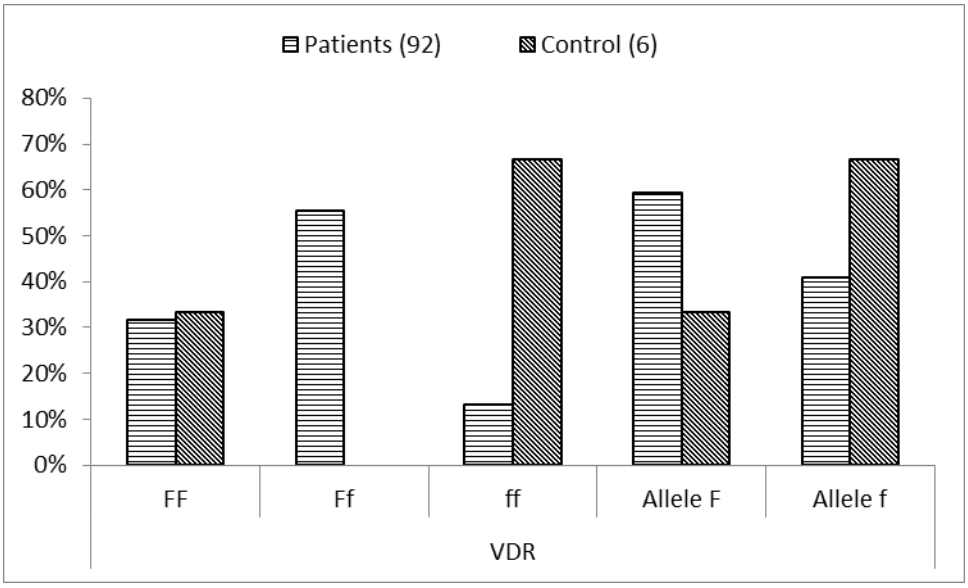

Figure (2): Illustrates the variants of alleles in CHCV patients and controls.

Table (2): Associations between SVR and the levels of serum vitamin D before and after treatment in CHCV patients.

Variables	SVR										P value
	Positive					Negative					
	Mean	SD	Median	Minimum	Maximum	Mean	SD	Median	Minimum	Maximum	
S. vit D.OH before treatment	*48.95*	*38.19*	*40.65*	*8.50*	*167.40*	*54.95*	*38.83*	*48.50*	*9.30*	*217.60*	*0.348 NS**
S. vit D.OH after treatment	*32.94*	*27.16*	*25.15*	*5.90*	*163.2*	*74.08*	*79.57*	*47.35*	*3.70*	*290.00*	*0.027 S**

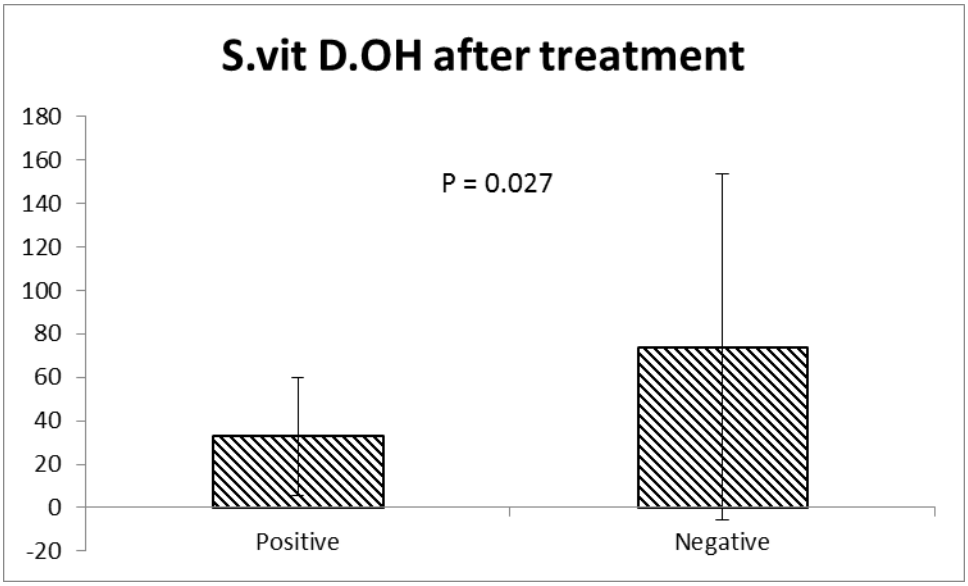

Figure (3): Illustrates that serum vit D levels in patients responder to hepatitis C virus treatment more than non responder.

Table (3): Comparison between s. vitamin D levels before and after CHCV patients treatment.

Variables	Mean	Standard Deviation	Median	Minimum	Maximum	P value
S.vit D.OH before treatment	*48.14*	*38.16*	*45.65*	*8.50*	*217.60*	*0.035 S **
S.vit D.OH after treatment	*50.74*	*56.18*	*31.15*	*3.70*	*290.00*	

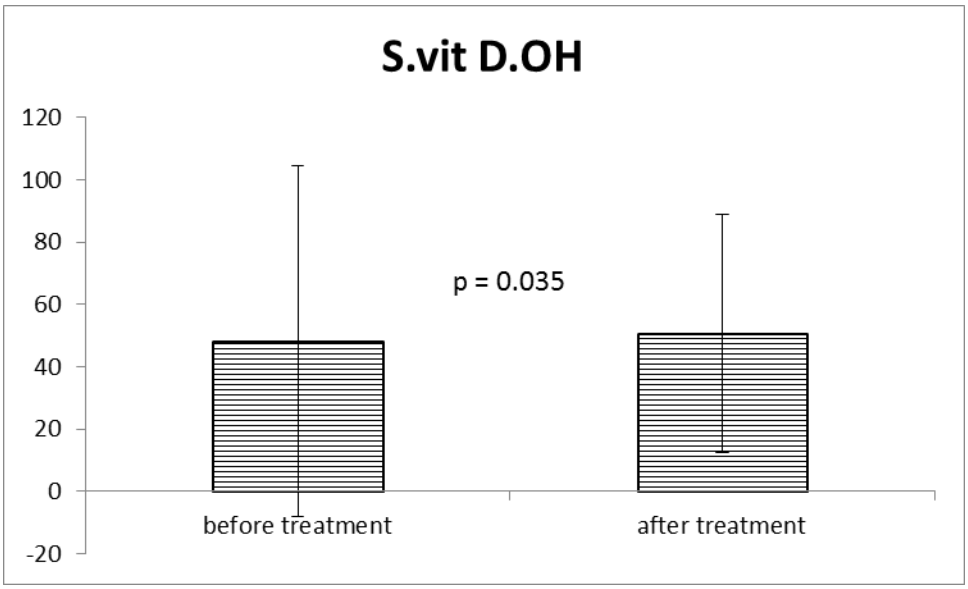

Figure (4): Levels of serum vitamin D levels in CHCV patients before and after treatment.

Table (4): Comparison between the levels of serum vitamin D after treatment in CHCV patients and the controls.

Variables	patients					Control					P value
	Mean	SD	Median	Minimum	Maximum	Mean	SD	Median	Minimum	Maximum	
s.vit D.OH after treatment	*50.74*	*56.18*	*31.15*	*3.70*	*290.00*	*20.20*	*9.15*	*20.10*	*9.70*	*36.40*	*0.157 NS**

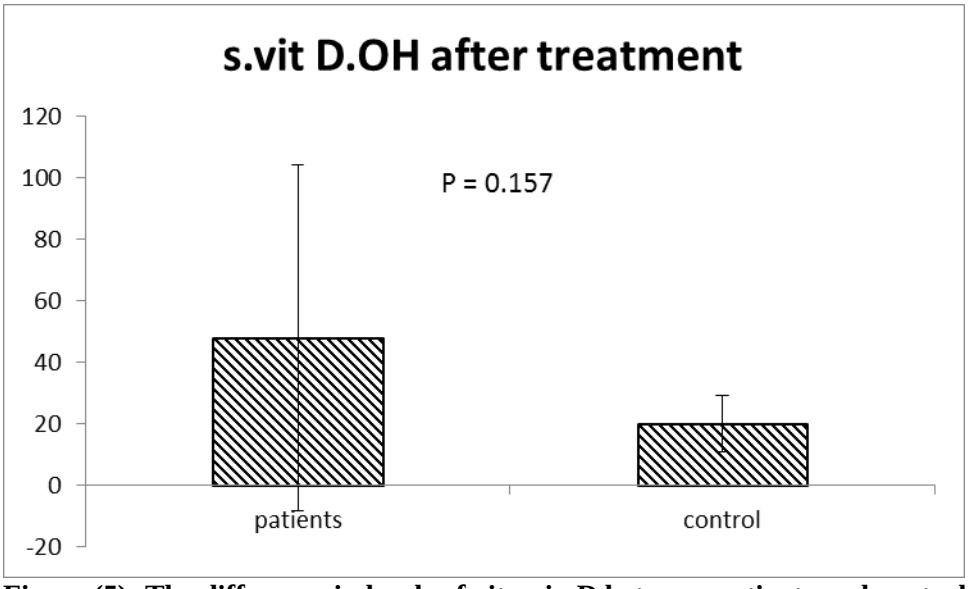

Figure (5): The difference in levels of vitamin D between patients and controls after treatment.

Table (5): Comparison between CHCV patients responder and non responder to treatment as regarding quantitative PCR before treatment.

Variables	SVR										P value
	Positive					Negative					
	Mean	SD	Median	Minimum	Maximum	Mean	SD	Median	Min	Maximum	
PCR before treatment	*2358089.47*	*2765355.02*	*1363500.00*	*959.00*	*10200000.00*	*4518911.93*	*13469510.95*	*578000.00*	*2.00*	*90900004.00*	*0.098 NS **

Table (6 a): The relationship between viral load and serum level of vit.D in CHCV patients.

Variables	Mean	Standard Deviation	Median	Minimum	Maximum
S.vit D.OH after treatment	*50.74*	*56.18*	*31.15*	*3.70*	*290.00*
PCR before treatment	*3720347.11*	*10840410.68*	*950770.50*	*2.00*	*90900004.00*

Table (6 b): The relationship between serum vitamin D level and viral load.

Variables		s.vit D.OH after treatment
PCR before treatment	Correlation Coefficient	*0.000*
	P value	*0.998 NS**
	N	*92*

Table (7): Distribution of the studied CHCV patients as regarding biopsy.

Variables		Count	%
SVR	positive	*34*	*37.0%*
	negative	*58*	*63.0%*
Biopsy	A1F1	*55*	*59.8%*
	A1F2	*22*	*23.9%*
	A1F3	*2*	*2.2%*
	A2F1	*4*	*4.3%*
	A2F2	*6*	*6.5%*
	A2F3	*1*	*1.1%*
	A3F3	*2*	*2.2%*

Table (8): Shows the comparison between responder and non responder to treatment as regarding CYP27B1 polymorphism and vitamin D receptor in CHCV patients.

Variables		SVR				P value	OR (95%CI)
		Positive (n=34)		Negative (n=58)			
		Count	%	Count	%		
CYP27B1	AA	*4*	*11.8%*	*8*	*13.8%*	*1*	*0.833 (0.231-3.005)*
	AC	*14*	*41.2%*	*19*	*32.8%*	*0.416*	*1.437 (0.598-3.450)*
	CC	*16*	*47.1%*	*31*	*53.4%*	*0.554*	*0.774 (0.331-1.808)*
	Allele A	*22*	*32.4%*	*35*	*30.2%*	*0.758 NS**	*1.107 (0.581-2.108)*
	Allele C	*46*	*67.6%*	*81*	*69.8%*		
VDR	FF	*13*	*38.2%*	*16*	*27.6%*	*0.289*	*1.625 (0.661-3.997)*
	Ff	*21*	*61.8%*	*30*	*51.7%*	*0.350*	*1.508 (0.636-3.571)*
	ff	*0*	*.0%*	*12*	*20.7%*	*0.003 S***	-------
	Allele F	*47*	*69.1%*	*62*	*53.4%*	*0.037 S**	*1.949 (1.037-3.663)*
	Allele f	*21*	*30.9%*	*54*	*46.6%*		

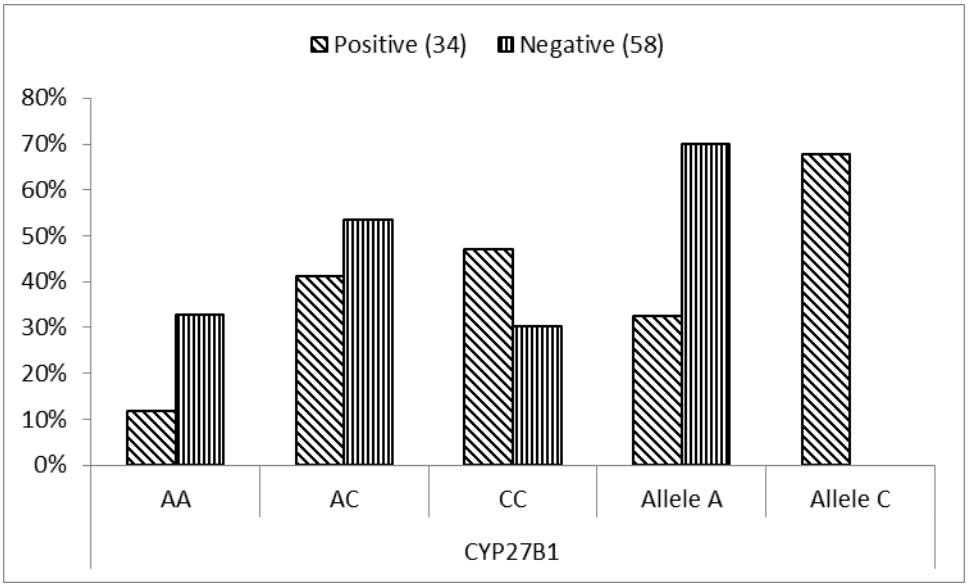

Figure (6): Illustrates the variants polymorphism in responder and non responder CHCV patients as regarding CYP27B1 alleles.

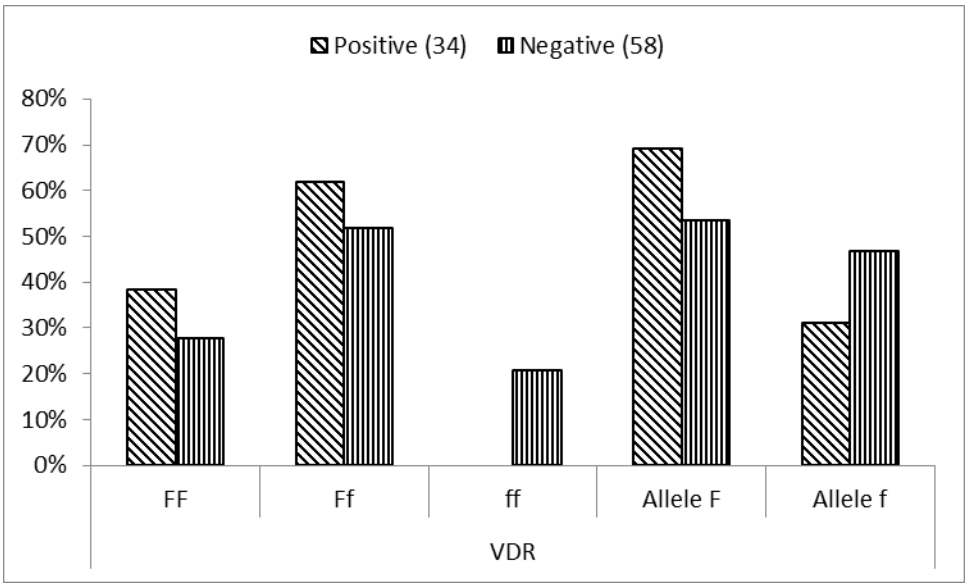

Figure (7): Illustrates the variants of VDR alleles in CHCV responder and non responder CHCV patients.

Table (9): Shows the relation between S. vit D and CYP27B1 polymorphism in CHCV patients.

Variables		CYP27B1			P value
		AA	AC	CC	
S.vit D.OH before treatment	Mean	*64.20*	*47.40*	*53.56*	*0.208 NS**
	Standard Deviation	*50.46*	*26.49*	*42.18*	
	Median	*53.70*	*48.00*	*42.60*	
	Minimum	*9.20*	*9.10*	*8.50*	
	Maximum	*217.60*	*128.00*	*177.90*	
S.vit D.OH after treatment	Mean	*69.86*	*55.41*	*37.50*	*0.065 NS**
	Standard Deviation	*75.34*	*57.92*	*47.72*	
	Median	*33.75*	*35.00*	*23.30*	
	Minimum	*6.70*	*8.10*	*3.70*	
	Maximum	*234.40*	*234.40*	*290.00*	

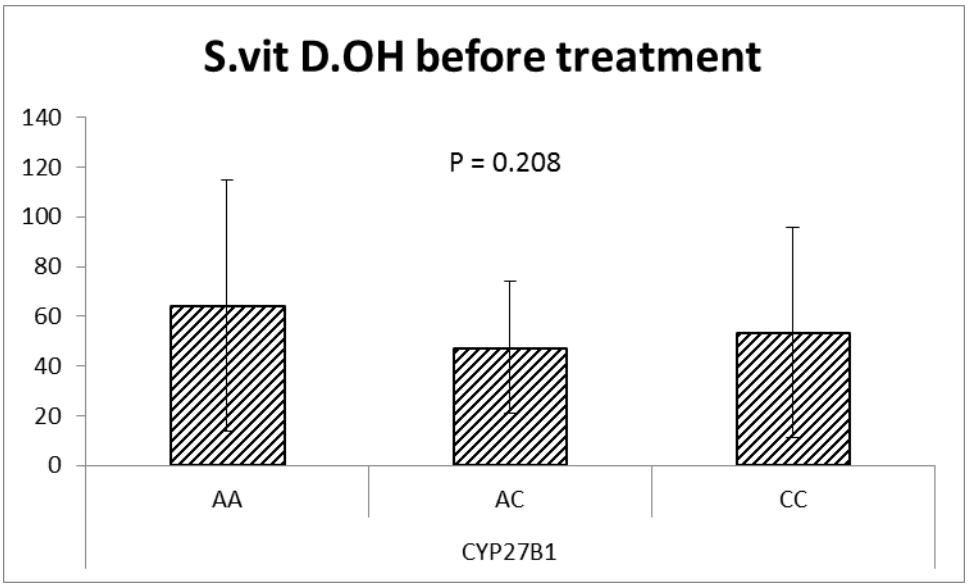

Figure (8): Illustrates the levels of CYP27B1 alleles as regarding s.vitamin D before CHCV treatment, increased level of AA then CC.

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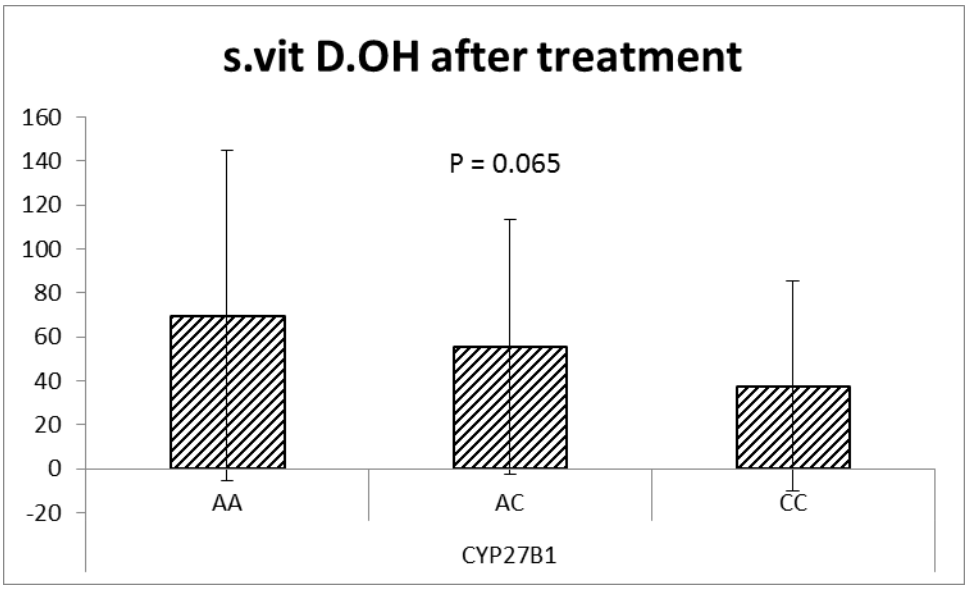

Figure (9): Illustrates the levels of CYP27B1 alleles as regarding s.vitamin D after CHCV treatment, increased AA, the AC.

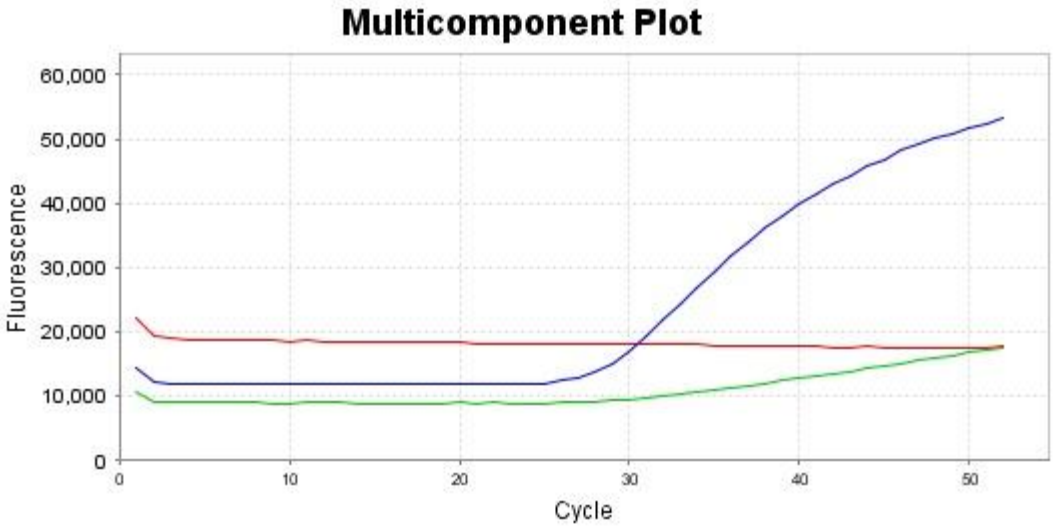

Figure (10): Illustrates the Genotype CYP27B1 1260 (CC). Code: The upper line of the curve represent C allele, the middle: reference, the lower: A allele.

Figure (11): Illustrates the Genotype CYP27B1 1260 (CA). Code: The upper line of the curve represent C allele, the middle: A allele, the lower: reference.

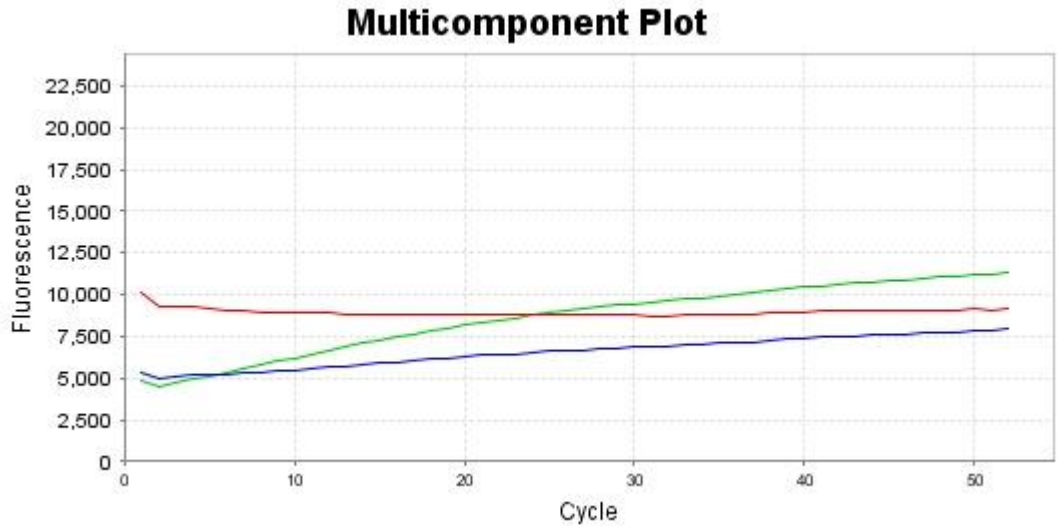

Figure (12): Illustrates the Genotype CYP27B1 1260 (AA). Code: The upper line of the curve represent A allele, the middle: reference, the lower: C allele.

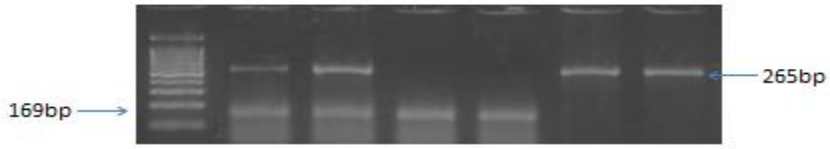

Figure (13): An agarose gel electrophoresis show VDR genotyping.
Lane M: 100 bp ladder, Lane 1&2: VDR genotype (Ff), Lane 3&4: VDR genotype (ff), Lane 5&6: VDR genotype (FF).