

# **Study of Vitamin D Receptor-FOK-I Gene Polymorphism in Chronic Hepatitis C Induced Hepatocellular Carcinoma Patients: A Case Control Study**

Hoda A.Nada<sup>1</sup>, Ayman Z. Elsamanoudy<sup>2</sup>, Hatem A.Elalfy<sup>3</sup>, Reham E. Mogahed<sup>4</sup>

Professor, Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Mansoura University, Egypt<sup>1</sup>

Associate Professor, Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Mansoura University, Egypt<sup>2</sup>

Associate Professor, Department of Tropical Medicine, Faculty of Medicine, Mansoura University, Egypt<sup>3</sup>

Master Student, Chemistry Department, Faculty of science, Port-Said University, Egypt<sup>4</sup>

**ABSTRACT:** Hepatitis C virus (HCV) infection is one of the major public health problems worldwide. The genomic actions of vitamin D are mediated through its binding to the Vitamin D Receptor (VDR), which allows it to modulate the expression of genes in a cell-and tissue-specific manner. The VDR directly or indirectly regulates the expression of more than 200 genes that influence cell, differentiation and apoptosis, as well as immunomodulation and angiogenesis. The VDR gene polymorphisms have been identified that may influence cancer development including HCC. The aim of study to evaluate the possible association between VDR gene polymorphism with (HCC) in top of chronic hepatitis C (HCV) patients. This study aims to investigate the possible association of VDR gene polymorphism and HCC development in patients with chronic HCV infection. 103 patients with HCC on top of chronic HCV infection, 44 non-viral HCC. 100 Healthy volunteers as a control group. The control group subjects are sero-negative and PCR negative for hepatitis C virus and HBs-Ag antibodies were analyzed for serum 1,25(OH)<sub>2</sub>D<sub>3</sub>, liver functions were determined and VDR-FOK1 gene polymorphism. Results revealed that individuals with F/F homozygote have a lower risk in HCC development, while others with f allele have a higher risk (with OR =2.58) and we found that vitamin D level was significant lower in HCV related HCC cases than normal control. We concluded that VDR genetic polymorphisms are significantly associated with the occurrence of HCV related HCC especially f allele carriers which could be considered as a risk factor of hepatocellular carcinoma in Egyptian patients. The FOKI C>T polymorphisms may be used as a molecular marker to predict the risk and to evaluate the disease severity of HCC in those infected with HCV.

**KEYWORDS:** Hepatitis C virus (HCV), HCC, VDR, FOKI

## **I. INTRODUCTION**

Hepatitis C virus (HCV) infection is one of the major public health problems worldwide [1]. Chronic HCV infection is characterized by a high rate of progression to fibrosis, chronic hepatitis, leading to cirrhosis and ultimately to hepatocellular carcinoma (HCC) [2]. Although the relationship between HCV and the development of HCC is well established, the pathogenetic mechanism of hepatocarcinogenesis, including host- and viral-related factors, is still unknown [3].

# International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 4, April 2016

It is prudent to affirm that differences in the incidence rates and the strong gender distribution in HCC are not entirely due to differences in the exposure to the causative agents [4,5]. Of great importance, genetic factors can also contribute, particularly gene polymorphisms of inflammatory cytokines and growth factor ligands and receptors [6].

Vitamin D is a prohormone with several active metabolites and acts as a hormone. It is recognized as a key player in calcium and bone homeostasis. In recent evidence that vitamin also regulates cell proliferation and differentiation and has anti-inflammatory, immunomodulatory and anti fibrotic properties including cell growth and differentiation, detoxification of xenobiotic and modulation of adaptive and innate immunity [7, 8] and antineoplastic effects through anti-proliferative action and programmed cell death [9]. Also, vitamin D can inhibit cancer cell invasion by interfering with specific steps such as angiogenesis and metastasis through decreasing the activity of certain proteases which degrade extracellular matrix and basement membrane [10]. HCC is usually asymptomatic in the early stages and tends to be invasive. Therefore, most patients are presented with an incurable disease at the time of detection which makes its early diagnosis critical for a good prognosis.

The vitamin D has widespread effects in the immune system [11];  $\alpha,25(\text{OH})_2\text{D}_3$  has been shown to suppress production of the interleukin (IL)-12, IL-2, tumor necrosis factor- $\alpha$  and  $\gamma$ -interferon. It has also been shown to activate expression of transforming growth factor- $\beta$ 1 and IL-4, thereby inhibiting T helper 1(Th 1)-type responses, and to induce regulatory T-cells [12]. Furthermore, the active form of vitamin D alter the development of Th1, Th17, and Th9 cells, which are implicated in the pathogenesis of different types of autoimmune diseases including the type 1 diabetes [13, 14]. These immunomodulatory effects may explain the reported protective effects of vitamin D in type 1 diabetes [15, 16] Also, direct effects for vitamin D on the normal function of beta cells have been described, with improved beta cell function and survival upon inflammatory or immune attack. In the current study, we aim to investigate the association of VDR gene polymorphisms with hepatocellular carcinoma (HCC) development in chronic hepatitis C patients.

## II. SUBJECTS AND METHODS

### A. Subjects :

This hospital-based case-control study included 147 HCC patients recruited prospectively from out and inpatient clinics of Tropical Medicine Department, Mansoura University, Egypt during the period from March 2011 to March 2014. The study was approved by the ethical committee of the faculty and informed consents were obtained from all subjects.

Subjects in this study were classified into patients 103 patients with HCC on top of chronic HCV infection, 44 non-viral HCC. 100 Healthy volunteers as a control group. The control group subjects are sero-negative and PCR negative for hepatitis C virus and HBs-Ag antibodies, also, abdominal ultrasound examination revealed nothing abnormal. HCC was diagnosed according to the diagnostic guidelines of the European Association for the Study of the Liver [17]. Exclusion criteria included are: Patients with liver diseases other than HCC such as (primary biliary cirrhosis, primary sclerosing cholangitis, toxic hepatitis, Wilson disease, tyrosinemia). Moreover, the following patients were also, excluded from the current study; patients with HCC but with no cirrhosis and those with AFP level less than 200 ng/dl, patients with liver metastasis as well as HIV infected patients.

**B. Samples collection:** Two mls venous blood samples were delivered to sterile collection tubes containing  $\text{K}_2\text{EDTA}$  (Stored as EDTA anticoagulated blood Samples at  $-70^\circ\text{C}$  for DNA extraction and genotyping of Vitamin D using the specific restriction enzyme (RFLP). Another 5 ml blood samples were delivered to Vacuum blood collection tubes and allowed to clot for 30 minutes and centrifuged at 7000 r.p.m for 10 minutes for serum separation then serum collected in other sterile tubes and stored at  $-70^\circ\text{C}$  until used to determine serum  $\alpha$ -fetoprotein, serum vitamin D level and serum liver function tests .

### C. Isolation of DNA:

Genomic DNA was extracted from EDTA whole blood using a spin column method according to the protocol using G-spin<sup>TM</sup> Total DNA Extraction Kit supplied by intron biotechnology, IBT-QMS-T1704 (R01-2012-01). The average DNA concentration was  $0.127 \pm 0.005 \mu\text{g}/\mu\text{l}$  determined by measuring the absorbance at 260 nm and 280 nm (Jenway, Genova Model, UK). All samples had a 260/280 nm absorbance ratio between 1.6 and 1.79. The integrity of the DNA was checked by electrophoresis on 0.8 % agarose gel stained with ethidium bromide using Gel electrophoresis apparatus.

# International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 4, April 2016

## D. Genetic polymorphism detection of the VDR gene [18]:

The DNA was extracted from peripheral blood leukocytes spin<sup>TM</sup> Total DNA Extraction Kit supplied by intron biotechnology, IBT-QMS-T1704 (R01-2012-01).

The C/T polymorphism in the first of two start codons (ATG) at the translation-initiation site of The VDR genotype was determined by polymerase chain reaction (PCR) amplification and was detected restriction length fragment polymorphisms (RFLP) described as Forward: 5'-AGCTGGCCCTGGCACTGACTCTGCTCT-3', Reverse: 5'-ATGGAAACACCTTGCTTCTTCTCCCTC-3'. PCR was carried out in 50  $\mu$ L final reaction volume using 2X PCR Master mix Solution Dream Taq thermo scientific( #K 1081 - 200rxns US patent NO 6,127,155. Applied biosystems, 850 Lincoln Centre Drive Foster city California ). The following mixture was prepared for each sample: 25  $\mu$ L PCR Master mix solution (2x), 1  $\mu$ L (20 pmole) of forward primer, 1  $\mu$ L (20 pmole) of reverse primer, 2  $\mu$ L (200ng) of genomic DNA and 21  $\mu$ L of double distilled deionizer water. Amplification was performed in a Thermal Cycler (TECHEN TC-312, Model FTC3102D, Barloworld Scientific Ltd. Stone, Stafford Shire St., 150 SA, UK) using the following program Initial Denaturation at 94°C for 5 minutes 35 cycles of: Denaturation at 94°C for 30 sec then, annealing at 61°C for 30 seconds then, extension at 72°C for 1 minute. Amplified samples were digested with the specific restriction enzyme; (Fast Digest FOKI for 100rxns #FD2144, Lot :00185288 ,thermo scientific 20,000 units /ml) according to the manufacturing instructions 1  $\mu$ L (2 units) of the Fast Digest enzyme (Thermo scientific) 10  $\mu$ L of amplified PCR product (1  $\mu$ g) 5  $\mu$ L (10X) of Fast Digest Green Buffer (20 mM Tris-acetate, 10 mM Magnesium acetate 50 mM Potassium acetate, 1 mM DTT (pH 7.9@25°C) 34.0  $\mu$ L of sterile distilled water to obtain 50  $\mu$ L total Reaction Volume incubated at 37°C in heat block for 5 min. This enzyme the digestion products were electrophoresed on a 3.0% agarose gel for 60 min, stained with ethidium bromide using Gel electrophoresis apparatus, visualized via Light UV Transilluminator (Model TUV-20, OWI Scientific, Inc. 800 242-5560, France) and photographed. FOKI restriction enzyme digestion products are : homogenous F/F shows one band at 265bp, F/f heterogenous genotype show the three bands (265, 196 & 69), and f/f shows two bands at (196, 69) bp.

**E. Estimation of serum Human (1,25 (OH)<sub>2</sub>D<sub>3</sub>) :** The authors used the commercially available Kit (Catalog Number: MBS733782 ,96 tests , San Diego, CA 92195-330 8 USA): This essay employs the quantitative sandwich ELISA technique. It was performed according to the manufacturer's instructions. The absorbance of each sample was read on plate ELISA Reader (Tecan, Sunrise, Austria) at 450 nm wave length.

## F. Measurement of serum AFP by ELISA Method :

The Immunospec AFP is a quantitative solid phase enzyme-linked immunosorbent assay (ELISA). AFP was determined using ELISA kit (Catalog No.E1-205) supplied from Immunospec Corporation, 7018 Owens mouth Ave. Suite 103 Canoga Park, CA, 91303, according to the manufacturer's instructions.

**G. Estimation of serum liver function :** Liver functions estimation was performed colorimetrically using the commercially available kits [19]. Alanine and aspartate amino transferase serum alkaline phosphate (ALP) were estimated according to the method [20], Total-Bilirubin, Direct Bilirubin [21] and Albumin (Alb) [22]. The kits were provided from Elitech diagnostics, Zone Industrielle 61500, Sees France.

**H. Serological determinations :** Serum markers of HCV infection were sought in serum using commercially available immunoenzymatic assays .(Abbott laboratories ,North Chicago IL and ortho Diagnostic systems ,Raritan,NJ).

**I. Statistical Analysis :** The data were expressed as mean, standard deviation ( $\pm$ SD) for the studied groups. Statistical analysis was performed using statistical package for social science (SPSS) program version 17 (SPSS Inc., Chicago, IL, USA). The studied groups were compared by Chi square (X<sup>2</sup>) test to evaluate Hardy-Weinberg equilibrium in allele and genotype frequencies in the study group. The association of VDR gene receptor polymorphisms with hepatocellular carcinoma with steatosis risk was estimated using odds ratios (OR) and 95% confidence intervals (95% CI) for the comparison of genotype and allele contrast. The difference was considered statistically significant when *P* value was less than 0.05.

# International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

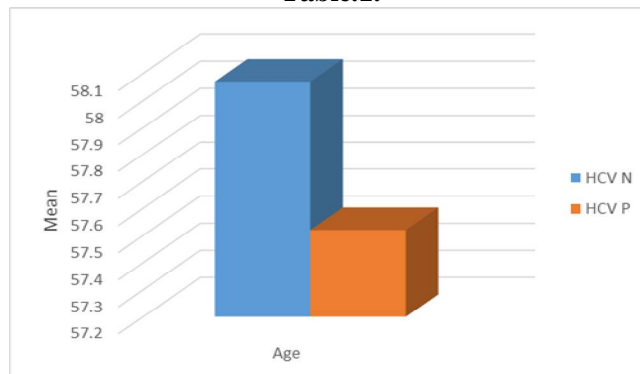
Vol. 5, Issue 4, April 2016

### III.RESULTS

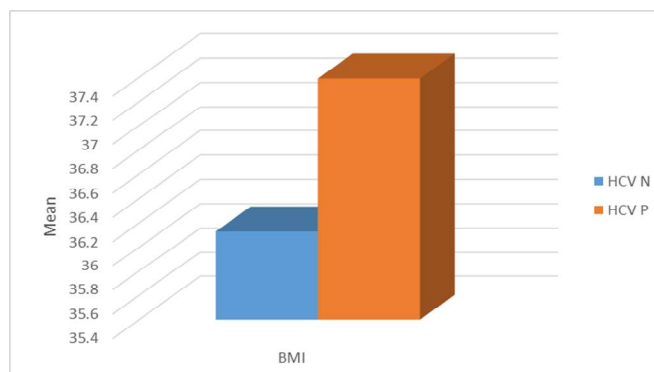
In table.1.and Fig(1.a),(1.b) represent the comparison between the studied HCC related HCV cases (103 subjects ) to HCC unrelatedHCV of the (44 subjects) . In positive HCV related HCC cases showed no statistically significant different in age ( $57.52 \pm 9.91$ ), ( $58.07 \pm 4.78$ ) respectively in negative HCV groups with  $p = 0.7$ , On the other hand , the results of BMI ( $37.39 \pm 5.51$ ), ( $36.13 \pm 5.09$ ), showed also no statistically significant different respectively with  $p = 0.19$ ,  $p=0.4$ ).

	HCV				P*
	N(n=44)		P(n=103)		
	Mean	±SD	Mean	±SD	
Age(years)	<b>58.07</b>	<b>4.78</b>	<b>57.52</b>	<b>9.91</b>	<b>0.7</b>
BMI(kg/m <sup>2</sup> )	<b>36.13</b>	<b>5.09</b>	<b>37.39</b>	<b>5.51</b>	<b>0.19</b>

**Table.1.**



**Fig.1.a.**



**Fig.1.b.**

**Table 1** Compare between HCC virus positive versus negative, P\*: Probability Test used: Mann-Whitney test , BMI: Body Mass ,  $p^* < 0.05$

**Fig .1.a.** Mean value of age in the different investigated groups, **Fig1.b.** Mean value of BMI in the different investigated groups

## International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 4, April 2016

**Table. 2.** showed the comparison between the studied positive HCV related HCC cases (103 subjects) group to the negative HCV unrelated HCC cases (100 subjects): in the liver function tests which are SGOT, SGPT, Albumin, ALP and Bilirubin (total and direct bilirubin). In the negative HCV related HCC individuals, the median value of SGOT activity was 62 IU/ml range = 32-146 IU/ml and that of positive HCV related HCC was 65 IU/ml range = 20-285 IU/ml the difference was not statistically

significant with  $p=0.5$ . In the negative HCV related HCC individuals, the median value of SGPT was 47 IU/ml range = 26-186 IU/ml and that of positive HCV related HCC was 54.53 IU/ml range = 20-195 IU/ml the difference was not statistically significant with  $p=0.77$ . In the negative HCV related HCC individuals the median value of t-bilirubin was 1.7 mg/dl range 0.9-4.1 mg/dl and that of positive HCV related HCC, the median value of t-bilirubin was 1.6 mg/dl range 0.5-25.1 mg/dl the difference was not statistically significant with  $p=0.6$ , also in the negative HCV related HCC individuals the median value of d-bilirubin was 0.85 mg/dl range was 0.25-3.6 mg/dl and that of positive HCV related HCC, the median value of d-bilirubin was 0.9 mg/dl range was 0.25-4.2 mg/dl, the difference was not statistically significant with  $p=0.18$ . In the negative HCV related HCC individuals the median value of albumin 2.7 g/dl range (1.9- 4) g/dl and that of positive HCV related HCC, the median value of albumin 2.8g/dl (1.9-5) g/dl, the difference was not statistically significant with  $p=0.1$  and in the negative HCV related HCC individuals the median value of ALP 313.47 u/l range (209.56-384.9) u/l and that of positive HCV related HCC, the median value of ALP 305.02 range (197.23-419.81) u/l the difference was not statistically significant with  $p=0.1$ .

Parameters	HCV						P*
	N(n=44)			P(n=103)			
	Median	Minimum	Maximum	Median	Minimum	Maximum	
SGOT(U/L)	62.00	32.00	146.00	65.00	20.00	285.00	0.5
SGPT(U/L)	47.00	26.00	186.00	54.53	20.00	195.00	0.77
T.Bilirubin (mg/dl)	1.70	0.90	4.10	1.60	.50	25.10	0.6
D.Bilirubin (mg/dl)	.85	.25	3.60	.90	.25	4.20	0.18
Albumin (g/dl)	2.70	1.90	4.00	2.80	1.90	5.00	0.1
ALP(U/L)	313.47	209.56	384.90	305.02	197.23	419.81	0.1

**Table.2.**

**Table .2.** Biochemical variables in positive HCV related HCC patients versus negative HCV related HCC, P\*: Probability Test used: Mann-Whitney test

**Table.3.** showed that tumor marker as AFP in positive HCV related HCC subjects the median value of AFP was 300ng/ml range 5-3150 ng/ml, and that of the cases HCV un related HCC subjects the median value of AFP was 50ng/ml range 26.6-124ng/ml the difference between these median values was statistically highly significant with  $p<0.0001$  and in serum vitamin D level in positive HCV related HCC the median value of serum vitamin D level was 25.102ng/ml range 12.001-73.447 ng/ml the difference between them was highly significant with  $p<0.0001$ .

## International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 4, April 2016

parameters	HCV						P* Value
	N (n=44)			P(n=103)			
	Median	Minimum	Maximum	Median	Minimum	Maximum	
AFP(ng/ml)	50.00	26.60	124.00	300.00	5.00	3150.00	<0.0001
ELISA of Vit D(ng/ml)	43.000	32.456	82.248	25.102	12.001	73.447	<0.0001

**Table.3.**

**Table .3.** Alpha fetoprotein (AFP) and serum level of vitamin D in positive HCV related HCC patients versus negative HCV related HCC, P\*: Probability Test used: Mann-Whitney test ,AFP : $\alpha$ -faetoprotein, P \* $<$  0.05

**In table.4. and fig4.a.** showed that the median values of serum vitamin D level versus the FOKI genotyping in HCV cases (positive and negative ).

In HCV un related HCC showed that the median of FF sub group 54ng/ml range 82.248- 32.456, Ff subgroup was 43ng/ml range 82.248-32.818, ff subgroup 41ng/ml range 59-33 ng/ml when the median values of serum vit D of the different genotypic groups were compared with each other , the results were not statistically significant with P=0.096.

In positive HCV related HCC showed that the median value of vitamin D in FF subgroup was 50.902 ng /ml range 16.783-73.447 ng/ml,Ff subgroup was 27.634 range 12.001-60.426 ng/ml and ff sub group was 22.691 ng/ml range 12.001- 48.25 ng/ml. when the median of FF and Ff of HCV positive patients were compared with each other ,the results was statistically significant with p=0.001 . on the other hand , the medians of FF versus FF was not statistically significant with p=0.092, the median of FF versus Ff was statistically significant with p<0.0001. the medians of FF versus ff and Ff versus ff, were statistically significant p<0.001,p=0.001 respectively.

On the other hand in positive HCV cases showed that FF 50.902 ng/ml (73.447 $\pm$ 16.783), Ff 27.634ng/ml (60.426 $\pm$  12.001), ff 22.691ng/ml (48.25 $\pm$ 12.001) show statistically significant decrease with p=0.001. in FF show no staticallyly significant with p=0.092,also Ff show statistically significant decrease with p<0.0001.On the other FF/ff, FF/Ff show statistically significant decrease with p=0.001in positive HCV related HCC to HCV un related HCC cases.

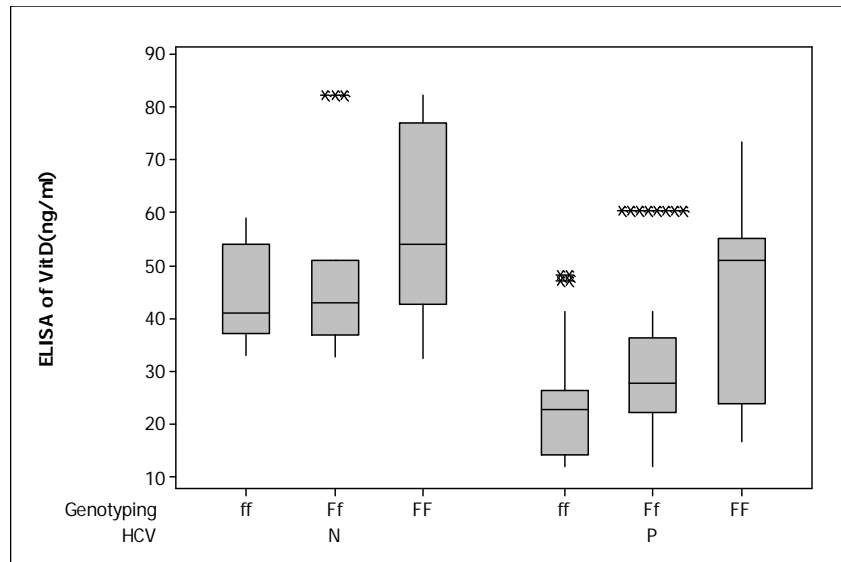
ELISA of Vit D			Genotyping			P1	
			FF	Ff	ff		
HCV	N	Median	54.000	43.000	41.000	0.096	
		Minimum	32.456	32.818	33.000		
		Maximum	82.248	82.248	59.000		
	P	Median	50.902	27.634	22.691 <sup>bc</sup>	0.001	
		Minimum	16.783	12.001	12.001		
		Maximum	73.447	60.426	48.250		
	P2			0.092	<0.0001	0.001	

**Table.4.**

## International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 4, April 2016



**Fig4.a.**

**Table.4.** Serum vitamin D level and its FOKI genotyping in HCV positive (P) and viral negative (N) patients, P1: Significance between genotyping FF, Ff and ff either in positive or negative HCV groups  
Test used: Kuskalwallis test followed by Mann-whitney for multiple comparisons

a: significance between FF and Ff, b: significance between FF and ff, c: significance between Ff and ff

P2: Significance between positive and negative HCV groups either in FF, Ff or ff  
Test used: Mann-whitney U test

**Fig4.a.** Box plot showing the distribution of serum vitamin D level with FOKI genotyping in positive HCV related HCC patients and negative HCV patients.

**Table.5.** and **fig.5.a** showed that serum level of vitamin D in the different FOKI allelic genotyping group HCV patients. In negative HCV related HCC, the median value of serum vitamin D level in F allele was [49 (range=32.46-82.25)], the median value of serum vitamin D level in f allele was [42 (range =32.82-82.25)], the difference between them was statistically significant with (p=0.02). On the other hand, in positive HCV related HCC the median value of serum vitamin D level in F allele was [31.97 (range =12-73.45)] and the median value of serum vitamin D level in f allele was [24.02 (range =12-60.43)], the difference between them was statistically significant decrease with p<0.0001. When the median value of serum vitamin D level of F allele in positive HCV related HCC were compared to the median value of serum vitamin D level of F allele in negative HCV related HCC groups the difference between them was statistically significant with p<0.0001, also when the median value of serum vitamin D level of f allele in positive HCV related HCC were compared to the median value of serum vitamin D level of f allele in negative HCV related HCC the difference between them was statistically significant decrease with p<0.0001.

ELISA of VitD (ng/ml)		Alleles						P1
		F			f			
		Median	Minimum	Maximum	Median	Minimum	Maximum	
HCV	N	49.00	32.46	82.25	42.00	32.82	82.25	0.02
	P	31.97	12.00	73.45	24.02	12.00	60.43	<0.0001
	P2	<0.0001			<0.0001			

**Table.5.**

# International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 4, April 2016

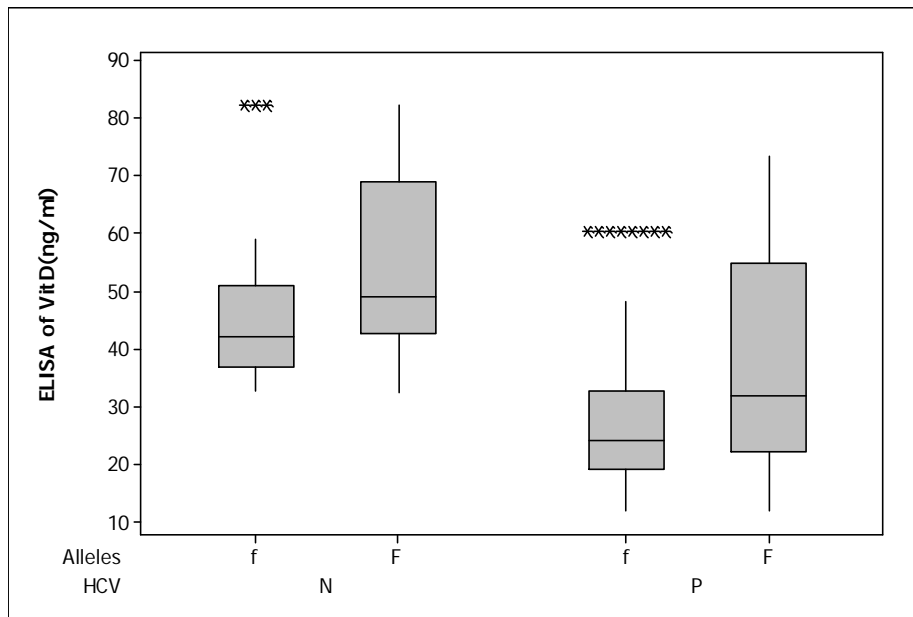


Fig.5.a.

**Table .5.** Association of Vitamin D serum level with FOKI allele in HCV viral positive (P) and viral negative (N) patients. P1: Significance between allele F and allele either in positive or negative HCV groups (Test used: Mann-whitney U test.

P2: significance between positive and negative HCV groups either in F or f allele groups Test used: Mann-whitney U test

**Fig.5.a.** Box plot showed the distribution of FOKI allele with serum vitamin D in HCV patients

## IV. DISCUSSION

Hepatocarcinogenesis is a complex and multi-factorial process, in which both environmental and genetic features interfere and contribute to malignant transformation [23]. The identification of genetic factors related to HCC susceptibility may improve our understanding of the various biological pathways involved in hepatocarcinogenesis and as well improve the scientific basis for preventative intervention. Numerous candidate-gene studies have reported associations between single nucleotide polymorphism and the development of HCC [24]. The present study revealed that the allele f of vitamin D receptor-FOKI genotype was significantly more common in patients with chronic HCV hepatitis when compared to the negative groups. These results are in agreement with previous studies which investigated the association of VDR gene polymorphism [25].

Hepatitis virus infection is associated with the increase of oxidative stress in liver cells and results in DNA changes in stability, thus increasing the risk of developing cirrhosis and/or HCC (24), also there are some studies provided evidence that genetic polymorphisms of certain genes may predict the HCC occurrence in hepatitis virus infection [26]. As the current study aimed to investigate the association of VDR gene polymorphism and HCC development in patients with chronic HCV infection. Falletti et al., [25] have demonstrated that VDR genetic polymorphisms are significantly associated with the occurrence of HCC in cirrhotic patients who underwent liver transplantation [25]. Chronic HBV-HCV co-infection is associated with a more rapid progression to liver cirrhosis. The evolution towards cirrhosis of HCV patients is determined by multiple factors related to both HCV strain and the biological and genetic parameters of the host [1]. As such, polymorphisms of vitamin D receptors (VDR) and vitamin D deficiency were reported in HBV patients [2,3] and recently in HCV patients [4,5]. In current study, we presented the frequencies of VDR genotypes in patients with and without HCV infection. We found a significant difference between patients with



## International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 4, April 2016

HCC on top of chronic HCV and patients with HCC without HCV with ( $p < 0.001$ ). This is supported by Falletti et al., (2010) [25]. In HCV genotype 1 – chronic hepatitis C (CHC) patients, low serum vitamin D levels are associated with the severity of liver fibrosis and severity necro-inflammatory activity by Petta et al., (2010) [26]. Vitamin D deficiency has been shown to be associated with several immune-mediated diseases, and susceptibility to infection and cancer. In fact, a 25(OH)D concentration  $< 50$  nmol/L (20 ng/mL) is an indication of vitamin D deficiency, whereas a 25(OH)D concentration of 51-74 nmol/L (21-29 ng/mL) is considered to indicate insufficiency [27,28].

A number of studies investigated the relations between serum vitamin D levels and various cancers such as cancer of the colorectal [29], esophagus [30], lung and ovaries [31], prostate [32] and pancreas [33], little epidemiologic data for vitamin D and liver cancer are available, despite the important role of the liver in metabolizing the circulating form of vitamin D [34]. HCC is now a rather common malignancy in Egypt which usually develops on top of liver cirrhosis secondary to viral infection [35]. It is three times more common in men than women, which could be explained by differences in exposure to risk factors [36]. The results of the present study were higher than in a study done in Egypt by Hammad et al., (2013) [37], who studied series of 1328 with HCC cases and they reported that HCC is significantly higher in men than women (77.7 and 22.3%, respectively).

In the current study, we found a significant association between HCC with FOKI-VDR gene polymorphism and FF as a reference. Our data showed that (ff) carriage had a significantly higher risk for development of HCC after adjustment with age, HCV infection, BMI and HOMA-IR with F allele as a reference. The OR of F allele carriage was  $< 0.0001$ .

Similarly, Lange et al., (2012) [36] measured the largest study of non-cirrhotic patients with chronic HCV infection. Vitamin D status was assessed in a cohort of 468 patients. Moreover, Mansoor et al., (2010) [38] made a study of prevalence and significance of vitamin D deficiency and insufficiency among apparently healthy adults. They found high prevalence of 25(OH) vitamin D deficiency 90% had low serum 25(OH)D levels (69.9% were deficient and 21.1% had insufficient levels of 25(OH)D among apparently healthy adults, hospital staff and health care professionals.

It is suggested that low vitamin D levels might be a risk factor for hepatocellular carcinoma. Liver steatosis is a feature of chronic hepatitis C virus (HCV) infection. HCV genotype 3 directly induces the highest degree and prevalence of steatosis (up to 80%), whereas HCV-related steatosis in non-3 genotypes is mainly associated with metabolic conditions. The role of vitamin D in the pathogenesis of hepatic diseases is actually of great interest. In the liver, vitamin D acts as an “immune-modulator” suppressing fibroblast proliferation and collagen production [39,40].

Novel studies demonstrated that vitamin D deficiency was associated with low rate of sustained virological response (SVR) in patients affected by hepatitis C virus (HCV) under interferon-alfa therapy. Furthermore, a recent intervention trial showed that vitamin D supplementation improves the probability of achieving a SVR following antiviral treatment in patients with recurrent hepatitis C [41].

Vitamin D deficiency is very common among patients with chronic liver disease (92%), and at least one-third suffer from severe vitamin D deficiency ( $< 12$  ng/ml). Serum vitamin D deficiency and the CYP27B1-1260 promoter polymorphism are more prevalent in patients with chronic hepatitis C and related to more fibrosis.

The anti-inflammatory and anti-fibrotic roles of vitamin D indicate that vitamin D has the potential to reduce HCV-mediated liver disease and, perhaps, to positively contribute to treatment outcome. It is well established that vitamin D plays an important antibacterial role by regulating cathelicidin expression in human monocytes. A number of studies now suggest that vitamin D may also have analogous effects, as evidenced by the fact that vitamin D and its metabolites can synergize with IFN treatment to directly inhibit HCV RNA replication in vitro [42].

Clinical evidence indicated that patients with CHC are always at higher risk of vitamin D deficiency by Gal-Tanamy, L et al., (2011) [43]. In a large scale study conducted by Petta et al., (2010) [26] serum 1,25(OH)<sub>2</sub>D in CHC was significantly lower than in healthy population.

Vitamin D is known to be stored into the adipocytes and serum 25(OH) vitamin D levels could be significantly influenced by body composition. We did not make direct measurements of body fatness but we measured BMI. An association between low 25(OH) vitamin D levels and the histological severity of NASH was suggested by Targher et al., (2007) [44] in patients with chronically elevated liver enzymes and hepatic steatosis detected by abdominal

# International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 4, April 2016

ultrasound examination, who underwent liver biopsy for suspected steatohepatitis.

The homozygous FokI(TT) and heterozygous (CT) polymorphism and vitamin D levels have independent effect on cancer development and are not synergistic in their actions .

## V.CONCLUSION

The current study demonstrates that VDR genetic polymorphisms are significantly associated with the occurrence of HCV related HCC especially f allele carriers which could be considered as a risk factor of hepatocellular carcinoma in Egyptian patients. The FOKI C>T polymorphisms may be used as a molecular marker to predict the risk and to evaluate the disease severity of HCC in those infected with HCV.

## REFERENCES

- [1] Mohd Hanafiah K, Groeger J, Flaxman AD., Wiersma ST., “ Globalepidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence,” *Hepatology* 57, 1333–1342,2013.
- [2] Hajarizadeh B, Grebely J., Dore GJ.,“ Epidemiology and natural history of HCV infection”. *Nat Rev Gastroenterol Hepatol* 10, 553–562,2013.
- [3] Massard J, Ratziu V, Thabut D, Moussalli J, Lebray P, Benhamou Y ..Poynard T., “ Natural history and predictors of disease severity in chronic hepatitis C”. *J Hepatol* 44(1 Suppl), S19–S24, 2006.
- [4] Hung CH, Lee CM, Wang JH, H.,u TH, Chen CH, Lin CY ..Lu SN., “ Impact of diabetes mellitus on incidence of hepatocellular carcinoma in chronic hepatitis C patients treated with interferon-based antiviral therapy”. *Int J Cancer*128, 2344–2352,2011:
- [5] Yu MW ., Chen CJ.,“ Hepatitis B and C viruses in the development of hepatocellular carcinoma”. *Crit Rev Oncol Hematol* 17, 71–91,1994.
- [6] Hung CH, Lee CM, Lu SN, Wang JH, Hu TH, Tung HD, Chen CH, ChenWJ, ., Changchien CS., “Long-term effect of interferon alpha-2b plus ribavirin therapy on incidence of hepatocellular carcinoma in patients with hepatitis C virus-related cirrhosis”. *J Viral Hepat* 13, 409–414, 2006
- [7] Stokes CS, Volmer DA, Grunhage Fand Lammert F “Vitamin D in chronic liver disease”. *Liver Int.*, 33:338-352, 2013
- [8] Kitson MT and Roberts SK D-livering the message,“ the importance of vitamin D status in chronic liver disease”. *J Hepatol.*, 57: 897-909,2012
- [9] Chiang KC, Yeh CN, Chen MF., Chen TC “Hepatocellular carcinoma and vitamin D”: a review. *J Gastroenterol Hepatol.*, 26: 1597–1603,2011.
- [10] Vanoirbeek E, Krishnan A, Eelen G, Verlinden L, Bouillon R, Feldman D and Verstuyf A., “The anti-cancer and anti-inflammatory actions of 1,25(OH)2D3”. *Best Pract Res Clin Endocrinol Metab.*, 25: 593–604, 2011.
- [11] von Essen MR, Kongsbak M, Schjerling P, Olgaard K, Odum N, Geisler C., “Vitamin D controls T cell antigen receptor signaling and activation of human T cells”. *Nat Immunol*; 11: 344-349, 2010.
- [12] Jones G, Strugnell SA, DeLuca HF ., “ Current understanding of the molecular actions of vitamin D”. *Physiol Rev.*;78(4):1193-231, 1998.
- [13] Zipitis CS, Akobeng AK., .,“ Vitamin D supplementation in early childhood and risk of type 1 diabetes a systematic review and meta-analysis”. *Arch Dis Child*;93:512–517, 2008
- [14] Baeke F, Takiishi T, Korf H, Gysemans C, Mathieu C ., “Vitamin D modulator of 427 the immune system. *Current Opinion in Pharmacology*”; 10 (4): 482–496, 2010.
- [15] Wolden-Kirk H, Overbergh L, Christesen HT, Brusgaard K, Mathieu C., “ Vita- 424 min D and diabetes: its importance for beta cell and immune function, *425 Molecular and Cellular*”. *Endocrinology*; 347 (1–2): 106–120, 2011
- [16] Hussein AG, Mohamed RH, Alghobashy A “A Synergism of CYP2R1 and CYP27B1 polymorphisms and susceptibility to type 1 diabetes in Egyptian children”. *Cellular Immunology*; 279: 42–45 , 2012
- [17] Onen IH, Ekmekci A, Eroglu M, Konac E, Yesil S ., Biri H,“ Association of genetic polymorphisms in vitamin D receptor gene and susceptibility to sporadic prostate cancer”. *Exp Biol Med (Maywood)*;233:1608–1614, 2008
- [18] H.Arai., K.I. Miyamoto,Y and Taketani et al “ A vitamin D receptor gene polymorphism in the translation initiation codon: effect on protein activity and relation to bone mineral density in Japanese women”. ,*Journal women ,Journal of Bone and Mineral Research* ,vol12,no6 pp.915-921,1997.
- [19] Reitman, S. .,Frankel S.,“ A colorimetric method for the Determination of Serum Glutamate Oxaloacetate and Glutamate Pyruvate Transaminase”. *Am. J. Clin.Path.*28:56- 61,1957..
- [20] DGKC.,“Standardization of Methods for 7measurement of activities in Biological Fluids”. *J.Klin. Chem. Klin. Biochem.* 10: 182,1972
- [21] Walters,M .,Gerade H “ Ultramicromethod for the determination of conjugated and total bilirubin in serum or plasma.”*Microchem J*; 15:231, 1970
- [22] Dumas BT, Watson WA and Biggs HG “Albumin standards and the measurement of serum albumin with bromocresol green”. *J. Clin Chim Acta*; 31(1):87-96,1976.
- [23] Bruix J .,Sherman M.,“ American Association for the Study of Liver Diseases Management of hepatocellular carcinoma”: an update. *Hepatology*; 53:1020–1022, 2011.
- [24] Nahon P, Zucman-Rossi J.,“ Single nucleotide polymorphisms and risk of hepatocellular carcinoma in cirrhosis”.*J Hepatol*;57 :663–674,2012.
- [25] Falletti E, Bitetto D, Fabris C, Cussigh A, Fontanini E, Fornasiere E, Fumolo E, Bignulin S, Cmet S, Minisini R.,“ Vitamin D receptor gene polymorphisms and hepatocellular carcinoma in alcoholic cirrhosis”. *World J Gastroenterol.* ;16 :3016–3024, 2010.

# International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 4, April 2016

- [26] Petta, C. Camma, C. Scazzone, "Low vitamin D serum level is related to severe fibrosis and low responsiveness to interferon-based therapy in genotype 1 chronic hepatitis C," *Hepatology*, 51(4), pp.1158-1167, 2010.
- [27] Dobnig H, Pilz S, Scharnagl H, Renner W, Seelhorst U, Wellnitz B, Kinkeldei J, Boehm BO, Weihrauch G, Maerz W, "Independent association of low serum 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels with all-cause and cardiovascular mortality". *Arch Intern Med*;168:1340–9., 2008.
- [28] Holick MF, "Vitamin D deficiency". *N Engl J Med*. ;357:266–81., 2007 .
- [29] Jenab M, Bueno-de-Mesquita HB, Ferrari P, van Duynhoven FJ, Norat T, Pischon T, Jansen EH, Slimani N, Byrnes G, Rinaldi S, Olsen A, Overvad K, Boutron-Ruault MC, Clavel-Chapelon F, Morois S, Kaaks R, Linseisen J, Boeing H, Bergmann MM, Trichopoulos A, Misirli G, Trichopoulos D, Berrino F, Vineis P, Panico S, Palli D, Tumino R, Ros MM, van Gils CH, Peeters PH, Brustad M, Lund E, Tormo MJ, Ardanaz E, Rodriguez L, Sanchez MJ, Dorronsoro M, Gonzalez CA, Hallmans G, Palmqvist R, Roddam A, Key TJ, Khaw KT, Autier P, Hainaut Pand Riboli E) "Association between pre-diagnostic circulating vitamin D concentration and risk of colorectal cancer in European populations" a nested case-control study. *BMJ*: 340: b5500., 2010..
- [30] Abnet CC, Chen Y, Chow WH, Gao YT, Helzlsouer KJ, Le Marchand L, McCullough ML, Shikany JM, Virtamo J, Weinstein SJ, Xiang YB, Yu K, Zheng W, Albanes D, Arslan AA, Campbell DS, Campbell PT, Hayes RB, Horst RL, Kolonel LN, Nomura AM, Purdue MP, Snyder K and Shu XO, "Circulating 25-hydroxyvitamin D and risk of esophageal and gastric cancer: cohort consortium vitamin D pooling project of rarer cancers". *Am J Epidemiol.*, 172: 94–106., 2010.
- [31] Ahn J, Peters U, Albanes D, Purdue MP, Abnet CC, Chatterjee N, Horst RL, Hollis BW, Huang WY, Shikany JM and Hayes RB, "Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial Project Team Serum vitamin D concentration and prostate cancer risk" a nested case-control study. *J Natl Cancer Inst.*, 100: 796–804, 2008.
- [32] Stolzenberg-Solomon RZ, Jacobs EJ, Arslan AA, Qi D, Patel AV, Helzlsouer KJ, Weinstein SJ, McCullough ML, Purdue MP, Shu XO, Snyder K, Virtamo J, Wilkins LR, Yu K, Zeleniuch-Jacquotte A, Zheng W, Albanes D, Cai Q, Harvey C, Hayes R, Clipp S, Horst RL, Irish L, Koenig K, Le Marchand L and Kolonel LN "Circulating 25-hydroxyvitamin D and risk of pancreatic cancer: cohort consortium vitamin D pooling project of rarer cancers". *Am J Epidemiol.*, 172: 81–93., 2010.
- [33] Albrechtsson E, Jonsson Tand Möller S, et al, "Vitamin D receptor is expressed in pancreatic cancer cells and a vitamin D3 analogue decreases cell number". *Pancreatol*;3:41-60., 2003.
- [34] Wang JB, Abnet CC, Chen W, Dawsey SM, Fan JH and Yin LY: Association between serum 25(OH) vitamin D, incident liver cancer and chronic liver disease mortality in the Linxian Nutrition Intervention Trials: a nested case-control study. *Br J Cancer*; 109:1997-2004., 2013.
- [35] Goma AI, Khan SA, Toledano MB, Waked I and Taylor-Robinson SD, "Hepatocellular carcinoma: Epidemiology, risk factors and pathogenesis. *World J Gastroenterol*"., 14(27): 4300-4308., 2009.
- [36] Lange CM, Miki D, Ochi H, Nischalke H-D and Bojunga J "Genetic Analyses Reveal a Role for Vitamin D Insufficiency in HCV-Associated Hepatocellular Carcinoma Development". *PLoS ONE*;8(5):e64053 2013., 2012.
- [37] Hammad LN, Abdelraouf SM, Hassanein FS, Mohamed WA and Schaalan MF, "Circulating IL-6, IL-17 and vitamin D in hepatocellular carcinoma: potential biomarkers for a more favorable prognosis? *J Immunotoxicol.*, 10: 380- 386., 2013.
- [38] Mansoor S, Habib A, Ghani F, Fatmi Z, Badruddin S, Mansoor S, Siddiqui I and Jabbar A., "Prevalence and significance of vitamin D deficiency and insufficiency among apparently healthy adults". *Clinical Biochemistry Journal*; CLB07508:5; 4c. ,2010.
- [39] Artaza JN, Norris KC, "Vitamin D reduces the expression of collagen and key profibrotic factors by inducing an antifibrotic phenotype in mesenchymal multipotent cells." *J Endocrinol*, 200:207-221., 2009.
- [40] Garcia de Leon, Model C, Montfort I, Tello Montes E, et al., "Hepatocyte production of modulators of extracellular liver matrix in normal and cirrhotic rat liver." *Exp Mol Pathol*, 80(1):97-108., 2006.
- [41] Gascon-Barré M, Demers C, Mirshahi A, Néron S, Zalzal S, Nanci A., "The normal liver harbors the vitamin D nuclear receptor in nonparenchymal and biliary epithelial cells". *Hepatology* ;37:1034–1042., 2003.
- [42] Han S, Li T, Ellis E, Strom S, Chiang JY. "A novel bile acid-activated vitamin D receptor signaling in human hepatocytes". *Mol Endocrinol*; 24:1151–1164. ,2010.
- [43] M. Gal-Tanamy, L. Bachmetov, A. Ravid, R. Koren, A. Erman, R. Tur-Kaspa, et al., Vitamin D an innate antiviral agent suppressing hepatitis C virus in human hepatocytes *Hepatology*, 54, pp. 1570–1579 ,2011.
- [44] Targher G, Bertolini L, Scala L, Cigolini M, Zenari L, Falezza G. et al., 00000 "Associations between serum 25-hydroxyvitamin D3 concentrations and liver histology in patients with non-alcoholic fatty liver disease". *Nutr Metab Cardiovasc Dis*, 2007.