S&S

Senses Sci 2019: 6 (3) 847-863
doi: 10.14616/sands- 2019-6-847863

Article

The Serum vitamin D deficiency and vitamin D receptor gene polymorphism as risk factors for hepatitis C virus related hepatocellular carcinoma: a case control study

Walaa N. Roushdy[1]*, Ahmed N. Abdel Aal[1], Ehsan M. Abdelrahman[1], Ahmed E. Zeid[2], Rasha A. Nassra[1]

[1] Medical Biochemistry Department, Alexandria Faculty of Medicine, Egypt

[2] Internal Medicine Department, Alexandria Faculty of Medicine, Egypt

*Correspondence: Walaa N. Roushdy. Medical Biochemistry Department, Alexandria Faculty of Medicine, Alexandria, Egypt. E-mail address: Walaa.roushdy@alexmed.edu.eg; walaanr@gmail.com.

Abstract. *Background:* Hepatocellular carcinoma (HCC) is the most frequent primary cancer of the liver. In Egypt, there is a doubling in the incidence rate in the past 10 years. The screening of HCC still relies on the serum alpha-fetoprotein (AFP) level, which is ineffective in detecting small tumors. Therefore, novel and reliable diagnostic biomarkers associated with increased risk of HCC would better define high-risk populations, helping to improve prevention and treatment strategies. Vitamin D is a steroid hormone implicated in inhibition of carcinogenesis. Genetic variations in the vitamin D receptor (VDR) gene have been reported to associate with increased risk of cancers. The aim of this study is to evaluate the role of serum vitamin D level and VDR polymorphism as predictors of HCC risk in the Egyptian hepatitis C virus (HCV) patients.
Methods: Venous blood samples were collected from 60 patients and 30 healthy subjects Patients were categorized into 2 groups: 30 patients with HCV-related HCC, and 30 patients with HCV-related cirrhosis without HCC. Serum levels of AFP and 25-OH D3 were measured using enzyme-linked immunosorbent assay (ELISA). DNA was extracted from whole blood and genotyping for VDR rs2228570 gene polymorphism was done using real time PCR, TaqMan assay.
Results: The mean serum vitamin D levels in HCC patients, cirrhotic patients, and control subjects were 15±7.78, 24.24±9.17 and 28.34±12.18 ng/ml, respectively. There was a significant decrease in the mean serum vitamin D level in HCC patients compared to cirrhotic patients (*p=0.001*) and

control subjects *(p<0.001*). Also, low serum vitamin D level was significantly correlated with advanced cancer stage (*p*<0.001). In HCC patients, the frequencies of VDR rs2228570 AA, AG and GG genotypes were 6.7%, 40% and 53.3% respectively. There was significant association of the VDR rs2228570 GG genotype with HCC cases (*OR=3.14*, *P=0.035*) when compared with controls.
Conclusion: Low serum vitamin D level and VDR rs2228570 polymorphism may contribute to increased susceptibility to HCV-related HCC.

Keywords: Hepatitis C virus, hepatocellular carcinoma, vitamin D deficiency, VDR, SNP.

Introduction

Hepatocellular carcinoma (HCC) is a major challenge in contemporary medicine, with an incidence of 1 million cases per year. It has a poor prognosis and most patients die within 1 year of diagnosis.[1]

In Egypt, HCC is the second most common cancer in men and the sixth in women. The most important risk factor for HCC development is cirrhosis of whatever cause, mostly chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections.[2] Egypt has one of the highest prevalence rates of HCV infection in the world. Viral hepatitis infections are associated with increased oxidative stress in hepatocytes. This leads to DNA changes, instability and genetic polymorphisms of various genes which may increase the risk of liver cirrhosis and/or HCC development.[3]

Current screening of HCC relies on clinical information, liver imaging and measurement of serum alpha-fetoprotein (AFP). AFP concentrations are directly correlated with tumor size, so it is not sufficient for early diagnosis (tumors <or=2cm). In addition, raised AFP concentrations in serum were found in many patients with non-malignant chronic liver disease. Tumors other than HCC may also have markedly increased AFP levels. So, the discovery of effective markers for early diagnosis of HCC could decrease the HCC-related cancer mortality worldwide.[4]

Vitamin D is a steroid hormone that regulates the expression of about 900 different genes, through binding to an intracellular nuclear transcription factor; the vitamin D receptor (VDR), causing its dimerization with the retinoid X receptor (RXR). Following translocation to the nucleus, the ligand-bound VDR–RXR complex binds to vitamin D response elements (VDREs) in the regulatory regions of the target genes. This causes the recruitment of co-activators or co-repressors, leading to positive or negative transcriptional regulation of gene expression.[5]

Vitamin D deficiency has become a major interest after the discovery of great extent of populations suffering with its various health consequences. Reports showed that most world's population are not getting sufficient amount of vitamin D due to the current lifestyle and environmental factors that limit sunlight exposure.[6]

Recent studies about vitamin D indicated that it has anticarcinogenic effects, mediated by different mechanisms including the regulatory effect on cell cycle, proliferation, apoptosis, invasion and angiogenesis.[5]

The discovery of VDR in many cell types along with the demonstration that 1,25(OH)$_2$D altered the function of these tissues has markedly increased the evidence of the anti-tumor effects of 1,25(OH)$_2$D.[7] More than four hundred and fifty polymorphisms within the VDR gene have been reported. These polymorphisms influence the binding of vitamin D to the VDR, affecting the VDR-mediated signaling pathways of vitamin D.[8] It has been found that SNPs in the VDR gene are associated with various tumors such as prostate,[9] breast,[10] ovary,[11] skin,[12] colon and rectum,[13] and kidneys.[14]

Vitamin D has many direct effects on the liver (e.g. anti-fibrotic and expression of detoxifying enzymes). Cirrhotic patients often have low serum vitamin D levels. The liver is the site of 25-OH vitamin D production from vitamin D.[15] In patients with cirrhosis, low vitamin D levels may be caused by the decreased number of hepatocytes, reduced exposure to sunlight, decreased adipose tissue, malabsorption of vitamin D and altered hydroxylation of vitamin D in the liver.[16] It was proved that low serum vitamin D levels are associated with increased liver fibrosis in patients with non-alcoholic steatohepatitis, and reported to be associated with high risk of HCC development.[17,18] Moreover, the serum vitamin D level had been reported to affect the natural course and treatment response of chronic hepatitis C.[19] Higher vitamin D concentrations were proved to be associated with better prognosis and improved outcomes of liver diseases.[5] Despite the reported mechanisms supporting the beneficial effects of vitamin D supplementation, the total benefits of its supplementation remain ambiguous.

It is known that the function of VDR is affected by single-nucleotide polymorphisms (SNPs) in VDR gene. There are several polymorphisms within the VDR gene. The most common polymorphisms are Taq-I (rs731236), Bsm-I (rs1544410), Fok-I (rs2228570), and Apa-I (rs7975232).[20] The *VDR* rs2228570 SNP G>A (C>T) results in the substitution of a methionine, encoded by ATG, for a threonine, encoded by ACG at position 1 of the protein (Thr1>Met). This leads to production of a three amino acids longer VDR protein, resulting in less transcriptional activity and decreased VDR potency. It has been assumed that a reduced activity of VDR could be associated with increased susceptibility to cancer risk and or more aggressive disease.[21]

VDR gene polymorphisms have been described in several chronic liver diseases such as liver cirrhosis,[22] autoimmune hepatitis,[23] primary sclerosing cholangitis[24] and primary biliary cirrhosis.[25] Moreover, a significant association between VDR gene polymorphisms and the occurrence of HCC has been reported in alcoholic liver cirrhosis in Caucasian subjects,[26] in HBV-infected patients[27] and even in patients with chronic HCV.[28] However, the mechanisms describing the possible association between VDR gene polymorphisms and HCC development are scarce and inconclusive. In the current study, the possible association between serum 25-Hydroxy- D3 (25-OHD) concentrations and VDR gene polymorphism and risk of HCC development in Egyptian HCV patients was investigated.

Material and methods

Sixty HCV infected patients recruited from the Hepatobiliary Unit, Department of Internal Medicine, Faculty of Medicine, University of Alexandria were included in the study. They were categorized into 2 groups: 30 patients with HCV-related HCC, and 30 patients with HCV-related cirrhosis without HCC. Also, 30 healthy subjects were included as a control group. The study was approved by the Ethics Review Board of the Faculty of Medicine, Alexandria University (IRB NO: 00007555) according to the declaration of Helsinki. A written informed consent was obtained from all subjects. The severity of liver disease was graded according to Child-Pugh classification.[29] The HCC stage was assessed according to the Barcelona-Clinic Liver Cancer (BCLC) staging system,[30] and HCC patients were classified according to BCLC stage into early (stages 0 and A), intermediate (stage B) and late HCC (stages C and D).

1. Sampling

Five ml venous blood were collected from every subject and divided into two aliquots. The first in centrifuge tube without anticoagulant to separate serum by centrifugation for 15 minutes at 8000 rpm for biochemical analysis. Hemolyzed samples were discarded. The second aliquot was transferred into EDTA tube for DNA extraction. All samples were stored at -20º C till analysis.
Routine laboratory investigations were performed including complete blood picture, serum alanine and aspartate aminotransferases (ALT and AST), total and direct bilirubin, albumin, gamma glutamyl transpeptidase (GGT), and prothrombin time (PT)/international normalized ratio (INR). Hepatitis virus markers were assayed including HCV antibody and hepatitis B surface antigen (HBsAg) using enzyme-linked immunosorbent assay (ELISA), and serum HCV RNA and HBV DNA levels using real time polymerase chain reaction.

2. Determination of serum levels of alpha-fetoprotein (AFP) and 25-OH D3:

Serum AFP and 25-OH D3 levels were measured using solid phase enzyme-linked immunosorbant assay (ELISA) following the manufacturer's instructions (Diagnostic Automation /Cortez Diagnostics, Inc. California, USA,[31] and Bioassay Technology Laboratory, Shanghai, China [32] respectively). Two non HCC cirrhotic patients showed high serum AFP levels, but HCC was excluded by triphasic CT and MRI.

3. Vitamin D receptor (VDR) SNP Genotyping Assay: [33]

DNA was purified from whole blood samples using a spin column protocol (QIAamp DNA Blood Mini Kit, Qiagen, Hilden, Germany).[34] NanoDrop 2000 (Thermoscientific; USA) was used to check DNA quality and quantity.

Genotyping for VDR single nucleotide polymorphism (rs2228570) was performed using 40x TaqMan® Predesigned SNP Genotyping Assay (Thermo Fisher Scientific, Waltham, Massachusetts, USA), diluted to a 20X working solution with nuclease free water. The reaction mix was composed of 1μL of 20X Assay Working Solution, 10μL of TaqMan® Genotyping Master Mix and 6μL of nuclease-free water. The final reaction volume per well was 20 μL (17 μL reaction mix + 3 μL DNA sample). The total reaction volume uses 20 ng of genomic DNA. The context sequence of VDR (rs2228570) SNP is: GGAAGTGCTGGCCGCCATTGCCTCC[A/G]TCCCTGTAAGAACAGCAAGCAGGCC. The A allele was detected with VIC® dye and the G allele with FAM™ dye.

Real time PCR was performed using Applied Biosystems StepOne™ Real-Time PCR System. The PCR cycles were as follows: 95˚C for 10 min, 40 cycles of denaturation at 95 ˚C for 15 sec each, annealing at 60ºC for 1 min, extension at 60ºC for 1 min. After the last PCR cycle, a final extension step for 2 min at 60ºC was performed.

Statistical analysis [35]

Data were analyzed using IBM Statistical Package for Social Sciences 20.0 (SPSS, Chicago, IL). Qualitative data were described using number and percent. Quantitative data were described using Range (minimum and maximum), mean, standard deviation and median. Comparison between different groups regarding categorical variables was tested using Chi-square test. When more than 20% of the cells have expected count less than 5, correction for chi-square was conducted using Fisher's Exact test or Monte Carlo correction.

The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-Wilk test and D'Agostino test, also Histogram and QQ plot were used for vision test. For normally distributed data, comparison between the three studied groups was analyzed using F-test (ANOVA) and Post Hoc test (Tukey HSD) for pair-wise comparisons, while for abnormally distributed data Kruskal Wallis test was used to compare between different groups and Mann-Whitney Test was assessed for pair-wise comparisons. Significance of the obtained results was judged at the 5% level.

The sensitivity and specificity of serum AFP and 25-OH vitamin D levels in discriminating cirrhotic and HCC patients were assessed by plotting a receiver-operating characteristic (ROC) curve and determining cut-off values. For SNP, the population of the studied sample was explored to find its equilibrium with Hardy-Weinberg equation (HWE) using the $\chi 2$ test. Odd ratio (OR) was used to calculate the ratio of the odds and 95% Confidence Interval (95%CI) of an event occurring in one risk group to the odds of it occurring in the non-risk group.

Results

1. Serum AFP and Vitamin D levels (ng/ml):

The serum level of AFP in HCC patients ranged between 9.7 and 1167 ng/ml with a mean of 398.45 ± 287.6 ng/ml, while in cirrhotic patients, it ranged between 9 – 250 ng/ml with a mean of 78.28 ± 84.93 ng/ml. In healthy (control) subjects, it varied from 2 to 8.5 ng/ml with a mean of 4.11 ± 1.87 ng/ml. The mean serum AFP level was significantly higher in HCC and cirrhotic patients compared to control subjects (p <0.001). Also it was significantly higher in HCC patients than in cirrhotic patients (p =0.001).

Serum vitamin D level ranged in HCC patients between 2.1 and 31.1 ng/ml with a mean value of 15±7.78 ng/ml, while in cirrhotic patients, it ranged between 7.6 and 40.1 ng/ml with a mean value of 24.24 ± 9.17 ng/ml. In control subjects, its range was between 6.3 and 52.3 ng/ml with a mean value of 28.34 ± 12.18 ng/ml. There was a significant decrease in the mean serum vitamin D level in HCC patients compared to cirrhotic patients and control subjects (p=0.001 p <0.001 respectively), while the difference between cirrhotic patients and control subjects did not reach statistical significance (p =0.272).

2. VDR rs2228570 Single Nucleotide Polymorphism (Table 1)

Vitamin D receptor (VDR) rs2228570 SNP genotyping assay showed that A was the minor allele with minor allele frequency (MAF) representing 38.3% in the studied population. Thirteen subjects in the study population carried the (A/A) genotype representing 14.4%, 37 subjects had the (G/G) genotype representing 41.1 % while 40 subjects carried the heterozygote (A/G) genotype representing 44.4 %.

As shown in table (1), statistical comparison between HCC patients and control subjects showed that the (G/G) genotype was associated with higher risk for HCC (p=0.035). While comparison between cirrhotic patients and control subjects showed that the (A/G) genotype was associated with higher risk for cirrhosis (p=0.038).

3. Risk analysis for VDR genotype with HCC (Table 1)

The association of all VDR rs2228570 genotypes with the risk of cirrhosis and HCC was analyzed using odds ratio (OR) with confidence interval of 95% (95%CI). When OR was studied in HCC patients versus (vs) cirrhosis group and vs control groups, VDR G/G genotype was found to be associated with an increased risk of HCC. While A/A genotype was associated with a decreased risk of HCC when studied vs cirrhosis. And A/G genotype was associated with a decreased risk of HCC when studied vs control. When OR was studied in cirrhosis patients vs control group, VDR G/G genotype was found to be associated with an increased risk of cirrhosis. While A/G genotype was associated with a decreased risk of cirrhosis.

Table (1): Comparison between the three studied groups according to VDR genotypes, with risk analysis using Odds ratio with 95%CI.

	HCC (n = 30)		Cirrhosis (n = 30)		Healthy subjects (n = 30)		p_1	p_2	p_3	OR1 (95% C.I)	OR2 (95% C.I)	OR3 (95% C.I)
	No.	%	No.	%	No.	%						
VDR genotype												
AA	2	6.7	7	23.3	4	14.3	0.145	0.671	0.317	0.23 (0.04 – 1.24)	0.46 (0.07 – 2.75)	1.98 (0.51 – 7.64)
AG	12	40.0	10	33.3	18	60.0	0.592	0.121	0.038*	1.33 (0.47 – 3.82)	0.44 (1.16 – 1.25)	0.33* (0.12 – 0.96)
GG	16	53.3	13	43.3	8	26.7	0.438	0.035*	0.176	1.49 (0.54 – 4.14)	3.14* (1.07 – 9.27)	2.10 (0.71 – 6.22)
Allele frequency												
A	16	26.7	27	45.0	26	43.3	0.036*	0.056	0.854			
G	44	73.3	33	55.0	34	56.7						

HCC: hepatocellular carcinoma

VDR: vitamin D receptor

P value for Chi square test for comparing between the studied groups

p_1: p value for comparing between HCC and Cirrhosis

p_2: p value for comparing between HCC and control

p_3: p value for comparing between Cirrhosis and control

*: Statistically significant at $p \leq 0.05$

OR1: Odd`s ratio (HCC vs Cirrhosis)

OR2: Odd`s ratio (HCC vs Control)

OR3: Odd`s ratio (Cirrhosis vs control)

CI: Confidence interval

4. Correlation between serum vitamin D level and Child Pugh class and BCLC stage in the HCC and cirrhosis groups: (Table 2, Figures 1,2)

In HCC patients, serum vitamin D level showed a significant negative correlation with the Child Pugh class (r_s= -0.440, *p=0.015*) and the BCLC stage (r_s= -0.801, *p* <0.001).

Table (2): Serum vitamin D level, Child-Pugh class and BCLC stage in HCC and cirrhosis groups.

Serum vitamin D (ng/ml)	**Child-Pugh class**			**H**	**p**
	A	**B**	**C**		
HCC group (n= 30)	**(n=14)**	**(n=13)**	**(n=3)**		
Range	3.50 – 31.10	3.20 – 23.40	2.10 – 10.10	6.789*	0.015*
Mean ± SD.	17.85 ± 7.94	13.99 ± 6.70	6.10 ± 4.0		
Median	15.95	13.80	6.10		
Cirrhosis group (n =30)	**(n = 9)**	**(n = 14)**	**(n = 7)**		
Range	8.10 – 35.20	11.0 – 40.10	7.60 – 39.20	2.209	0.331
Mean ± SD.	20.90 ± 9.60	26.29 ± 8.18	24.43 ± 10.59		
Median	22.70	26.65	25.60		
	BCLC stage			**H**	**p**
	Early (stages 0,A) (n=8)	**Intermediate (Stage B) (n=12)**	**Late (Stages C,D) (n=10)**		
HCC group (n= 30)					
Range	15.60 – 31.10	3.50 – 23.50	2.10 – 14.80	17.668*	<0.001*
Mean ± SD.	23.36 ± 5.07	15.44 ± 5.76	7.79 ± 3.78		
Median	21.90	15.35	7.60		

HCC: hepatocellular carcinoma

BCLC: Barcelona-Clinic Liver Cancer

H: H for **Kruskal Wallis test,** Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Dunn's for multiple comparisons test)**

p: p value for comparing between the three categories

*: Statistically significant at p ≤ 0.05

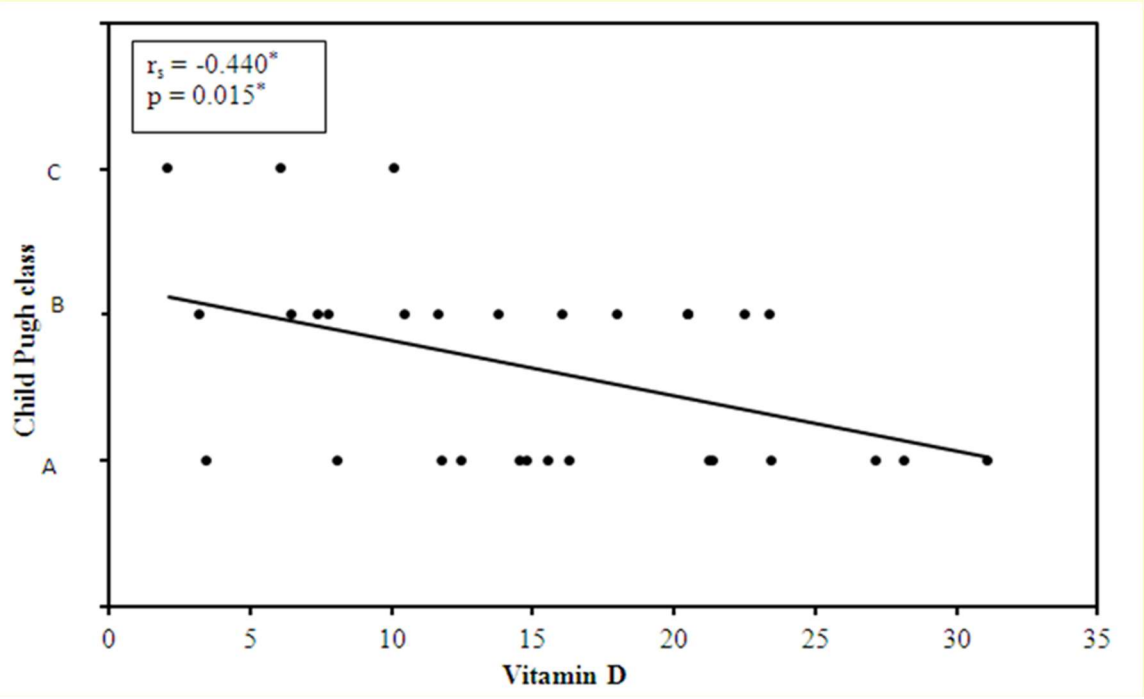

Figure (1): Correlation between serum vitamin D level and Child-Pugh class in HCC patients.

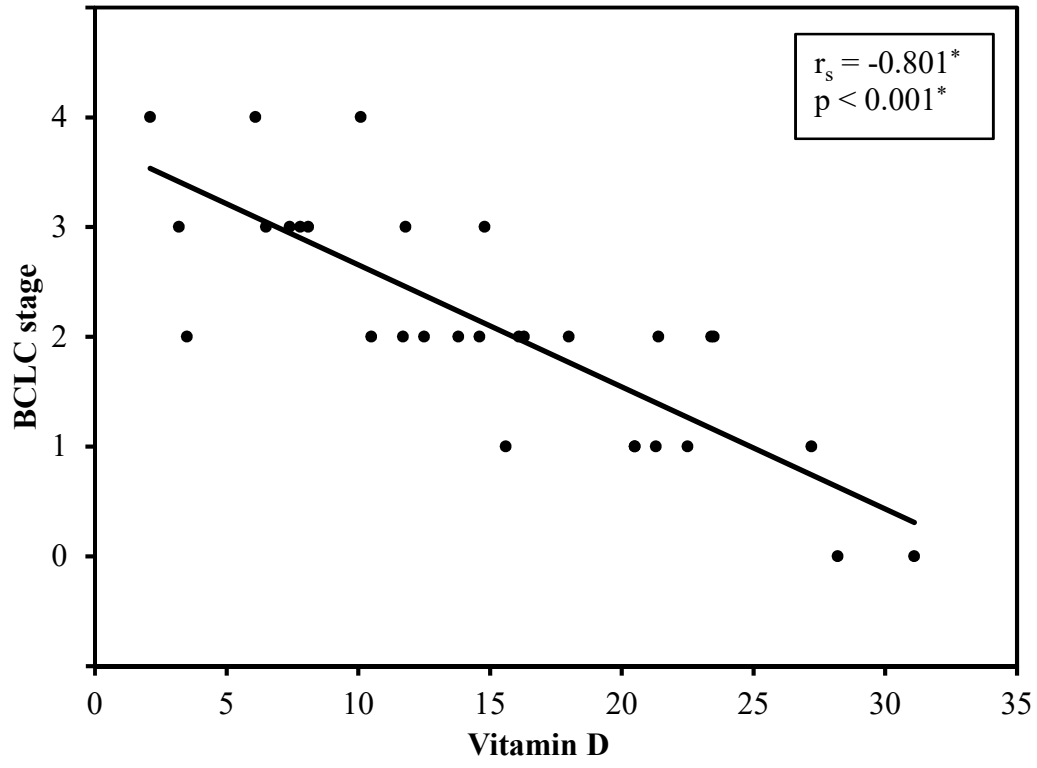

Figure (2): Correlation between serum vitamin D level and BCLC stage in HCC patients.

5. Diagnostic performance of AFP and vitamin D serum levels in HCC cases vs cirrhosis (Figure 3)

Receiver-Operating Characteristic (ROC) curve analysis was applied to assess the diagnostic performance of serum AFP and vitamin D in HCC patients versus the cirrhosis group. It revealed that serum AFP at cut-off level of 45 ng/ml could significantly predict the occurrence of HCC with a diagnostic sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of 90%, 63.33%, 71.1%, and 86.4% respectively. While for serum vitamin D, sensitivity was 83.33%, specificity 66.67%, PPV 71.4% and NPV 80% at cut-off level of 22.5 ng/ml. Combined together, serum AFP and vitamin D diagnostic sensitivity, specificity, PPV and NPV were 93.33%, 76.67%, 80% and 92% respectively.

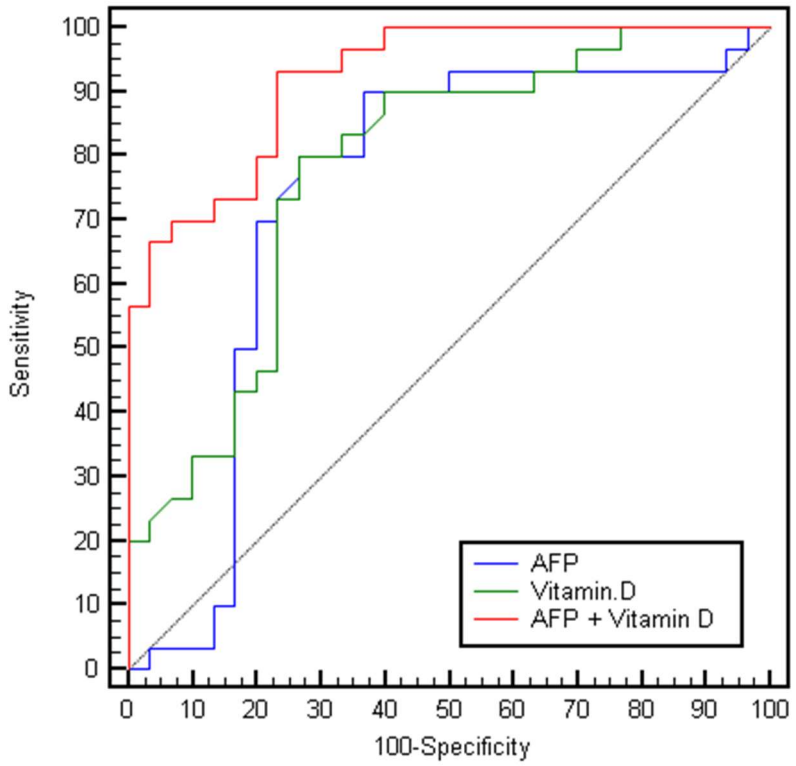

Figure (3): ROC curve for serum AFP and vitamin D to diagnose HCC (predict HCC cases vs cirrhosis)

Discussion

Hepatocellular carcinoma (HCC) is one of the commonest malignant tumors worldwide and the third leading cause of cancer death.[36] The prognosis of HCC is mostly poor, because of late detection at an advanced, non-resectable stage. Thus, more effective screening strategies should be used for early detection of HCC in the high risk population.[37]

Most of the effects of active vitamin D in many tissues are mediated by VDR. Vitamin D has potent antitumor effects. In addition, it was reported that the VDR plays an important role in regulating cell proliferation, differentiation and induction of apoptosis.[38]

It has been reported that several single nucleotide polymorphisms (SNPs) in the VDR gene are associated with various malignancies,[9-14] but these observations are conflicting. However, the association of vitamin D and HCC is still inconclusive.[39]

In the current study, there was a significant decrease in the mean serum vitamin D level in HCC patients compared to non HCC cirrhotic patients and healthy controls, while the difference between non HCC cirrhotic patients and healthy controls did not reach statistical significance. Moreover, the correlations between serum vitamin D level and VDR gene polymorphism and the severity of chronic liver diseases and the HCC stage were also investigated in the current study.

There was a significant negative correlation between serum vitamin D level and the Child Pugh class and the BCLC stage in HCC patients. The ROC curve was applied to assess the diagnostic performance of serum AFP and vitamin D levels in differentiating between cirrhotic patients with and without HCC. It revealed that combined serum AFP and vitamin D at cut off values of 45 ng/ml and 22.5 ng/ml respectively, showed the best sensitivity and specificity in detecting HCC (AUC=0.921, P<0.001 and 95%CI=0.858-0.984). Statistical comparison between HCC and control groups showed that the (G/G) genotype was associated with higher risk for HCC. While comparison between cirrhosis and control groups showed that the (A/G) genotype was associated with lower risk for cirrhosis.

Similar to our finding in Egyptian patients, Peng et al. investigated the association between VDR gene polymorphisms and HBV-related HCC risk in a Chinese population. This study found that rs2228570 TT and TC genotypes were associated with a significant increased HBV-related HCC risk when compared with the wild-type CC homozygote.[27]

In addition, a previous study investigated the association between several VDR gene polymorphisms and HCC development and severity in Egyptians with Chronic Hepatitis B (CHB). Patients carrying FokI TT genotype had significantly higher risk for HCC. Also, FokI TT genotype was associated with advanced cancer stage, and lymph node metastasis. On the other hand, other SNPs of VDR gene at TaqI, BsmI and ApaI loci were not associated with HCC development.[40] These findings are in accordance to those obtained by Yao et al. who revealed that FokI polymorphism could be used as a molecular marker to predict the risk of HCC development and severity in patients infected with HBV. [41]

On contrary to our findings, a previous study assessed the relationship between VDR FokI C>T polymorphism and HCC in Egyptian patients with chronic liver diseases (CLD). VDR FokI genotypes and allele frequencies were neither significantly associated with HCC, HCV nor were risk for progression of chronic liver disease into decompensated liver disease.[42] Similar result were obtained by Falleti et al. who studied the association between VDR gene polymorphisms and HCC in alcoholic cirrhosis and found that FokI C>T polymorphism was non-significantly distributed between HCC and non HCC patients.[26]

The discrepancies in the results of such studies usually reveal ethnic differences since Fan et al. found the distribution of VDR FokI, genotypes significantly differed between Chinese and Caucasian populations.[23]

Several studies also investigated different VDR gene polymorphisms and serum vitamin D level in HCC patients. The association between the VDR gene polymorphisms (BsmI, ApaI, and TaqI) and HCC risk and severity in Egyptian patients with chronic hepatitis C has been investigated and showed that the prevalence of HCC was significantly higher in patients carrying ApaI CC genotype, who also showed advanced stage of liver cirrhosis and lower serum 25-OH D3 concentrations. Also, an evidence for contribution of reduced serum levels of 25-OH D3 to HCV-related HCC was demonstrated in this study. Thus, the determination of VDR genetic variations in HCV patients could help in identification of patients at risk of HCC development. On the other hand, BsmI and TapqI polymorphisms showed no significant associations with the development and severity of the disease.[43]

Another study investigated the possible association between both plasma vitamin D levels and VDR gene ApaI polymorphism and HCC in a Chinese population with chronic HCV infection. The study showed that both were significantly associated with rapid fibrosis progression rate and the presence of cirrhosis. Patients with HCC had a higher frequency of ApaI CC genotype as compared to patients with chronic hepatitis and cirrhosis and control subjects.[44] VDR gene ApaI polymorphism also could genetically predispose to low serum 25-OHD3 levels.[45] Similarly, a study on the association between VDR gene ApaI polymorphism and HCV-related HCC development in Egyptian population revealed significant statistical difference between patients with HCC and those with liver cirrhosis and control group. This result suggested that VDR gene ApaI polymorphism could be associated with increased risk of HCV-related HCC development in Egyptian population.[46]

Also, it had been previously reported that reduced serum levels of 25-OH D3 were linked to the HCC development; nevertheless, the causal relationships remained unclear because of the small sample size of these studies or the false positive associations due to impaired liver functions at the date of HCC.[47]

In an Italian study, BsmI GG genotype and TaqI TT genotype were associated with higher HCC occurrence rate among patients who underwent liver transplantation due to liver cirrhosis caused by HCV, HBV or alcoholic liver disease.[26]

However, Huang et al. demonstrated that VDR gene polymorphisms at BsmI, ApaI, and TaqI were associated with distinct clinical phenotypes of hepatitis B carriers in Taiwanese patients but not with the risk of HCC suggesting a limited role of VDR gene polymorphisms in hepatocarcinogenesis.[48] However, a biochemical evidence clearly indicated that HCC cells respond to the inhibitory effect of vitamin D and its analogs.[49] Moreover, it had been reported that the antiproliferative effects of vitamin D against HCC cells correlate with intracellular VDR level.[50]

The limitation in the current study is in its nature as a hospital based study with limited financial resources, relatively small sample size, and with no previous information about the VDR polymorphisms available at the time of the study. The cohort consisted of only Egyptian patients and thus results could not be generalized to other population, particularly ethnic differences have been shown in the allelic frequencies in VDR polymorphisms.[20]

Conclusion

Based on the results of the present study, it can be concluded that low serum vitamin D level and VDR rs2228570 polymorphism may contribute to increased susceptibility to HCV-related HCC, a finding that could have a potential preventive and therapeutic applications in the future. Serum vitamin D level could be a potential biomarker for early diagnosis of HCC especially when combined with AFP.

Large population-based prospective studies with ethnically diverse populations are warranted to further elucidate the impact of VDR SNPs on HCC susceptibility. Also, further controlled clinical trials are justified to evaluate the impact of vitamin D supplementation on HCC risk and overall survival in patients with chronic HCV.

References

1. Guthle M, Dollinger MM. Epidemiology and risk factors of hepatocellular carcinoma. Der Radiologe. 2014;54(7):654-9.
2. Soliman AS, Hung CW, Tsodikov A, et al. Epidemiologic risk factors of hepatocellular carcinoma in a rural region of Egypt. Hepatol Int. 2010;4(4):681-90.
3. Nault JC. Pathogenesis of hepatocellular carcinoma according to aetiology. Best Pract Res Clin Gastroenterol. 2014;28(5):937-47.
4. Marrero JA. Screening tests for hepatocellular carcinoma. Clinics in liver disease. 2005;9(2):235-51, vi.
5. Krishnan AV, Feldman D. Mechanisms of the anti-cancer and anti-inflammatory actions of vitamin D. Annu Rev Pharmacol Toxicol. 2011;51:311-36.
6. Azarpeykan S, Dittmer KE, Gee EK, et al. Influence of blanketing and season on vitamin D and parathyroid hormone, calcium, phosphorus, and magnesium concentrations in horses in New Zealand. Domest Anim Endocrinol. 2016;56:75-84.
7. Peehl DM, Skowronski RJ, Leung GK, Wong ST, Stamey TA, Feldman D. Antiproliferative effects of 1,25-dihydroxyvitamin D3 on primary cultures of human prostatic cells. Cancer Res. 1994;54(3):805-10.
8. Bai YH, Lu H, Hong D, Lin CC, Yu Z, Chen BC. Vitamin D receptor gene polymorphisms and colorectal cancer risk: a systematic meta-analysis. World J Gastroenterol. 2012;18(14):1672-9.
9. Atoum MF, AlKateeb D, AlHaj Mahmoud SA. The Fok1 vitamin D receptor gene polymorphism and 25(OH) D serum levels and prostate cancer among Jordanian men. Asian Pac J Cancer Prev. 2015;16(6):2227-30.
10. Shaikh F, Baig S, Jamal Q. Do VDR Gene Polymorphisms Contribute to Breast Cancer? Asian Pac J Cancer Prev. 2016;17(2):479-83.

11. Mohapatra S, Saxena A, Gandhi G, Koner BC, Ray PC. Vitamin D and VDR gene polymorphism (FokI) in epithelial ovarian cancer in Indian population. J Ovarian Res. 2013;6(1):37.
12. Orlow I, Reiner AS, Thomas NE, et al. Vitamin D receptor polymorphisms and survival in patients with cutaneous melanoma: a population-based study. Carcinogenesis. 2016;37(1):30-8.
13. Budhathoki S, Yamaji T, Iwasaki M, et al. Vitamin D Receptor Gene Polymorphism and the Risk of Colorectal Cancer: A Nested Case-Control Study. PLoS One. 2016;11(10):e0164648.
14. Yang C, Li J, Li Y, et al. The vitamin D receptor gene ApaI polymorphism is associated with increased risk of renal cell carcinoma in Chinese population. Sci Rep. 2016;6:25987.
15. Arteh J, Narra S, Nair S. Prevalence of vitamin D deficiency in chronic liver disease. Dig Dis Sci. 2010;55(9):2624-8.
16. Pappa HM, Bern E, Kamin D, Grand RJ. Vitamin D status in gastrointestinal and liver disease. Curr Opin Gastroenterol. 2008;24(2):176-83.
17. Barchetta I, Angelico F, Del Ben M, et al. Strong association between non alcoholic fatty liver disease (NAFLD) and low 25(OH) vitamin D levels in an adult population with normal serum liver enzymes. BMC Med. 2011;9:85.
18. Fedirko V, Duarte-Salles T, Bamia C, et al. Prediagnostic circulating vitamin D levels and risk of hepatocellular carcinoma in European populations: a nested case-control study. Hepatology. 2014;60(4):1222-30.
19. Petta S, Camma C, Scazzone C, et al. Low vitamin D serum level is related to severe fibrosis and low responsiveness to interferon-based therapy in genotype 1 chronic hepatitis C. Hepatology. 2010;51(4):1158-67.
20. Uitterlinden AG, Fang Y, Van Meurs JB, Pols HA, Van Leeuwen JP. Genetics and biology of vitamin D receptor polymorphisms. Gene. 2004;338(2):143-56.
21. Gnagnarella P, Pasquali E, Serrano D, Raimondi S, Disalvatore D, Gandini S. Vitamin D receptor polymorphism FokI and cancer risk: a comprehensive meta-analysis. Carcinogenesis. 2014;35(9):1913-9.
22. Ramos-Lopez O, Martinez-Lopez E, Roman S, Fierro NA, Panduro A. Genetic, metabolic and environmental factors involved in the development of liver cirrhosis in Mexico. World J Gastroenterol. 2015;21(41):11552-66.
23. Fan L, Tu X, Zhu Y, et al. Genetic association of vitamin D receptor polymorphisms with autoimmune hepatitis and primary biliary cirrhosis in the Chinese. J Gastroenterol Hepatol. 2005;20(2):249-55.
24. Kempinska-Podhorodecka A, Milkiewicz M, Jablonski D, Milkiewicz P, Wunsch E. ApaI polymorphism of vitamin D receptor affects health-related quality of life in patients with primary sclerosing cholangitis. PLoS One. 2017;12(4):e0176264.
25. Fang F, Wang J, Pan J, Su GH, Xu LX, Li G. Relationship between vitamin D (1,25-dihydroxyvitamin D3) receptor gene polymorphisms and primary biliary cirrhosis risk: a meta-analysis. Genet Mol Res. 2015;14(1):981-8.
26. Falleti E, Bitetto D, Fabris C, et al. Vitamin D receptor gene polymorphisms and hepatocellular carcinoma in alcoholic cirrhosis. World J Gastroenterol. 2010;16(24):3016-24.

27. Peng Q, Yang S, Lao X, et al. Association of single nucleotide polymorphisms in VDR and DBP genes with HBV-related hepatocellular carcinoma risk in a Chinese population. PLoS One. 2014;9(12):e116026.
28. Hung CH, Chiu YC, Hu TH, et al. Significance of vitamin d receptor gene polymorphisms for risk of hepatocellular carcinoma in chronic hepatitis C. Transl Oncol. 2014;7(4):503-7.
29. Nakagawa S, Hayashi H, Nitta H, et al. Scoring system based on tumor markers and Child-Pugh classification for HCC patients who underwent liver resection. Anticancer Res. 2015;35(4):2157-63.
30. Adhoute X, Penaranda G, Bronowicki JP, Raoul JL. Usefulness of the HKLC vs. the BCLC staging system in a European HCC cohort. J Hepatol. 2015;62(2):492-3.
31. Hirai H, Miyaji Tr, Nihon Gan Gakkai. Alpha-fetoprotein and hepatoma. Baltimore,: University Park Press; 1973. viii, 320 p. p.
32. de Lyra EC, da Silva IA, Katayama ML, et al. 25(OH)D3 and 1,25(OH)2D3 serum concentration and breast tissue expression of 1alpha-hydroxylase, 24-hydroxylase and Vitamin D receptor in women with and without breast cancer. J Steroid Biochem Mol Biol. 2006;100(4-5):184-92.
33. Wrzosek M, Jakubczyk A, Wrzosek M, et al. Association between Fok I vitamin D receptor gene (VDR) polymorphism and impulsivity in alcohol-dependent patients. Mol Biol Rep. 2014;41(11):7223-8.
34. Scherczinger C, Bourke M, Ladd C, Lee H. DNA extraction from liquid blood using QIAamp. Journal of Forensic Science. 1997;42(5):893-6.
35. Kotz S BN, Read CB, Vidakovic B. Encyclopedia of statistical sciences. 2nd ed. Hoboken, N.J. Wiley-Interscience; 2006.
36. Kitamura Y, Shirahata A, Sakuraba K, et al. Aberrant methylation of the Vimentin gene in hepatocellular carcinoma. Anticancer research. 2011;31(4):1289-91.
37. Stefaniuk P, Cianciara J, Wiercinska-Drapalo A. Present and future possibilities for early diagnosis of hepatocellular carcinoma. World J Gastroenterol. 2010;16(4):418-24.
38. Gu H, Wang X, Zheng L, et al. Vitamin D receptor gene polymorphisms and esophageal cancer risk in a Chinese population: a negative study. Med Oncol. 2014;31(2):827.
39. Gandini S, Gnagnarella P, Serrano D, Pasquali E, Raimondi S. Vitamin D receptor polymorphisms and cancer. Adv Exp Med Biol. 2014;810:69-105.
40. Mohammed MA, Sheta T, Omar NM, Mohammed SA. Vitamin D Receptor Gene Polymorphisms as a predictive risk factor for Hepatocellular Carcinoma development and severity in chronic hepatitis B. Int J Cancer Res 2017;13(1):26-35.
41. Yao X, Zeng H, Zhang G, et al. The associated ion between the VDR gene polymorphisms and susceptibility to hepatocellular carcinoma and the clinicopathological features in subjects infected with HBV. Biomed Res Int. 2013;2013:953974.
42. Yasmine M, Fatma K, Ashraf K. FokI polymorphism in vitamin D receptor gene and its association with hepatocellular carcinoma in Egyptian patients with chronic liver disease. Meta Gene. 2019;19:104-10.

43. Mohammed MA, Mohammed SA, Deiab AG. The Significance of Vitamin D Receptor Gene Polymorphisms for Susceptibility to Hepatocellular Carcinoma in Subjects Infected with Hepatitis C Virus. Gastroenterol Hepatol. 2017;7(4):00246.
44. Baur K, Mertens JC, Schmitt J, et al. Combined effect of 25-OH vitamin D plasma levels and genetic vitamin D receptor (NR 1I1) variants on fibrosis progression rate in HCV patients. Liver Int. 2012;32(4):635-43.
45. Abdelsalam A, Rashed L, Salman T, Hammad L, Sabry D. Molecular assessment of vitamin D receptor polymorphism as a valid predictor to the response of interferon/ribavirin-based therapy in Egyptian patients with chronic hepatitis C. J Dig Dis. 2016;17(8):547-53.
46. Rawhia HE, Mohamed SM, Belal AM, Yasmen AE. Study of Apa-I vitamin D receptor gene polymorphism in patients with hepatocellular carcinoma. Menoufia Medical Journal 2017;30:619-25.
47. Lange CM, Miki D, Ochi H, et al. Genetic analyses reveal a role for vitamin D insufficiency in HCV-associated hepatocellular carcinoma development. PLoS One. 2013;8(5):e64053.
48. Huang YW, Liao YT, Chen W, et al. Vitamin D receptor gene polymorphisms and distinct clinical phenotypes of hepatitis B carriers in Taiwan. Genes Immun. 2010;11(1):87-93.
49. Pourgholami MH, Morris DL. 1,25-Dihydroxyvitamin D3 in lipiodol for the treatment of hepatocellular carcinoma: cellular, animal and clinical studies. J Steroid Biochem Mol Biol. 2004;89-90(1-5):513-8.
50. Bandera Merchan B, Morcillo S, Martin-Nunez G, Tinahones FJ, Macias-Gonzalez M. The role of vitamin D and VDR in carcinogenesis: Through epidemiology and basic sciences. J Steroid Biochem Mol Biol. 2017;167:203-18.