The Role of the Vitamin D Receptor Gene Polymorphisms in Hepatocarcinogenesis in Cirrhotic Patients Infected With Chronic Hepatitis C Virus

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Abstract: Background and study Aim: Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide and the third most common cause of cancer death. Early diagnosis of HCC is of great importance in order to offer the possibility of curative treatment. several single nucleotide polymorphisms have been described in the VDR gene, and some polymorphisms are associated with tumor occurrence. In this study, we investigated the possible association between the VDR gene polymorphisms and HCC in Egyptian post hepatitis c cirrhotic patients. **Patients and method**: The study was carried out in Tropical Medicine Department in Tanta University Hospital on 50 individual, including 20 post hepatitis C cirrhotic patients with HCC, 20 post hepatitis C cirrhotic patients without HCC, 10 healthy individuals as control. All patients were subjected to history, clinical examination, image findings and laboratory investigations, Vitamin D receptor (VDR) genotype was determined by polymerase chain reaction (PCR) amplication and restriction length fragment polymorphisms. **Results**: our results showed that: ApaI CC genotype was present in 70% in Hcc patients, 35% in cirrhotics, 40% in control. APaI CA genotype represent 20% of Hcc patients, 55% of cirrhotics, 20% of control, while AA genotype represent 10% of Hcc patients, 10% of cirrhotics and 40% of control. **Conclusion**: VDR ApaI polymorphismplays a role in the development of HCC among chronic hepatitis C patients.

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1. Introduction:

Hepatitis C virus (HCV) infection is one of the major public health problems worldwide ***(MohdHanafiah et al., 2013)***.

Chronic HCV infection is characterized by a high rate of progression to fibrosis, chronic hepatitis, leading to cirrhosis and ultimately to hepatocellular carcinoma (HCC) ***(Hajarizadeh et al., 2013)***.

Early detection is critically important because the most effective treatment for HCC is surgical resection or ablation therapy when the tumour is small ***(Bolondi et al., 2005).***

The relationship between HCV and the development of HCC is well established, 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 ***(Hung et al., 2006).***

Of great importance, genetic factors can also contribute, particularly gene polymorphisms of inflammatory cytokines and growth factor ligands and receptors ***(Bataller et al., 2003).***

Vitamin D is involved in the metabolism of skeleton as a systemic hormone but also has important roles in the regulation of host immune responses, fibrogenesis and development of cancer through vitamin D receptor (VDR) ***(Holick, 2007).***

Previous data have suggested that vitamin D levels may influence cancer development. In particular, several single nucleotide polymorphisms have been described in the VDR gene, and some polymorphisms are associated with tumor occurrence ***(Köstner et al., 2009)***.

For instance, VDR polymorphisms have been related to cancers of the breast, prostate, skin, colon-rectum, bladder and kidney, although with conflicting observations ***(Köstner et al., 2009)***.

VDR polymorphisms have also been investigated in the context of some chronic liver diseases, such as chronic hepatitis B, primary biliary cirrhosis and autoimmune hepatitis ***(Huang et al., 2010).***

In a recent published study, VDR polymorphism may be used as a molecular marker to predict the risk and to evaluate the disease severity of HCC in patients with chronic hepatitis B ***(Yao et al., 2013).***

A significant association of VDR ApaI polymorphism with the development of HCC in chronic HCV infection may help to identify those who

are at high risk of developing HCC ***(Hung et al., 2014).***

2. Patients and Methods

The study was carried out in Tropical Medicine Department in Tanta University Hospital on 50 individual.

They will be divided into 3 groups:-

Group (I): including 20 post hepatitis C cirrhotic patients with HCC.

Group (II): including 20 post hepatitis C cirrhotic patients without HCC.

Group (III): including 10 healthy individuals as control.

Inclusion criteria:

- Post hepatitis C cirrhotic patient with and without HCC.

Exclusion criteria

- Malignancy other than HCC.
- Co-infection with HBV and HIV.
- Cirrhosis due to causes other than chronic hepatitis C virus infection.

Clinical and laboratory assessment:

All patients were subjected to the following: history taking, thorough clinical examination, liver function tests, complete blood picture, kidney function tests, blood sugar, Polymerase chain reaction for HCV-RNA (by Real Time PCR), abdominal ultrasonography, Alpha fetoprotein and abdominal triphasic CT for HCC patients.

Specific Laboratory investigation: The Vitamin D receptor (VDR) genotype was determined by polymerase chain reaction (PCR) amplication and restriction length fragment polymorphisms.

Statistical analysis:

Once data was collected, a code sheet was developed. Organization, tabulation, presentation and analysis of data were performed by using SPSS V21 of IBM, USA (Statistical Package for Social Studies version 21). Numerical data was presented as mean and standard deviation (SD). For parametric quantitative data, Student t-test and ANOVA (one way analysis of variance) were used for statistical analysis and for non-parametric data; Kruskal-Wallis test was used. Categorical data was presented as number and percentage and the chi-squared test was used for statistical analysis. When the chi-squared test was not appropriate, the Mont Carlo exact test was applied. Multivariate analysis (logistic regression) was performed to predict factors associated with risk of HCC in patients with chronic HCV infection. The level of significance was adopted at $p<0.05$.

3. Results:

Table (1): Distribution of VDR genotypes among the studied groups

VDR genotypes	Control (n=10)		HCC (n=20)		LC (n=20)		Statistics	P value
	N	%	n	%	n	%		
CC	4	40	14	70	7	35	MCET	0.019*
Adjusted residual (Z score)	-0.7		2.3		-1.7			
P value	0.4795		0.0209*		0.0832			
CA	2	20	4	20	11	55		
Adjusted residual (Z score)	-1.0		-1.7		2.6			
P value	0.2960		0.0879		0.0104*			
AA	4	40	2	10	2	10		
Adjusted residual (Z score)	2.3		-0.9		-0.9			
P value	0.0206*		0.34470		0.34470			
C vs. A allele	**10:10**		**32:8**		**25:15**			

- ApaI CC genotype was present in 70% in group I, 35% in group II, 40% in group III.
- APaI CA genotype represent 20% of group I, 55% of group II, 20% of group III, while AA genotype represent 10% of group I, 10% of group II and 40% of group III.

Table (2): Univariate and multivariate analysis of factors associated with risk of HCC

Variables	Univariate analysis		Multivariate analysis	
	Odds ratio (95% CI*)	P value	Odds ratio (95% CI)	P value
Age	1.12(0.3-2.1)	0.826	0.929(0.8-1.03)	0.165
Gender	2.8(0.8-10.5)	0.058	4.039(0.56-29.008)	0.165
Smoking	1.8(0.9-3.4)	0.240	1.76(0.25-12.24)	0.569
Low platelets	0.96(0.49-1.9)	0.907	0.211(0.037-1.198)	0.079
Apal CC genotype	2.3(1.1-5.1)	0.021*	17.3(1.7-78.6)	0.017*

95% CI*=95% confidence interval univariate analysis revealed that carrying of APaI CC genotype was a factor significantly associated with developing HCC. Logistic regression showed that the carriage of APaI CC genotype was the independent predictor.

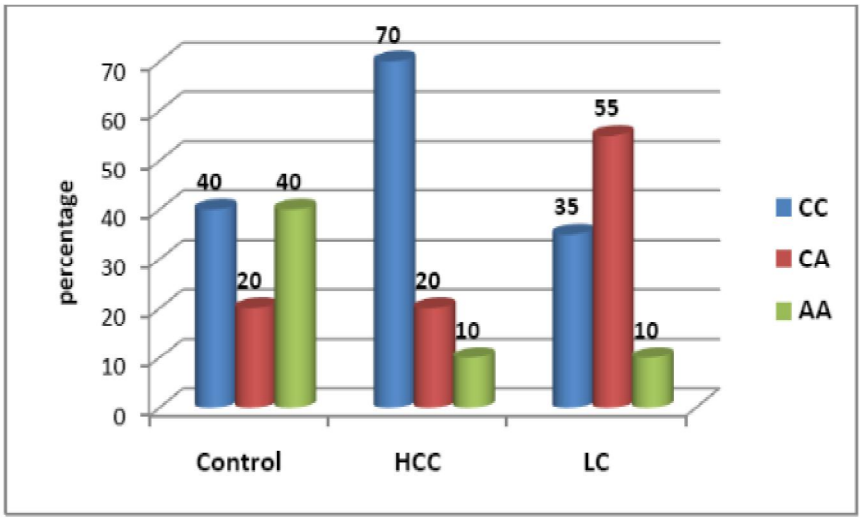

Figure (1): Distribution of VDR genotypes among the studied groups

Table (3): Association between VDR genotypes and AFP and platelets in the studied groups

Variables	VDR genotypes			Statistic	P value
	CC (n=25)	CA (n=17)	AA (n=8)		
AFP: Mean+ SD Median (IQR) Range	190.05±303.7 47.6(4.1-222.5) (2.7-1000)	19.2±27.5 5.3(3.6-27.6) (2.4-80)	13.03±16.1 6.3(4.7-17.2) (2.6-50.6)	Kruskal-Wallis test	0.038*
	CC & CA ((P value =0.015*) CC & AA ((P value =0.049*)				
Platelets: Mean+ SD Range	142520±13789.3 (48000-320000)	123823.5±20401.3 (440000-380000)	210375±27145.7 (70000-295000)	F=3.655	0.033*
T	AA & CA (P value =0.010*) AA & CC (P value =0.032*)				

IQR= Inter quartile range F= one way ANOVA * denotes statistically significant p<0.05
-AFP was significantly higher in subjects with CC genotype in correlation with CA and AA genotypes
As regard platelet, there is significant difference between the three genotypes,

4. Discussion:

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide and the third most common cause of cancer death ***(Dai et al., 2014).***

The burden of HCC has been increasing in Egypt with a doubling in the incidence rate in the past 10 years ***(El-Shenawy et al., 2012).***

Early diagnosis of HCC is of great importance in order to offer the possibility of curative treatment ***(Waly et al., 2012).***

Hepatocarcinogenesis is a complex and multi-factorial process, in which both environmental and genetic features interfere and contribute to malignant transformation ***(Nahon et al., 2012).***

Previous data have suggested that vitamin D levels may influence cancer development. In particular, several single nucleotide polymorphisms have been described in the VDR gene, and some polymorphisms are associated with tumor occurrence ***(Köstner et al., 2009).***

VDR polymorphisms have been related to cancers of the breast, prostate, skin, colon-rectum, bladder and kidney, with conflicting observations ***(Köstner et al., 2009).***

In this study, we investigated the possible association between the VDR gene polymorphisms and HCC in Egyptian post hepatitis c cirrhotic patients.

We found that ApaI CC genotype was present in 70% in group I, 35% in group II, 40% in group III. APaI CA genotype represent 20% of group I, 55% of group II, 20% of group III, while APaI AA genotype represent 10% of group I, 10% of group II and 40% of group III. these results were in agreement with ***(Hunget al., 2014)*** who reported that 71% of patiens of HCC had ApaI CC genotype, 26% had ApaI CA genotype and 3% had ApaI AA genotype, and suggested a significant association of VDR ApaI polymorphism with the development of HCC in chronic HCV infection.

Baur et al., (2012) have demonstrated that ApaI CC genotype is significantly associated with a rapid fibrosis progression rate and with the presence of cirrhosis in patients with chronic hepatitis C.

Falleti et al., (2010) have demonstrated that VDR genetic polymorphisms are significantly associated with the occurrence of HCC in cirrhotic patients who underwent liver transplantation.

However, this relationship is more specific for patients with an alcoholic etiology.

As regard signs of decompansation, ascites was predominant in CA genotype. This is due to high prevelance of CA genotype in cirrhotic patients, and our patients were mostly child C.

AFP was significantly higher in subjects with CC genotype in correlation with CA and AA genotypes, as both AFP and CC genotype polymorphism were significantly higher in HCC patients.

As regard platelet, we found that the most lower mean values were related to CA genotype, then CC genotype followed by AA genotype, as majority of our cirrhotic patients were mostly child C.

Furthermore, stepwise logistic regression analyses revealed that ApaI CC genotype was an independent factor, suggesting that the ApaI CC polymorphisms may play a role in the development of HCC among chronic hepatitis C patients.

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