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Association between VDR Gene Polymorphism and HBV Related HCC

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Abstract: *Hepatocellular carcinoma (HCC) accounts for 85% to 90% of primary liver cancers. The majority of these cases are related to chronic viral hepatitis. Several VDR gene single nucleotide restriction(SNP) have been described in association with neoplastic and non-neoplastic diseases. The aim of this study was to explore the association between VDR polymorphism and development of HCC and liver cirrhosis in chronically HBV infected patients. The study included 152 patients with chronic HBV and 50 healthy subjects as controls. HBV Patients were divided into three groups (59 chronic HBV infected patients without cirrhosis, 48 chronic HBV with cirrhosis and 55 chronic HBV with HCC).For all patients liver enzymes and serum AFP were measured. DNA was extracted from peripheral whole blood. VDR polymorphism was detected by the polymerase chain reaction technique followed by restriction fragment length polymorphism. The genotype frequencies of VDR FokI C>T polymorphism were significantly different among the studied groups. HCC patients and HBV patients with cirrhosis had higher prevalence of FokI TT genotype than HBV patients without cirrhosis and controls. The VDR FokI TT polymorphisms was associated with significant increased risk of HBV-related HCC however, due to the marginal significance and study limitations, larger well-designed epidemiological studies are recommended to confirm these findings.*

Keywords: *VDR polymorphism, HCC, HBV*

I. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common malignancy and the third leading cause of cancer mortality worldwide[1].It represents 85% to 90%of primary liver cancers(2). Infection with hepatitis B virus (HBV) and hepatitis C virus (HCV), fungal aflatoxin exposure and alcohol intake are considered the major risk factors [2,3]. Epidemiological studies showed variable genetic factors may influence the susceptibility to cancer[4,5,6]. Identification of these genetic factors may help to elucidate the complex process of hepato-carcinogenesis and improve the preventative intervention. Overall, 75–85% of HCC cases are attributable to persistent viral infection of HBV, specially in the developing countries (1). However, a small percent of lifelong chronic carriers of HBV eventually develops HCC. This finding suggests the importance of genetic variation as a risk factor for HBV-related HCC [7].The clinical outcomes of HBV infection vary from asymptomatic carrier to chronic necro-inflammatory liver disease and hepatocellular carcinoma. The progress of the liver disease is defined according to the interaction between host immune response and virus. The mechanisms that lead to the viral clearance or become chronic aren't well known. The host's susceptibility to infectious diseases or severity of the infection cannot be attributed only to the virulence of the microbial agent[8]. HBV pathogenesis is a very complex progress that could be directed by many factors related to both the virus and the host. For example, a recent study showed that HBe antigen positivity was more common in identical than non-identical twins, which suggested that the genetic factors of the host can affect the progress of the disease [8].Many genes were reported to have a role in HBV pathogenesis such as tumor necrosis factor-alpha, interleukin-6, interferon-γ, tumor growth factor-β and certain cellular receptors as chemokine receptor 5, vitamin D receptor(VDR) and estrogen receptor [9].Vitamin D is a fat soluble seco-steroid which is related to many biological functions as bone metabolism, regulation of immune response, cell proliferation and cell differentiation [10]. Vitamin D exert its affects as a systemic hormone, immune responses regulator through VDR[11,12,13,14].VDR is an intracellular hormone receptor that specifically binds the biologically active form of vitamin D and interacts with specific nucleotide sequences of target genes to produce a variety of biological effects. Also VDR may interacts with other cell-signaling pathways that influence cancer development. The VDR gene is highly polymorphic and is located on chromosome 12q12–q14 [15].Several single nucleotide polymorphisms (SNP) have been described in the VDR gene in association with neoplastic and non-neoplastic diseases. VDR polymorphisms were observed in relation to cancers of the breast, prostate, skin, colon, bladder and kidney[16]. Furthermore, VDR polymorphisms can influence the prognosis of prostate and breast cancer, renal cell carcinoma and malignant melanoma[17].This study was conducted to explore the VDR polymorphism in development of HCC and hepatic cirrhosis in chronic hepatitis B patients.

II. Subjects and methods

The study included 152patients from Menoufia university hospitals, In addition to 50 healthy controls.Patients were divided into three groups; 59 patients with chronic HBV infections not complicated with hepatic cirrhosis, 48 patients with chronic HBV infections complicated with cirrhosis and55with chronic HBV complicated byHCC. Chronic HBV infection was diagnosed by detection of hepatitis B surface antigen (HBsAg),then confirmed by real time PCR testing for HBV DNA. Liver cirrhosis was detected by abdominal ultrasonic examination while, HCC was screened for by both abdominal ultrasonic examinations and determination of alpha-fetoprotein (AFP) level then confirmed by triphasic CT.

Blood samples: 4 ml of blood from each patient were collected in two tubes.1 ml in heparinized tubes for DNA extraction, 3 ml were collected in plain tubes for the separation of serum. Sera were separated and stored at -20^{o}C.

Biochemical measurements:

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyletransferase (GGT), albumin, urea, bilirubin (total and direct) and creatinine were measured in serum by routine enzymatic methods (Spinreact, Spain). Serum AFP concentration was measured by ELISA assay according to the manufacturer's recommendation using Elecsys 2010 (Roche Diagnostics, USA). HBV markers were measured by commercially available ELISA kits (Bio-Rad, USA).

DNA Extraction and Genotyping:

Genomic DNA was extracted from whole peripheral bloodby DNA purification kit (Qiagen, UK) according to the Manufacturer's instructions. For the detection of the VDR polymorphisms, the polymerase chain reaction (PCR) technique was applied and followed by restriction fragment length polymorphism assays. The total volume of 25μL reaction mixture containing 1 U DNA Taq polymerase 16 μL buffer, 50 ng genomic DNA, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L primers, and 0.08 mmol/L dNTPs (Promega USA). The primer sequences are shown in table (1).PCR protocols were as follows: for *FokI* C>T; initial denaturation at 95°C for 2 min followed by of 40 cycles at 95℃ for 30 s, 61℃ for 30 s and 72℃ for 1 min. In a total volume of 25 µL, (18) For *BsmI* and *ApaI*, the DNA template was denatured at 95°C for 2 min. followedby 40 cycles of 45 s at 94°C, 45 s at optimum temperature (67°C for *ApaI* and 60°C for *BsmI*), 1 min at 72°C. then final extension step at 72°C for 2 min was added after the last PCR cycle[19].10 μL of the PCR productswere digested overnight with 2U of by allele-specific restriction endonucleases (New England Biolabs, Hitchin, UK) using the buffers andtemperatures recommended by the manufacturers. Digestion with the *BsmI* (820 bp) resulted in two fragments (645 bp and 177 bp) and digestion with *ApaI* (745 bp) produced two fragments of 531 bp and 214 bp when the restriction site was present. While, digestion with the *FokI* (157bp) resulted in two fragments of 121bp and 36 bp. All the PCR products and restriction fragments were sized by electrophoresis on a 2%agarosegel stained with ethidiumbromide[19].

Table (1) the sequences of primers for PCR amplification VDR genes

SNP	**The primer**	Base change
FokI	Sense, 5´-AGCTGGCCCTGGCACTGACTCTGCTCT-3´	C>T
	Antisense,5´-ATGGAAACACCTTGCTTCTTCTCCCTC-3´.	
BsmI	Sense, 5´-CAACCAAGACTACAAGTACCGCGTCAGTGA-3´	G>A
	Antisense, 5´-AACCAGCGGGAAGAGGTCAAGGG3-3´.	
ApaI	Sense, 5´- CAGAGCATGGACAGGGAGCAA3 -3´	G>T
	Antisense, 5´-GCAACTCCTCATGGCTGAGGTCTC -3´.	

Statistical analysis:

Data were analyzed using SPSS version 16 (SPSS Inc., Chicago, IL, USA). Normality of distribution was assessed using kolmogorov-Smirnov Z test. Quantitative variables were expressed in means ± S.Ds. Qualitative variables were expressed in frequencies and percentages. Univariate analysis was performed by kruskal-Wallis test for quantitative data and by chi-square test (X^2) or Fisher exact test (where appropriate) for qualitative data. Multinomial logistic regression was performed to determine independent predictors for different outcomes. Statistical significance was set at 0.05 level.

III. Results

Demographic and clinical parameters:

The demographic and laboratory parameters of the study population are presented in tables (2) and (3). There was no significant difference between different groups in their age. However, The HBV patients with HCC had a higher significant proportion of smokers compared with the other three groups (P<0.05). In addition, HBV patients with HCC and the other three groups without HCC had statistical significant different laboratory results for bilirubin (total and direct), AST, ALT, ALB and GGT, (P<0.001). Serum AFP levels were compared among different VDR genotype carriers in HCC patients. It was found that HCC patients with *FokI* TT genotype had a much higher AFP level than those with CT and CC (*P*< 0.01 versus CC and P<0.05versus CT, respectively). The AFP levels were similar among *BsmI* and *ApaI* genotype carriers (data not shown in the tables). Results showed variation in AFP levels among different groups where the maximal level was detected in HCC group followed by HBV patients with cirrhosis (Mean ± SD were 781 ± 328.4 and 141± 41ng/ml respectively).

Genotype and allele distributions of the VDR:

The genotype frequencies of VDR gene among the studied subjects are presented in table (4). The genotype frequencies of VDR *FokI* C>T polymorphism were significantly different among the studied groups.HCC patients and HBV patients with cirrhosis had higher prevalence of *FokI*TT genotype(38.1% and 31.2% respectively) than HBV patients without cirrhosis and controls (15.2% and12%, respectively).Regarding allele comparison, HCC subjects had significantly (*P*<0.05) higher T allele frequency than controls. Regarding SNPs of *BsmI* and *ApaI*, their genotype and allele frequencies there was no significant difference (*P*<0.05)between HCC and other three groups.

In multinomial logistic regression (table 5) the predictors for developing HBV without cirrhosis were AST and Total bilirubin while the predictors for developing HBV with cirrhosis were albumin, total bilirubin and smoking. Finally, predictors for developing HCC were ALT, AST, albumin, Total bilirubin, *FokI*, T allele and smoking.

Table (2): Demographic data of the studied groups.

	Control group (n = 50)	**HBV without cirrhosis (n = 59)**	**HBV with cirrhosis (n = 48)**	**HBV with HCC (n = 55)**
Age (Mean± SD)	56.8 ± 5.6	57.4 ± 6.2	58.7 ± 5.5	57.2 ± 6.5
Gender - Male - Female	33 (66.0.7%) 10 (33.0%)	39 (78.0%) 20 (22.0%)	31 (64.0%) 17 (36.0%)	42 (76.0%) 21 (24.0%)
Smoking - Yes - No	27 (54.0%) 23 (46.0%)	30 (50.8%) 29 (49.2%)	27 (56.2%) 21 (43.8%)	44 (80%) 11 (20%)

Table (3): Biochemical laboratory parameters among different studied groups.

Parameters	**Control group (n = 50)**	**HBV without cirrhosis (n = 59)**	**HBV with cirrhosis (n = 48)**	**HBV with HCC (n = 55)**
AFP (ng/ml)	3.1 ± 1.3	7.6 ± 7	142.7 ± 41.4[a]	786.9 ± 331.4[a,b]
ALT (IU/L)	25 ± 3	112.6 ± 37.3 [a]	42 ± 31.3	55.6 ± 13.2
AST(IU/L)	22 ± 6	73.1 ± 37.3 [a]	42 ± 37.4[a]	60.4 ± 20.3[a]
GGT (IU/L)	15.2 ± 5.1	28.1 ± 7.1 [a]	58 ± 11.1 [a]	105 ± 32.5[a,b]
Albumin (g/dl)	4±1	3.6±0.4	2.6±0.6	1.7±0.7[a,b]
T-Bil. (mg/dl)	0.8 ± 0.3	2.5 ± 1.7 [a]	6.5 ± 1.6[a]	15.1 ± 7.4[a,b]
D-Bil. (mg/dl)	0.1 ± 0.03	0.5 ± 02 [a]	2 ± 0.7[a]	5.5 ± 1.4[a,b]

[a]Significant difference from control group, (X^2 test P value < 0.05).

[b]Significant difference from cirrhosis group, (X^2 test P value < 0.05).

Table (4): the genotype frequencies of VDR genes among different studied groups.

Genotypes	**Control group (n = 50)**	**HBV without cirrhosis (n = 59)**	**HBV with cirrhosis (n = 48)**	**HBV with HCC (n = 55)**
FokI	21(42%)	23(39%)	12(25%)	11(20%) [a]
CC	23(46%)	27(45.8%)	21(43.8%)	23(41.8%)
CT	6(12%)	9(15.2%)	15(31.2%)[a]	21(38.1%)[a]
TT	65(65%)	73(61.6%)	45(46.9%)	45(40.9%)
C allele	35(35%)	45(38.1%)	51(53.1%)	65(59.1%)[a]
T allele				
BsmI	19(38%)	27(45.7%)	21(43.7%)	24(43.6%)
GG	24(48%)	25(43.4%)	20(41.7%)	23(41.8%)
GA	7(14%)	7(11.8%)	7(14.6%)	8(14.5%)
AA	62(62%)	79(66.9%)	62(64.6%)	71(64.5%)
G allele	38(38%)	39(33.1%)	34(35.4%)	39(35.5%)
A allele				
ApaI	14(28%)	21(35.6%)	15(31.3%)	13(23.6%)
GG	23(46%)	28(47.5%)	20(41.7%)	28(50.9%)
GT	13(26%)	10(16.9%)	13(27.0%)	14(25.4%)
TT	51(41%)	70(59.3%)	50(52.1%)	54(49.1%)
G allele	49(49%)	48(40.7%)	46(47.9%)	56(50.9%)
T allele				

[a]Significant difference from control group, (X^2 test, P value < 0.05).
[b] Significant difference from localized HCC group, (X^2 test, P value < 0.05).

Table (5): Multinomial logistic regression for comparison between HBV groups and control.

variables	**HBV without cirrhosis (n = 59)**		**HBV with cirrhosis (n = 48)**		**HBV with HCC (n = 55)**	
	OR	P value	OR	P value	OR	P value
AFP (ng/ml)	---	---	1.2	0.997	2.3	0.993
ALT (IU/L)	2.1	0.881	---	---	1.08	0.0001
AST(IU/L)	1.1	0.0001	---	---	1.06	0.0001
GGT (IU/L)	4.5	0.908	2.05	0.958	4.7	0.892
Albumin (g/dl)	---	---	0.20	0.0001	0.003	0.0001
T-Bil. (mg/dl)	4.1	0.001	2.1	0.003	7.7	0.0001
D-Bil. (mg/dl)	1.1	0.975	1.2	0.986	2.03	0.966
FokI	---	---				
CC			0.66	0.430	0.15	0.001
TT			0.78	0.588	0.29	0.022
T allele	---	---	---	---	2.9	0.008
Smoking	1.1	0.743	0.32	0.011	0.29	0.005

Blank areas are for insignificant relations in univariate analysis.

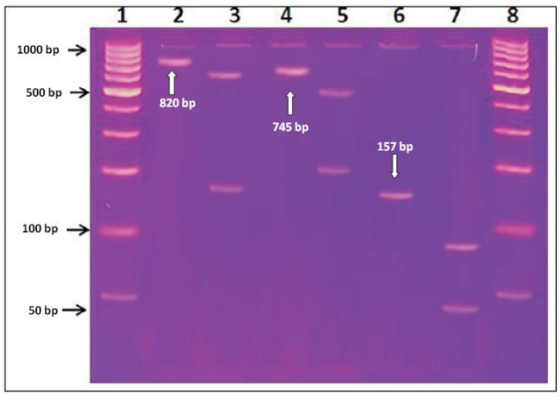

Fig.1: Gel electrophoresis of PCR products and restriction fragments: Lane 2 shows *BsmI* PCR product (820 bp) and in lane 3 restriction fragments (645 bp and 177 bp). Lane 4 shows *ApaI* PCR product (745 bp) and in lane 5 restriction fragments (531 bp and 214). Lane 6 shows *FokI* PCR product (157 bp) and in lane 7 restriction fragments (121 bp and 36).

IV. Discussion

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third leading cause of cancer-related death worldwide [1], accounting for 85% to 90% of primary liver cancers [20]. A better understanding of the mechanism involved in HCC is essential to develop new preventions and modify the existing therapies. It is established that HCC is a complex disease for which the underlying causes remain exactly unclear. In this study the VDR gene polymorphism was studied to determine whether the genetic factors are related to the occurrence of HBV-related HCC. Viral hepatitis may increase of oxidative stress in hepatocyte that leads to DNA changes and instability and increase the risk of developing cirrhosis and HCC [21,22]. Some previous studies provided evidence that genetic polymorphisms of certain genes including VDR gene may involved the HCC occurrence in HBV infected patients [23,24,25,26].Our study showed that only the *FokI* C>T polymorphism were associated with the HCC susceptibility in patient with chronic HBV infection; the other SNPs, including *BsmI* and *ApaI* were not related with the development of either HCC or liver cirrhosis. The HCC and liver cirrhosis patients had a significantly higher rate of FokI C>T polymorphism, suggesting that this SNP in the VDR gene at *FokI* locus may increase HCC risk through enhancing the occurrence of liver cirrhosis. The *FokI* fragment SNP is located in the coding region of the VDR gene the responsible for production of a VDR variant protein which is three amino acids longer and functionally less effective than the regular one [27]. Arai *et al*.,[28] demonstrated that *FokI* C/C had 1.7-fold greater vitamin D reporter activation function than *FokI* T/T genotype. They has been hypothesized that this less effective function could be associated with either an increased susceptibility to cancer risk or to a more aggressive disease.

Our result are in accordance with Peng*et al*.,[7] who reported that VDR FokI C>T polymorphisms may contribute to increased susceptibility to HBV-related HCC in the Chinese population. Also Vogel *et al*..[29]reported a significant increase of the *FokI* C>T SNP in autoimmune hepatitis patients and primary biliary cirrhosis indicating a genetic link of VDR polymorphisms to autoimmune liver diseases. Also, Fan *et al*.,[30] reported a significant difference in *FokI* polymorphism between autoimmune hepatitis patients and controls. On the other hand, Falleti *et al*.,[18] reported that GG genotype of BsmI was significantly associated with occurrence of HCC in the Italian patients. Although many factors may be account for the discrepancies among these studies, the ethnic differences should be predominately considered since Fan *et al*.,[30] found the distribution of *FokI*, *BsmI* and *ApaI* gene types significantly differed between Chinese and Caucasian healthy populations. The prognostic role of *FokI* C>T polymorphism in cancer patients has been reported. Hama *et al*.,[31] reported the VDR *FokI* TT genotype was associated with a poor progression and low survival rate in patients with squamous cell carcinoma. In contrast, the other polymorphisms (*BsmI* and *ApaI*) showed no significant associations with the survival rate in this carcinoma. In this study, no prognostic analyses wasn't available however, it was noted that the TT genotype carriage of *FokI* C>T polymorphism was associated with more advanced tumor stage and worse tumor differentiation. In addition, the TT carriers had higher serum AFP level than CC and CT carriers. Some limitation in this study should be mentioned; first, no information was available regarding vitamin D intake and circulating vitamin D levels of the studied subjects. Also, information about alcohol intake that may influence liver health status were not available due to social and religious causes. Second, the study included only subjects with HBV infection hence, due to the fact that HCV infection is another major cause for HCC in our country, future study with larger sample size including HCV subjects is warranted.

V. Conclusion

In conclusion, this study suggested that the VDR *FokI* TT polymorphisms were associated with a significantly increased risk of HBV-related HCC. These results may suggest that FokI C>T polymorphisms could be used as a molecular risk for development of in those infected with HBV. Due to the marginal significance and study limitations, larger well-designed epidemiological studies and functional evaluations are recommended to confirm these findings.

References

[1]. Jemal A, Bray F, Center MM, Ferlay J, Ward E, et al. Global cancer statistics. CA Cancer J Clin 2011; 61: 69–90.
[2]. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology 2007;132: 2557–2576.
[3]. Kuper H, Tzonou A, Kaklamani E, Hsieh CC, Lagiou P, et al. Tobacco smoking, alcohol consumption and their interaction in the causation of hepatocellular carcinoma. Int J Cancer 2000; 85: 498–502.
[4]. Wang PR, Xu M, Toffanin S, Li YL, Lovet JM, Russell DW. Induction of hepatocellular carcinoma by in vivo gene targeting. Proceedings of the National Academy of Sciences of the United States of America, 2012; 109 (28):11264–11269.
[5]. Ma NF, Lau SH, Hu L, Dong SS, Guan XY. Hepatitis B virus X gene in the development of hepatocellular carcinoma. Hong Kong Medical Journal 2011; 17(6): 44–47.
[6]. Cillo C, Schiavo G, Cantile M et al. The HOX gene network in hepato- cellular carcinoma. International Journal of Cancer 2011 129(11): 2577–2587.

[7]. Peng Q, Yang S, Lao X, Li R, Chen Z, Wang J, Qin X, Li S. Association of Single Nucleotide Polymorphisms in VDR and DBP Genes with HBV-Related Hepatocellular Carcinoma Risk in a Chinese Population. PLOS ONE 2014;26:1–17.
[8]. Lin TM, Chen CJ, Wu MM, Yang CS, Chen JS, Lin CC, K wang TY, et al. Hepatitis B virus markers in Chinese twins. Anticancer Res. 1989;(3): 737-741.
[9]. Sheron N, Lau J, Daniels H, Goka J, Eddleston A, Alexander GJ, Williams R. Increased production of tumour necrosis factor alpha in chronic hepatitis B virus infection. J Hepatol. 1991;12(2):241-245.
[10]. 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 Res2013; 6: 37.
[11]. Holick MF. Vitamin D deficiency. N Engl J Med 2007; 357: 266–281.
[12]. Peterlik M and Cross HS. Vitamin D and calcium deficits predispose for multiple chronic diseases. Eur J Clin Invest 2005;35, 290–304.
[13]. Köstner K, Denzer N, Müller CS, Klein R, Tilgen W, and Reichrath J. The relevance of vitamin D receptor (VDR) gene polymorphisms for cancer: a review of the literature. Anticancer Res 2009;29: 3511–3536.
[14]. Tang C, Chen N, Wu M, Yuan H, and Du Y. Fok1 polymorphism of vitamin D receptor gene contributes to breast cancer susceptibility: a meta-analysis. Breast Cancer Res Treat 2009; 117: 391–399.
[15]. Zhou H, Xu C, Gu M. Vitamin D receptor (VDR) gene polymorphisms and Graves' disease: a meta-analysis. ClinEndocrinol 2009; 70: 938–945.
[16]. Raimondi S, Johansson H, Maisonneuve P, Gandini S. Review and meta-analysis on vitamin D receptor polymorphisms and cancer risk. Carcinogenesis 2009; 30: 1170-1180.
[17]. Köstner K, Denzer N, Müller CS, Klein R, Tilgen W, Reichrath J. The relevance of vitamin D receptor (VDR) gene polymorphisms for cancer: a review of the literature. Anticancer Res 2009; 29: 3511-3536.
[18]. Falleti E, Bitetto D, Fabris C et al. Vitamin D receptor gene polymorphisms and hepatocellular carcinoma in alcoholic cirrhosis. World Journal of Gastroenterology 2010; 16(24):3016–3024.
[19]. Yao X, Zeng H, Zhang G, Zhou W, Yan Q, Dai L, and Wang X. The association between the VDR gene polymorphisms and susceptibility to hepatocellular carcinoma and the clinic-pathological features in subjects infected with HBV. Biomed Res Int2013; 953974.
[20]. Chen Y, Liu W, Sun T, Huang Y, Wang Y, et al. 1,25-Dihydroxyvitamin D promotes negative feedback regulation of TLR signaling via targeting microRNA-155-SOCS1 in macrophages. J Immunol 2013; 190: 3687–3695.
[21]. Tan Y J. Hepatitis B virus infection and the risk of hepatocellular carcinoma. World Journal of Gastroenterology 2011; 17(44): 4853–4857.
[22]. Jin Y, Abe K, Sato Y, Aita K, Irie H, Shiga J. Hepatitis B and C virus infection and p53 mutations in human hepatocellular carcinoma in Harbin, Heilongjian Province, China. Hepatology Research 2002; 24 (4):379–384.
[23]. Migita K, Maeda Y, Abiru S et al. Polymorphisms of interleukin-1β in Japanese patients with hepatitis B virus infection. Journal of Hepatology 2007; 46(3):381–386.
[24]. Al-Qahtani A, Al-Ahdal M, AbdoA et al. Toll-like receptor 3 polymorphism and its association with hepatitis B virus infection in Saudi Arabian patients. Journal ofMedicalVirology2012; 84(9): 1353–1359.
[25]. Kim JH, Yu SJ, ParkBL et al. TGFBR3 polymorphisms and its haplotypes associated with chronic hepatitis B virus infection and age of hepatocellular carcinoma occurrence. Digestive Diseases 2011; 29 (3):278–283.
[26]. Chan KY, Wong CM, Kwan J S et al. Genome-wide association study of hepatocellular carcinoma in Southern Chinese patients with chronic hepatitis B virus infection. PLoS One, 2011; 6 (12): Article ID e28798.
[27]. Whitfield GK, Remus LS, Jurutka PW et al. Functionally relevant polymorphisms in the human nuclear vitamin D receptor gene. Molecular and Cellular Endocrinology, 2001; 177(1-2):145–159.
[28]. Arai H, Miyamoto K I, Taketani Y 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 of Bone and Mineral Research, vol. 12, no. 6, pp. 915–921, 1997.
[29]. Vogel A, Strassburg CP, and Manns MP. Genetic association of vitamin D receptor polymorphisms with primary biliary cirrhosis and autoimmune hepatitis. Hepatology2002 ;35(1):126–131.
[30]. 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. Journal of Gastroenterology and Hepatology 2005; 20(2):249–255.
[31]. Hama T, Norizoe C, Suga H et al., "Prognostic significance of vitamin Dreceptor polymorphisms in head and neck squamous cell carcinoma," PLoS One 2011; 6 (12) Article ID e29634, 2011.