ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Evaluation of Fok-I polymorphisms of VDR gene in Iraqi patients with colorectal cancer

Salih M. Al-Khafaji
Department of Anatomy &Histology, College of Medicine, University of Kufa, Najaf, Iraq.

Manuscript Info

Manuscript History:

Received: 11 February 2015
Final Accepted: 22 March 2015
Published Online: April 2015

Key words:

Colorectal Cancer, Polymorphisms, Vitamin D receptor.

Corresponding Author

Salih M. Al-Khafaji

Abstract

Colorectal cancer is the most important cause of cancer death in human in many parts of the world. The vitamin D receptor (VDR) proto-oncogene has been suggested to be involved in the regulation of cell proliferation and differentiation in colorectal cells.

Objective: The study was designed to determine a possible relationship between vitamin D receptor (VDR) gene polymorphisms and the risk of colorectal cancer.

Methods : We investigated the VDR Fok-I gene polymorphisms in 55 colorectal patients and 60 matched healthy control. The study performed by polymerase chain reaction –based restriction fragment polymorphisms (RFLP).

Results : The VDR Fok-I (TT) genotype distribution was significantly increased in patients compared with healthy controls($P < 0.05$) , and the carriers of VDR-Fok-I genotype (CT) also significantly increased in patients compared with control($P < 0.05$) . The allele T is associated the risk for disease 34(31%) compared with the healthy control 17 (14%) OR, 2.71, CI 95%, (1.41- 5.20), ($P < 0.05$).

Conclusion: We suggest that VDR Fok-I might affect the development of colorectal cancer.

Introduction:

INTRODUCTION

Colorectal cancer (CRC) is the third most common tumor and the fourth most common cancer –related causes of death in both gender . In 2002 , the registered incidence of colorectal cancer in Europe was 371, 706 (5,1% of all cases) and 203 , 296 death(2.81% of all cancer related death) . (**1)**

In Iraq and according to the Iraqi cancer registry reports, the colonic cancer represented about 4.7% of all malignant primary tumor registered during the period from 1995- 1997, while rectal malignancy registered represented about 3.4%. (**2)**

In 2014 , the incidence of colorectal carcinoma was 4.89% of whole body malignancy and it is the seventh causes of death from cancer **. (3)** Colorectal cancer incidence and mortality vary markedly between ethnicities, it has related to age, nutrition, and extent of wintertime ultraviolet (UV) radiation of the latitude of patients place of residence.

It has been proposed that some categories of external agents, including physical, chemical and biological carcinogens, may contributed to the development of this disease, and the role of these factors in carcinogenesis would depend largely on genetic factors. Correspondingly, a recent study showed that insufficient levels of vitamin D may result in colorectal cancer. (4**)**. The relevance of vitamin D receptor (VDR) gene restriction fragment length

polymorphisms for various types of cancer has been investigated by a at number of studies. It has been suggested that VDR polymorphisms may impact both the risk of cancer occurrence and prognosis. Furthermore, genetic variations in genes controlling vitamin D activity would be hypothesized to play an important role in determining susceptibility to colorectal cancer.

Polymorphisms in VDR have been studied widely in studies of CRC,(**5**)these studies have mostly focused on few selected variations, including FOKI .The VDR gene is located on chromowsome12q 12-q14. More than 100 single nucleotid polymorphisms (SNPs) have been described within 67076bp sequence. Eight exons (2-9) and six alternatively spliced regions (1a-1f) are distributed in functionally relevant areas, including the promoter region (fig). Molecular variants of the VDR gene may be related to the development of colon cancer.(**6),(7),(8).**

The purpose of this study was to investigate the association of the VDR gene rs2228570 C> T polymorphisms in Iraqi population.

Materials and methods

Between June 2014 and February 2015 , a total of 55 male patients with different stage of colorectal cancer ,with age ranged from 45-88 years , with a mean 63.2 and ±SD 11.62 ,and 60 healthy control, with a mean of 62.65and±SD 12.82, who did not have any disease .

The patients were diagnosis as CRC and confirmed by histopathological diagnosis from oncology unit in Al –Sadder medical city. The specimens were taken after obtaining informed consent, the medical ethics committee of kufa medical college was obtained for the study.

VDR gene polymorphisms was studied by the Fok I polymorphisms of VDR gene and DNA was extracted from peripheral blood leukocytes using ReliaPrep TM blood gDNA Miniprep System promega. According to the manufacturer's instructions. VDR FokI genotype was analyzed using PCR- RFLP. The DNA was amplified by polymerase chain reaction using primers described by Harris et al. [14]. The primers used for PCR–RFLP were Forward 5′- AGCTGGCCCTGGCACT GACTCTGCTCT -3′ and Reverse 5′- ATGGAAACACCTTGCTTC TTCTCCCTC-3′ resulting in a PCR product of 275 bp. The amplification was accomplished with a 25 µl reaction mixture containing 5 µl of (10-100) ng template DNA, 1.5 µl 1.5 pmol of each primer, 12.5 µl master mix contains of (2.5 µl 10 mM dNTPs, 1.5 µl of 20 mM MgCl2, 0.3 µl of 5 U/ µl Taq polymerase with 2.5 µl of 10X Taq Buffer) (Promega, USA). The reaction volume completed by addition of nuclease free water. PCR conditions were as follows: Initial denaturation at 94°C for 6 minutes followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 60°C for 45 seconds, extension at 72°C for 45 seconds and final extension at 72°C for 5 minutes. The amplicons were digested with 5 units of FokI enzyme (New England Biolabs, USA) by incubating at 37°C for 1 hour. The FF genotype (homozygote of common allele) shows only one band of 273. The ff genotype (homozygote of infrequent allele) generates two fragments of 198 and 75 bp. The heterozygote displays three fragments (273, 198, and 75 bp), visualized on 2.0 % agarose gel containing ethidium bromide (Figure 1).

Statistical analyses.

Statistical analyses were performed using the SPSS software package (revision 20 Inc., Chicago ,USA) Data are expressed as means ±SD .Differences in distribution of genotype or alleles between patients and control were tested using the Chi- square statistic. Odds ratios (ORs) and 95% confidence intervals (95% CI) were calculated to estimate the risk colorectal cancer. Values of $P > 0.05$ were considered statistically significant**.**

Results

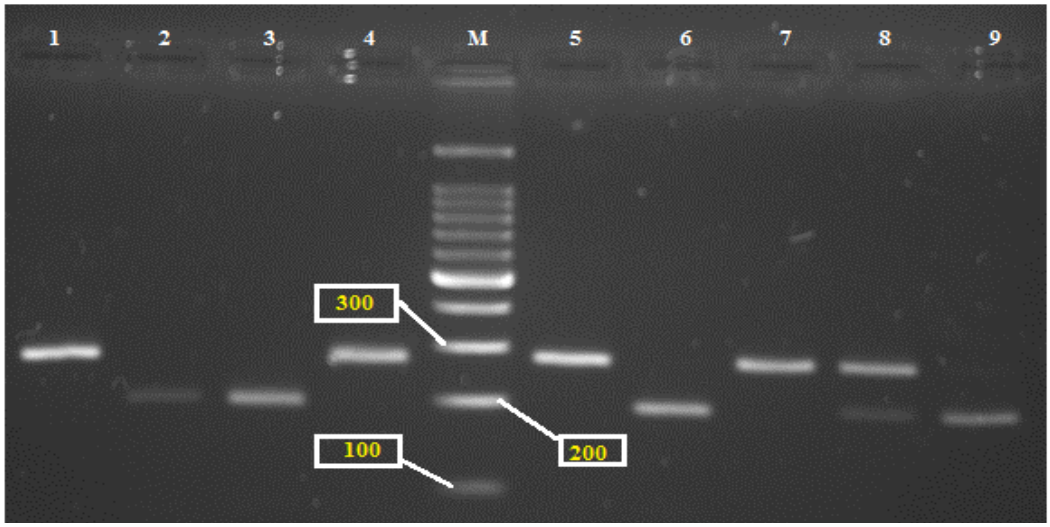

Fig 1. The FokI restriction digested PCR product profile of VDR gene. Lane M; DNA Marker; Lane1,4,5, and 7 show CC genotype, Lane 8 shows CT genotype, Lane 2,3,6 and 9 show TT genotype.

In the polymorphisms study, the distribution of genotype frequency of VDR gene in CRC patients and control individuals were examined by statistical analysis shown in table **(1).**

1-The frequencies of the alleles and the genotypes were in hardy-Weinberg equilibrium among the patients and the controls X^2 = 0.01, P Value =0.63 and X^2 =0.27, P Value =0.74 respectively.

2- VDR gene polymorphism (VDR), genotype and allele frequencies in CRC patients and healthy control in heterozygous genotype (CT) may be increase the risk of CRC (OR = 2.39, 95% CI= 1.06-5.38, P value = 0.039).

While homozygous genotype (CC) was highly significant compared to healthy control group (OR = 9.70, 95% CI= 1.11-85.68,P value = 0.035).

Table 1 Allele frequency and genotype of SNPs Analysis in patients with colorectal cancer and control group.

SNPs Genotype/Allele frequency	CRC Patients No.55	Healthy control No.60	OR	(95%CI)	P Value
CC	27(49%)	44(73%)	1.00	reference	------
CT	22(40%)	15(25%)	2.39	1.06-5.38	0.035
TT	6 (11%)	1 (2%)	9.70	1.11-85.68	0.039
C	76(69%)	103(86%)	1.00	reference	-------
T	34(31%)	17 (14%)	2.71	1.41- 5.20	0.002

OR= Odds ratio, CI=Confidence interval, SNP= Single Nucleotide Polymorphisms

Discussion

The reduction in proliferation and differentiation of human colon cancer cells are accompanied with high levels of serum 25-OH vitamin D **9.** The VDR polymorphisms play are important factors in the assessment of colorectal cancer risk, possibly indicating vitamin D and calcium as preventive measures **10**. Vitamin D is effect agent for the prevention of colorectal cancer through mechanisms mediated by the VDR 11 and 12 . These findings led to the question of whether variants in VDR gene influence an individual's susceptibility to colorectal cancer development. In our study , there was strong association of the C>T polymorphisms of the VDR gene in patients with CRC and healthy control in Iraqi population , these findings indicated that the VDR gene rs (T) allele was risk for CRC patients (31%), compared with healthy control (17%). Similarly, (TT) genotype frequency in CRC patients 6 (11%)was significant difference from healthy group 1 (2%).

The VDR multiple polymorphisms have been examined for the association with VDR in several populations . In this manner, our results corresponded with those reported in the literature for various populations. Indeed, the expression of the nuclear vitamin D receptor (VDR), which is involved in regulating cell growth and proliferation may contribute to the development of CRC .(13) The finding that genetic variation in vitamin D pathway contribute to the risk supports a role for vitamin D in colon cancer etiology (8). On the other hand recent studies demonstrate that there is no association between VDR polymorphisms in 3 ' UTR of VDR gene and patients with colorectal adenoma, compared with healthy controls in a USA and Turkish populations (14,15) . up to our knowledge this study is the first investigation of this relationship in Iraqi population we observed an association between CRC and VDR gene polymorphism, suggesting an evidence that differences in the oncogenic properties of the VDR gene,(T) allele could confer a genetic predisposition to colorectal carcinogenesis . Finally further studies in different population are needed to reach to a final conclusion.

References:

1.Globocan, IARC International Agency for research on cancer (2002)

2. Results of Iraqi cancer Registry 1995-1997, Iraqi cancer board, ministry of health, Baghdad –Iraqi 1999.

3.Hamid Yah , Sharif F.A. Alawachi . Incidence of rate , Pattern and time trend of registered Cancer in Iraqi (1991-2008). Open access Library Journal, 2014, 1-6.

4. Holf,P.RDiary Food and prevention of colon cancer Human Studies. The journal of the American college of nutrition. 18, 3795- 3915, 1999.

5. Miyamoto,K., kesterson, R.A., Yamamoto,H., Taketani,K., Nishiwaki,E., Tstsumi, S., Inoue,Y., Morita,K., Takeda,E., and Pike, J.W. Structural organization of human vitamin D Receptor chromosomal gene and its promotor, Molecular Endocrinology. 11, 1105-1179, 1997.

6. Slattery ML, Neuhausen SL, Hoffman M, et al. Dietary calcium , vitamin D , VDR genotypes and colorectal cancer .Int J Cancer 111:750-756,2004.

7. Slattery ML, Yakumo K, Hoffman M, Neuhausen SL. Variants of the VDR gene and risk of colon cancer (USA). Cancer causes control 12: 359- 364, 2001.

8. Wong HC, Seow A, Arakawak K,etal. Vitamin D receptor start codon polymorphisms and colorectal Cancer risk: Effect modification by dietary calcium and fat in singapora Chinese. Carcinogenesis24: 1091-1095, 2003.

9. Flugge J, Krusckopf S, Gold Metal. Vitamin D receptor haplotypes protect againist development of colorectal cancer. Eur. J. Clin. Pharmacol. 63:997-1005, 2007.

10. Gong YL, Xie DW, Deng ZL, et al. Vitamin D receptor gene Tru9I polymorphisms and risk for incidental sporadic colorectal adenomas .World J Gastroenterol 11:479404799, 2005.

11. Ban Y, Taniyama M, Ban Y.Vitamin D receptor gene polymorphisms is associated with Graves disease in Japanese population. J,Clin Indocrinolo. Metabo 85: 4639-4643, 2000.

12.Peter U, Hayes RB, Chatterjee N, et al. Circulating vitamin D metabolites, polymorphism in vitamin D receptor and colorectal adenoma risk. Cancer Epidemiol. Biomarkers prev 13: 546-552, 2004

13. Park K, Woo M, Nam j, Kim JC. Start codon polymorphisms in the vitamin D Receptor and colorectal cancer risk. Cancer Lett 237: 199- 206, 2006.

14. Peters U, McGlynn KA, Chatterjee N, et al. Vitamin D, calcium, and vitamin D receptor polymorphism in colorectal adenomas. Cancer Epidemiol Biomarkers Prev 10: 1267-1274, 2000.

15. Gran MV, Baron JA, Sandler RS, et al. Vitamin D, Calcium Supplementation, and colorectal adenomas: Results of randomized trial .J Natl. Cancer Inst. 95:1765-1771, 2003.