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Vitamin D receptor gene polymorphism and its relation to cancer colon occurrence

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Abstract

Cancer colon is the second most prevalent cancer for females and the third for males. Vitamin D's cellular impacts are achieved by 1,25 (OH) 2D binding to the Vitamin D receptor (VDR). This study aimed at assessing the relation between vitamin D receptor gene polymorphism and cancer colon. This case-control study included 50 colorectal cancer (CRC) cases which were candidates for colonoscopy and 50 controls with normal colonoscopy. The study was conducted in Suez Canal University hospitals. All cases were diagnosed with colonoscopy and confirmed with histopathology. Blood samples from study subjects were used for detection of vitamin D receptor FokI polymorphism. We found that more than two thirds of patients were males. Around half of the cases were over 60 years old. Most of the study participants were overweight (26%), obese (53%), non-alcoholics (99%), and non-smokers (72%). However, about one third of the patients were diabetic (31%). Noticeably, none of these factors was significantly variant among CRC group and normal colonoscopy group ($p< 0.05$). The most common presentation among cases with colon cancer was constipation (80%). Of the 100 studied cases, 74% had left-sided colon cancer, with a 66% of them were resectable. The odds ratio of VDR polymorphism between the cases and control groups was high (3 with 95% CI (0.3-31)), however it did not reach statistical significance ($p= 0.3$). Most of the cases with VDR polymorphism had colon cancer (75%). In conclusion, based on our findings, there was no correlation between colorectal cancer and vitamin D receptor gene polymorphism.

Keywords: Cancer Colon, Vitamin D, Gene Polymorphism

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Introduction

World-wide, colorectal cancer (CRC) is the second most prevalent cancer for females and the third most frequently reported by males. Its prevalence is higher in Australia, New Zealand, Europe, and the United States of America (USA) 10 times than in Africa and Asia.[1] The prevalence of CRC in Egypt is 6.5%. Furthermore, according to the World Health Organization (WHO) predictions, in 2040 the number of new CRC cases will exceed 3 million and over 1.5 million deaths will be recorded worldwide.[2]

CRC is related to age and lifestyle, with few occurrences caused by hereditary anomalies.

Other risks involved nutrition, obesity, smoking, and lack exercise.[3] Nutritional risk factors included red meat, processed meat and alcohol drinking, and inflammatory bowel disease (IBD). Screening for CRC is advised to be done in old age (50-75 years) as it prevents and reduces the mortality rate. Small polyps can be removed during colonoscopy while in large polyps or tumors, biopsy is obtained for histopathology.[4]

The biologically active form of vitamin D3, 1α, 25 (OH) 2D3 (1,25D3), is obtained by 25-hydroxylation of vitamin D3 in the liver and 1α-hydroxylation in the kidney, liver, or other tissues. Hydroxylation of 25 (OH) D3 by CYP27B1 yields the hormonally active form 1, 25 (OH) 2D3, which is metabolized to fewer active metabolites by CYP24A1.[5] The active form 1, 25D3 suppresses the development of tumor cells by stimulation of cyclin-dependent kinase inhibitors such as p21, p27, and cystatin D and by inhibition of proliferative genes, including c-my and cyclin D1. Moreover, 1,25D3 can upregulate miR-627, targeting the histone demethylase domain Jumonji containing protein 1A, and therefore inhibiting CRC cell growth in-vitro and in-vivo through epigenetic control.[6] The mechanism of 1,25D3 is the regulation of intestinal epithelium homeostasis and acts as anti-neoplastic because it is capable of interfering with the signaling of Wnt/β-catenin and inhibiting inflammation.[7]

Chronic inflammations have demonstrated a predisposition to tumor development, a remarkable example being IBD linked with an increased incidence of colon cancer.[8] Furthermore, colon cancers that are unrelated to IBD seem to be caused by inflammatory cells. It has been observed that taking nonsteroidal anti-inflammatory drugs (NSAIDs) regularly decreases colon cancer mortality and limits adenomas in familial adenomatous polyposis (FAP) individuals with the adenomatous polyposis coli (Apc) gene mutation.[9] The preventive function of vitamin D in CRC has drawn great interest after Garland and Garland's 1980 study,[10] which showed that the colon cancer death rate was higher in the regions receiving comparatively low solar UV radiation.[10]

Common differences in the VDR gene have been suggested to impact VDR development and activity, which can cause different effects of vitamin D across people.[11] Even though the VDR gene is big (>100 kb) with several single nucleotide polymorphisms (SNPs) found there, a greater part of common SNPs in VDR related to colorectal cancer have been recorded in a few reports.[12] A study by Levin et al., 2012, revealed the connection between the VDR varieties, SNP ID rs 7968585, and low vitamin D and bad clinical outcomes, including cancer.[13] Consequently, our study aimed to assess the relation between vitamin D receptor gene polymorphism and cancer colon which could improve the outcome of colorectal cancer in those populations.

Subjects and Methods

This case control study was conducted in Suez Canal University hospitals during the period from September 2019 to November 2020. The study included 100 patients who were candidate for colonoscopy. Selected patients were adults, from both sexes and presented with abdominal pain, flatulence, diarrhea and/or constipation, weight loss, rectal bleeding, unexplained anemia, with or without occult blood in stool. Based on the endoscopic and histopathological findings, the study subjects were divided in two groups, a group of cancer colon and a control group that showed normal colonoscopy findings.

The study excluded all patients who had advanced illnesses preventing them from undergoing colonoscopies like advanced liver cirrhosis, uncontrollable heart failure, uncontrollable pulmonary disorders, severe sepsis, coagulopathy or had one or more of risk factors to cancer colon occurrence as like inflammatory bowel diseases, family history of colorectal cancer and/or one of colonic polyposis syndromes.

Methods

1. Personal interviewing: All patients were inquired regarding sociodemographic data (age, sex, residence, marital status), their complaints

and comorbidity. A thorough clinical examination was performed

2. Colonoscopy: Preparation for colonoscopy was done according to the European society of gastrointestinal endoscopy (ESGE), by using polyethylene glycol with split dose method accompanied with food fasting and transparent fluids for 24 hours before procedure.[14] Sedation: some of patients were under general anesthesia and others were conscious sedation. The colonoscopy system used was of Pentax product EPM-3500 Video Processor (India). During the procedure, biopsies were taken from cases with suspicious masses. Tissues were fixed in 10% formalin overnight, followed by washing, dehydration with gradual increasing concentration of ethyl alcohol and then clearing by xylene. This is followed by wax impregnations and the tissues were embedded in paraffin. From each block, sections of 3µm thickness were submitted, mounted to glass slide, stained by hematoxylin and eosin (H&E) and examined by an independent qualified pathologist.

3. Vitamin D receptor FokI polymorphism genotyping:

-DNA extraction: Whole blood samples (about 2ml) were collected in ethylenediamine-tetraacetic acid (EDTA) tubes from the study participants. The genomic DNA was extracted from the buffy coat by a commercial kit (QIAamp DNA Mini Kit, Qiagen, Hilden, Germany). The DNA purification was achieved according to the manufacturer's instructions.[15]

-Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP): The PCR-RELP reaction mixture volume used was 20 µl. The reaction was mixed in an amplification PCR tube, consisted of: 1µL of purified DNA with a concentration of approximately 50-150 ng/µl, 10 µL of Master Mix (Thermo Fisher Scientific, USA), 2 µL of both forward and reverse Fok1 primers, and 7 µL of nuclease-free water. The sequences of the Fok1 primers were:

Forward: 5-AGCTGGCCCTGGCACTGACTCTGGCTCT-3 and, Reverse: 5-ATGGAAACACCTTGCTTCTTCTCCCTC-3.

Amplification of the extracted DNA was performed in a thermal cycler according to the following protocol: 5 min of initial denaturation at 95 °C followed by 40 cycles each of denaturation at 95 °C for 30 s, extension at 61 °C for 30 s and final extension at 72 °C for 10 min.

The amplified genomic DNA product was digested by restriction enzymes "Fast Digest Fok-I" (Thermo Fisher Scientific, USA), electrophoresed and visualized on a 2.5% agarose gel. The 267-bp PCR product was digested with FokI and resulted in two fragments: 197-bp and 70-bp. The interpretation of the genotyping was analyzed as following: when the restriction site was absent the allele was designated F and designated f when the restriction site was present. According to the size and number of fragments, three genotypes (FF, Ff, and ff) were determined as FF (homozygote) was present at 267 bp, Ff (heterozygote) at 267, 198, 69 bp and ff (homozygote) at 198, 69 bp.[16]

Statistical analysis

Data were analyzed using the software SPSS for windows version 15 (SPSS Inc., Chicago, IL, USA). Data were expressed as means±SD (quantitative data) or numbers and percentages (qualitative data). Categorical variables were compared by using chi-square test ($\chi 2$). Comparison of genotypes frequencies and association with colorectal cancer were examined for statistical significance using $\chi 2$. Odds ratios (ORs) with 95% confidence intervals (CIs) were also calculated. A statistically significant test was considered at $p < 0.05$.

Results

The study included 100 participants, 50 cases with CRC and 50 controls with normal colonoscopy. The mean age of the studied participants was 46.6±15.7. Of the study subjects, 64 were males, 77 married and about two thirds (59.0%) from rural areas. Compared to the control group, CRC cases were older, with higher frequency of males (66%), married (88%) and 70% from rural areas (Table 1).

Table 1. Demographic data of the studied groups.

Variable		Cases (n=50) n (%)	Control (n=50) n (%)	*p* value
Age	20-39	8 (16)	10 (20)	NS
	40-59	20 (40)	21 (42)	
	>60	22 (44)	19 (38)	
Sex	Male	33 (66)	31 (62)	NS
	Female	17 (34)	19 (38)	
Marital status	Married	44 (88)	33 (66)	NS
	Single	6 (12)	17 (34)	
Residence	Urban	27 (54)	26 (52)	NS
	Rural	23 (46)	24 (48)	

P > 0.05 is not significant (NS).

Concerning the risk factors and comorbidities, many of all studied participants were obese (53%) while overweight was present in 26%, followed by normal body built (18%) and underweight (3%). Alcohol drinking was recalled by one case while current smoking and diabetes mellitus were recalled by 31% each. No significant differences were noted between cases and controls regarding the BMI, smoking, alcohol drinking, or diabetes mellitus (Table 2).

Table 2. Distribution of the studied groups according to risk factors for cancer colon and comorbidities.

Variable		Cases (n=50) n (%)	Control (n=50) n (%)	*p* value
BMI	Underweight	2 (4)	1 (2)	NS
	Normal	8 (16)	10 (20)	
	Overweight	11 (22)	15 (30)	
	Obese	29 (58)	24 (48)	
Smoking	Yes	15 (30)	13 (26)	NS
	No	35 (70)	37 (74)	
Alcohol	Yes	1 (2)	0 (0)	NS
	No	49 (98)	50 (100)	
Diabetes mellitus	Yes	15 (30)	16 (32)	NS
	No	35 (70)	34 (68)	

P > 0.05 is not significant (NS).

Regarding cases with colorectal carcinoma, the most common presentation was constipation (80%), followed by abdominal pain (70%), bleeding (36%), weight loss (24%) and anemia (20%). There were significant differences between cases and the control group in all clinical presentation except anemia (Table 3). Of the CRC cases, approximately three fourths of the cases (74%) had left-sided colon cancer, with a 66% resectability rate.

Table 3. Distribution of the studied groups according to clinical presentations.

Variable		Cases (n=50) n (%)	Control (n=50) n (%)	p value
Constipation	Yes	40 (80%)	27 (54%)	0.006
	No	10 (20%)	23 (46%)	
Abdominal pain	Yes	35 (70%)	19 (38%)	0.001
	No	15 (30%)	31 (62%)	
Bleeding	Yes	18 (36%)	5 (10%)	0.002
	No	32 (64%)	45 (90%)	
Weight loss	Yes	12 (24%)	0 (0%)	<0.001
	No	38 (76%)	50 (100%)	
Anemia	Yes	10 (20%)	5 (10%)	NS
	No	40 (80%)	45 (90%)	

P > 0.05 is not significant (NS).

Polymorphism incidence in colon cancer

As shown in Table 4, the odds ratio of VDR polymorphism between the cases and control groups was high (3 with 95% CI: 0.3-31), however, the difference did not reach statistical significance (*p*= 0.3).

Table 4. Distribution of genotype and allele frequencies of VDR gene polymorphism *FokI* (rs2228570) in CRC patients and normal controls.

VDR gene polymorphism (FokI)(rs2228570)	CRC patients (n=50)	Normal control (n=50)	OR (95%CI)	*p*-value
Genotypes				
FF	21 (42%)	28 (56%)	1.00	
Ff	26 (52%)	21 (42%)	1.65 (0.74-3.70)	NS
Ff	3 (6%)	1 (2%)	4.00 (0.39-41.23)	
F/F-F/f	47 (94%)	49 (98%)	1.00	NS
f/f	3 (6%)	1 (2%)	3.13 (0.31-31.14)	
Alleles				
F	68 (68%)	77 (77%)	1.57 (0.84-2.95)	NS
F	32 (32%)	23 (23%)		

OR: odds ratio, CI: Confidence interval. *P* > 0.05 is not significant (NS).

Discussion

To the best of our knowledge, this is the first study which aimed to evaluate the relation between vitamin D receptor gene polymorphism and CRC occurrence in an Egyptian population. In the study, we focused on patients presented with symptoms or signs suggesting colorectal cancer. The study included 100 patients, divided into a case group and a normal control group (50 patients in each group). There was no difference in patients' characteristic between the study groups.

Also, there was no significant difference in risk factors including BMI, smoking an alcohol intake and DM in both study groups. In addition,

our study offered little assistance regarding relation between vitamin D receptor gene and CRC risks. These results agreed with those of Takeshige et al., 2019[15] which was conducted among Japanese population and showed no relation between the vitamin D receptor and CRC.

Our study showed that there was no correlation between FokI genotypes or alleles and susceptibility of CRC. However, there was a significant positive correlation between the age and colorectal cancer. On the other hand, a study reported association of vitamin D deficiency with increased risk of CRC and mortality.[17] VDR polymorphism was revealed as a significant factor among cancer individuals with the ff homozygous mutant genotype. Patients with stage IV CRC had this mutant genotype. The result suggested that there may be higher mortality among patients with homozygous mutant genotypes compared with heterozygous ones.[17]

Another research study revealed the pleiotropically biological activity of vitamin D with complex anti-cancer effects, including suppression of cells, invasion, apoptosis, cell cycle arrest and differentiation simulations [18]. Furthermore, a study hypothesized that vitamin D receptor could be a possible mediator which might alter the effects of vitamin D. The vitamin D receptor interferes with b-catenin and prevents the deregulation of b-catenin in majority of CRC.[19]

In conclusion, our study data indicated that there was no association between the colorectal cancer and vitamin D receptor gene polymorphism among the Egyptian population. However, there was a significant positive correlation between the age and CRC.

Author Contributions

WO, MGS, AAH, NE, and AG; Conception and design of the study. WO, AAH; Acquisition of data: laboratory or clinical/literature search. WO, MGS, and NE; Analysis and interpretation of data collected. WO, MGS, AAH, NE, and AG; Drafting of the article and/or critical revision. WO, MGS, AAH, NE, and AG; Final Approval.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was reviewed and approved by the Research Ethics Committee at the Faculty of Medicine, Suez Canal University, (ID number 3508#).

Informed consent

The aim and benefits of the study were explained individually to each participant and after their approval, an informed consent was obtained from each participant before being included in the study.

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