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Serum vitamin D receptor (VDR) levels as a potential diagnostic marker for colorectal cancer

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Serum vitamin D receptor (VDR) levels as a potential diagnostic marker for colorectal cancer

ABSTRACT

Colorectal cancer (CRC) is one of the leading causes of mortality and morbidity worldwide, and there has been a significant increase in the incidence of CRC in recent decades. Therefore, there is an urgent need to identify blood biomarkers that can be used for early diagnosis. It is not yet clear whether the level of vitamin D and its receptor, vitamin D receptor (VDR), in the blood are helpful factors in the diagnosis of CRC. Therefore, the study focuses on determining the VDR serum level's contribution and other chemical parameters to the risk of CRC. A total of 189 Saudi participants (66 CRC patients and 123 control patients) aged 20-80 years old were enrolled in this case-control study. A serum sample was collected from each participant, and the levels of VDR and other bone profile tests were determined using ELISA or chemiluminescent assays. P values < 0.05 were considered statistically significant. The results showed a highly significant reduction in the levels of total vitamin D ($P < 0.0001$), VDR ($P < 0.0001$), vitamin D_3 ($P < 0.05$), and calcium ($P < 0.0001$) in the serum of CRC patients compared to the controls. However, the alkaline phosphatase level was higher in CRC patients compared to the controls ($P < 0.0001$). None of the blood markers showed a significant correlation to the progression of CRC ($P >$

0.05). More investigation is needed to elucidate different physiological processes that can be affected by these blood biomarkers, therefore changing the carcinogenesis of CRC.

Keywords: Vitamin D receptor, Calcium, Alkaline phosphatase, Colorectal cancer, Bone biomarkers

1. Introduction

In previous decades, colorectal cancer (CRC) was the least common type of cancer and could be described as rare; however, it recently has become the third leading cause of cancer-related death in developed countries (Kuipers et al., 2015). The global incidence of CRC is projected to increase by 60 percent as early as 2030, which translates into 2.2 million new CRC cases and 1.1 million deaths (Aziz and Allah-Bakhsh, 2018). In Saudi Arabia, CRC is considered the second most common type of cancer, ranked first and third among the male and female population, respectively, and its incidence is increasing over time (Saudi Cancer Registry, 2017). The elevation in the CRC occurrence percentage can be attributed to many factors, including high life expectancy, smoking, obesity, a lack of physical activity, malnutrition, and genetic factors (Takeshige et al., 2015). A number of CRC-related factors require more research and awareness to find an appropriate examination for the population. Because the rate of carcinogenesis of CRC is very high and the pre-clinical stage is too long, more early screening methods are required. In the meantime, the most important treatment regimen for CRC includes the application of chemotherapy and/or radiotherapy after/before the extraction of the tumor based on the stage of the cancer (Welch and Robertson, 2016).

A high number of injuries and deaths are caused by CRC, making it the focus of scientists and researchers in recent years. Many studies have been conducted about the role of vitamin D and its receptor, vitamin D receptor (VDR), alongside calcium in certain types of cancer treatment or prevention, particularly CRC. However, it is not yet clear whether the level of vitamin D in the blood and calcium uptake are considered helpful factors in the pathogenesis of CRC (Jenab et al., 2009). Vitamin D is essential for balancing the calcium and also plays a

4

role in modifying the cell cycle kinetics in colon cells, rectum cells, and other cells. It is well-known that vitamin D performs its function via its receptor, VDR. VDR is a member of the nuclear receptor superfamily that is found in several types of cells, including colorectal epithelial cells (Zhu et al., 2017). This binding enables the transactivation of target genes that promote cellular differentiation, induce apoptosis, inhibit angiogenesis and proliferation, and regulate calcium absorption in the intestines (Jenab et al., 2009; Zhu et al., 2017).

Vitamin D, calcium (Ca), alkaline phosphatase (ALP), magnesium (Mg), parathyroid hormone (PTH), phosphorous (P), and VDR affect several hallmarks of cancer, including the promotion of differentiation, adhesion, the activation of estrogen, the prevention of proliferation and inflammation, and the reduction of the contrast potential (Aggarwal and Kállay, 2016). Understanding these interactions will therefore enhance the potential for cancer prevention. Population studies have mainly investigated and focused on the effect of VDR mutations and the Ca sensing receptor in reducing the risk of colorectal cancer. The results in these studies were contradictory, as some found that there was no relationship and others found a modest reduction in colorectal cancer risk. Due to the inconsistent results and contradictions in the research, it is necessary to perform more studies to reveal the functions of VDR and other related parameters in CRC tumorigenesis (Jenab et al., 2009; Takeshige et al., 2015; Zhu et al., 2017). To the best of our knowledge, fewer studies were conducted in Saudi Arabia to investigate this relationship. Therefore, the current study focused on determining the contribution of VDR serum levels and other chemical parameters to the CRC risk via comparing these blood parameter levels to normal controls and correlating them with clinical CRC stages.

5

2. Materials and methods

2.1 Study design

A total of 189 Saudi participants (66 CRC patients and 123 controls) aged 20-80 years old were enrolled in this case-control study from September 2018 to April 2019. The purpose of the study was explained to all participants and written consent was obtained. The research committee of the Biomedical Ethics Unit at the Faculty of Medicine, King Abdulaziz University (KAU) approved this study (Reference No. 379-17). Demographic data, family cancer history, lifestyle, and the medical risk factors of the participants were ascertained close to the time of blood collection and before diagnosis. For CRC participants, additional information such as tumor subsite, clinical stages, and treatment regimen was collected through written questionnaires. Regarding the controls they must be free of any type of cancer at the time of diagnosis and they were selected with the CRC cases based on their laboratory tests. Blood samples (5 ml) were drawn from each participant into red top vacutainers containing no anticoagulants. All CRC patients' samples were obtained from oncology clinics at King Abdulaziz University Hospital (KAUH), and the control samples were collected from the blood bank at King Fahad General Hospital-Jeddah, Saudi Arabia. The practical experiments were performed at the Cancer and Mutagenesis Unit at King Fahd Medical Research Centre and the Biochemistry Laboratory at KAUH, Jeddah, Saudi Arabia.

2.2 Measurement of serum levels of 1,25-dihydroxy vitamin D

The level of total vitamin D $(1,25(OH)_2D_3)$ was measured in the serum using a commercial LIAISON® 25 OH Vitamin D Total Assay Kit (Reference number: 310600, DiaSorin, USA). This kit is a direct competitive chemiluminescent immunoassay (CLIA) for quantitative determination of total 25 OH vitamin D in serum. During the first incubation, 25 OH vitamin D

was dissociated from its binding protein and bound to the specific antibody on the solid phase in the 96-well plate. After 10 minutes, the tracer (vitamin D linked to an isoluminol derivative) was added. After 10 minutes of incubation, the unbound material was removed with a wash cycle. Subsequently, the starter reagents were added to initiate a flash chemiluminescent reaction. The light signal was measured by a photomultiplier as relative units (RLU) and was inversely proportional to the concentration of 25 OH vitamin D present in calibrators, controls, or samples. The LIAISON® analyzer automatically calculates the concentration of 25 OH vitamin D in the sample. The concentration is expressed in ng/ml, with an assay range of 4 ng/ml to 150 ng/ml. Each sample was run in duplicate for more reliability.

2.3 Measurement of VDR serum level

The VDR level in the serum was determined using a Human Vitamin D Receptor (VDR) ELISA Kit (Catalog number: SG-10743, SinoGeneClon Biotech Co., Ltd, China). This Sandwich ELISA kit is designed for the quantitative measurements of human VDR in direct steps in the sample. First, a purified VDR antibody was adopted to coat a 96-well microtiter plate, making a solid-phase antibody. The serum samples that contained VDR were then added to the corresponding wells. The VDR antibody was then combined with labeled horseradish peroxidase to form an antibody-antigen–enzyme antibody complex. Finally, after washing the wells completely, TMB substrate solution was added to each well until the TMB substrate became blue in color. The reaction was terminated by the addition of a stop solution, and the color change was measured at a wavelength of 450 nm. The concentration of VDR in each sample was then determined by comparing the optical density of the samples to the standard curve. Each sample was repeated in

duplicate for reliable measurements. The detection range of the kit is from 0.1 ng/ml to 8 ng/ml, with a sensitivity of 0.05 ng/ml.

2.4 Measurement of the serum level of bone profile tests

Levels of Ca, ALP, Mg, PTH, and P were measured directly in serum samples using a colorimetric assay on a Dimension VISTA 1500 Intelligent Lab System analyzer (Instrument ID: DV310677, Siemens Healthcare GmbH, Germany).

2.4.1 Measurement of serum levels of 25(OH) D_3

Serum concentrations of 25(OH) D_3 were measured using chemiluminescent assays. The Elecsys Vitamin D total II assay (Reference number: 07464215 190, Roche Diagnostics GmbH, Mannheim, Germany) employs a vitamin D binding protein (VDBP) labeled with a ruthenium complex[a)] as a capture protein to bind 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2. The assay was performed in three steps. In the first incubation step, 20 µl of serum sample was incubated with pretreatment reagent 1 and 2, which released 25-hydroxyvitamin D from the VDBP. In the second incubation step, the pretreated sample was incubated with the ruthenium-labeled VDBP, resulting in the formation of a complex between the 25-hydroxyvitamin D and the ruthenylated VDBP. In the third incubation step, after the addition of streptavidin-coated microparticles and 25-hydroxyvitamin D labeled with biotin, the unbound ruthenylated-labeled VDBPs become occupied. A complex consisting of the ruthenylated VDBP and the biotinylated 25-hydroxyvitamin D was formed and became bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture was aspirated into the measuring cell, where the microparticles were magnetically captured onto the surface of the electrode. The unbound substances were removed using ProCell/ProCell M. With the application of a voltage to the

electrode, a chemiluminescent emission was initiated, which was measured by a photomultiplier. The results were determined via a calibration curve, which is an instrument specifically generated by two-point calibration and a standard curve provided via the reagent barcode. The detection range is 3-100 ng/mL or 7.5-250 nmol/L.

2.4.2 Measurement of parathyroid hormone (PTH) serum levels

The electrochemiluminescence immunoassay (ECLIA) on the Cobas E601 immunoassay analyzer was used to measure serum levels of PTH. The Elecsys assay (Reference number: 11972103 122, Roche Diagnostics GmbH, Mannheim, Germany) for determining intact PTH employs a sandwich test principle in which a biotinylated monoclonal antibody reacts with the N-terminal fragment (1-37), and a monoclonal antibody labeled with a ruthenium complex[a)] reacts with the C-terminal fragment (38-84). The antibodies used in this assay react with epitopes in amino acid regions 26-32 and 37-42 on the PTH polypeptide chain. The assay was done in multiple steps. In the first incubation step, 50 µL of serum, a biotinylated monoclonal PTH-specific antibody, and monoclonal PTH-specific antibody labeled with a ruthenium complex formed a sandwich complex. In the second incubation step, after streptavidin-coated microparticles were added, the complex bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture was aspirated into the measuring cell, where the microparticles were magnetically captured onto the surface of the electrode. The unbound substances were removed with ProCell/ProCell M. With the application of a voltage to the electrode, the chemiluminescent emission was excited, which was measured by a photomultiplier. The detected range for PTH in serum or plasma was 1.20-5000 pg/mL or 0.127-530 pmol/L, with a lower detection limit of 1.20 pg/mL (0.127 pmol/L).

2.5 Statistical analysis

All statistical analyses were performed on GraphPad Prism Version 5.00 (San Diego California, USA). The comparison between two parameters was performed using an unpaired t-test, and a one-way analysis of variance (ANOVA) test was performed for comparisons between more than two parametric groups. P values < 0.05 were considered statistically significant.

3. Results

3.1 Demographic and clinical characteristics of participants

In this study, 189 participants were included. The CRC patients (n = 66) were composed of 50 males and 16 females, representing 75.76 and 24.24 percent, respectively. The controls (n = 123) were divided according to their gender into 104 males and 19 females, representing 84.55 and 15.45 percent, respectively. The analysis of demographic data for the study groups (Table 1) revealed that the majority of the CRC patients were aged 50-59 years old, while the controls were mainly aged 40-49 years old. The weight of the CRC patients (73.17 ± 1.94) was significantly lower ($P < 0.0001$) than the controls (83.85 ± 1.43) as a result of the chemotherapy-related loss of appetite. However, the body mass index (BMI) comparison between each subgroup showed an insignificant difference between CRC patients and the controls ($P > 0.05$) (Table 1). The CRC patients involved in this study were clinically classified according to the TNM staging system. Most patients were in stage IV and had high-grade metastatic secondary tumors, mainly in the liver (n = 34, 51.51%), followed by stage III CRC (n = 18, 27.27%). The

remaining CRC patients were diagnosed with low-grade CRC tumors (n = 14, 21.22% for stage I

and II combined) (Table 1).

Table 1 The demographic and clinical data of colorectal cancer (CRC) patients and healthy
controls

	CRC Patients (n = 66)	Healthy controls (n = 123)	*P* value
Age (years)			
30-39	5	61	
40-49	15	36	
50-59	25	22	Not applicable
60-69 years	9	4	
70-79 years	9	0	
80-89 years	3	0	
Weight (kg)	73.17 ± 1.94 (66)	83.85 ± 1.43 (123)	< 0.0001***
Body mass index (kg/m^2)			
lean (< 18.5 kg/m^2)	16.24 ± 0.89 (5)	17.9 ± 0.0 (1)	Not applicable
Normal weight (18-25 kg/m^2)	22.93 ± 0.39 (23)	22.61 ± 0.60 (14)	0.64
Overweight (25-30 kg/m^2)	27.00 ± 0.39 (16)	27.53 ± 0.20 (56)	0.23
Obese (> 30 kg/m^2)	33.23 ± 0.53 (22)	34.38 ± 0.68 (52)	0.30
CRC Stage (TNM staging system)			
Stage I (n) %	(7) 10.61%		
Stage II (n)%	(7) 10.61%		
IIA	(1)		
IIB	(3)		
IIC	(3)		
Stage III (n)%	(18) 27.27%	Not applicable	Not applicable
IIIA	(7)		
IIIB	(11)		
IIIC	(0)		
Stage IV (n)%	(34) 51.51%		
IVA	(16)		
IVB	(18)		

11

3.2 Determination of vitamin D serum levels and other bone profile tests in CRC patients and controls.

The total vitamin D levels were measured for all study participants. According to the vitamin D level, the CRC patients and controls were classified into two major classes: the insufficient total vitamin D group (if total vitamin D level was < 30 ng/ml) and the sufficient total vitamin D group (if total vitamin D level was ≥ 30 ng/ml) (Table 2). The analysis by unpaired t-test revealed a highly significant reduction ($P < 0.0001$) of total vitamin D levels in CRC patients (36.00 ± 3.32, n = 66) compared to healthy controls (50.10 ± 2.23, n = 123). In the insufficient total vitamin D group, the CRC patients had insufficient levels of vitamin D compared to healthy controls (18.89 ± 1.11, n = 31 versus 24.34 ± 0.85, n = 26, $P = 0.0004$, respectively) (Table 2). The comparison between the serum levels of VDR showed that CRC patients had a very low concentration of VDR in their serum compared to healthy controls (CRC patients: 0.76 ± 0.31 versus controls: 4.08 ± 0.43, $P < 0.0001$).

Other biochemical parameters such as vitamin D_3 (25 $(OH)D_3$), Ca, and ALP showed a significant difference between CRC patients and controls. Regarding the serum level of vitamin D_3 and Ca (Table 2), data showed a significant difference in the level between the two groups; CRC patients had lower levels of vitamin D_3 (33.06 ± 1.98) and Ca (2.21 ± 0.02) in their serum compared to controls (vitamin $D_3 = 38.74 \pm 1.19$ and Ca 2.32 ± 0.02, $P = 0.01$ and $P < 0.0001$, respectively). Interestingly, ALP levels showed a highly significant increase in CRC patients compared to controls. The mean concentration of ALP in CRC patients was 109.9 ± 7.73, compared to 84.29 ± 2.37 in controls ($P < 0.0001$) (Table 2).

Table 2 Comparison of serum level of total vitamin D and bone profile tests between CRC patients and controls

Biochemical test	CRC Patients Mean ± SEM (66)	Controls Mean ± SEM (123)	*P* value
Total vit D (ng/ml)	36.00 ± 3.32	50.10 ± 2.23	< 0.0001***
< 30 ng/ml	18.89 ± 1.11 (n = 31)	24.34 ± 0.85 (n = 26)	0.0004***
≥ 30 ng/ml	53.68 ± 4.88 (n=35)	57.14 ± 2.43 (n = 97)	0.50
VDR (ng/ml)	0.76 ± 0.31	4.08 ± 0.43	< 0.0001***
25 (OH)D_3 (nmol/L)	33.06 ± 1.98	38.74 ± 1.19	0.01*
PTH (pmol/L)	4.49 ± 0.28	5.05 ± 0.19	0.11
Ca (mmol/L)	2.21 ± 0.02	2.32 ± 0.02	< 0.0001***
Mg (mmol/L)	0.89 ± 0.01	0.92 ± 0.01	0.10
P (mmol/L)	1.02 ± 0.03	1.00 ± 0.02	0.49
ALP (U/L)	109.9 ± 7.73	84.29 ± 2.37	< 0.0001***

Total vit D: total vitamin D; VDR: vitamin D receptor; 25 (OH)D_3: 25-hydroxy vitamin D_3; PTH: Parathyroid hormone; Ca: Calcium; Mg: Magnesium; P: phosphorous; ALP: alkaline phosphatase,*P* value was calculated with unpaired t test; *P* < 0.05*, *P* < 0.001***

3.3 Relationship between total vitamin D serum levels and other biochemical tests with CRC stages

The mean of each biochemical test was calculated for each stage, then a comparison a with one-way ANOVA test was performed. The results (Table 3) revealed that none of the measured biochemical parameters in this study were related to CRC progression, indicating that these blood tests can be used in the diagnosis of CRC. However, alone, they do not have any significant correlation with progression. When a comparison was made with the controls using a one-way ANOVA test, the results showed a highly significant difference in total vitamin D, VDR, and Ca levels (*P* = 0.0038, *P* < 0.0001, and *P* = 0.0015, respectively) between high-grade CRC patients, particularly stage III, and controls. However, ALP was higher in low-grade stage II CRC patients compared to the healthy controls (*P* = 0.0008) (Table 3).

Table 3 Comparison between bone profile tests and different TNM clinical stages of CRC

Biochemical tests	Stage I CRC patients	Stage II CRC patients	Stage III CRC patients	Stage IV CRC patients	Controls	P value
Total vit D (ng/ml)	36.34 ± 8.49	52.96 ± 19.75	30.43 ± 3.93	35.69 ± 4.53	50.10 ± 2.28	$0.0038**$
VDR (ng/ml)	0.49 ± 0.13	0.34 ± 0.07	0.28 ± 0.09	0.39 ± 0.10	4.08 ± 0.43	$< 0.0001***$
25 $(OH)D_3$ (nmol/L)	33.86 ± 6.50	32.67 ± 5.59	35.43 ± 3.97	31.65 ± 2.79	38.74 ± 1.19	0.13
PTH (pmol/L)	4.58 ± 0.94	3.75 ± 0.75	3.84 ± 0.37	4.98 ± 0.44	5.05 ± 0.19	0.18
Ca (mmol/L)	2.21 ± 0.11	2.25 ± 0.06	2.18 ± 0.03	2.22 ± 0.02	2.32 ± 0.02	$0.0015**$
Mg (mmol/L)	0.81 ± 0.04	0.87 ± 0.03	0.89 ± 0.02	0.91 ± 0.02	0.92 ± 0.01	0.22
P (mmol/L)	0.93 ± 0.03	1.05 ± 0.04	1.04 ± 0.06	1.03 ± 0.04	1.00 ± 0.02	0.71
ALP (U/L)	94.29 ± 18.52	120.1 ± 35.95	98.65 ± 9.55	117.6 ± 12.01	84.29 ± 2.37	$0.0008***$

Total vit D: total vitamin D; VDR: vitamin D receptor; 25 $(OH)D_3$: 25-hydroxy vitamin D_3; PTH: Parathyroid hormone; Ca: Calcium; Mg: Magnesium; P: phosphorous; ALP: alkaline phosphatase, P value was calculated using one-way ANOVA test, P value > 0.05 (not significant), $P < 0.05*$, $P < 0.01**$, $P < 0.001***$

4. Discussion

CRC is a wide-spread tumor with a high mortality rate worldwide (Bray et al., 2018). An epidemiologic study revealed that CRC can be developed from an interaction among genetic, epigenetic, and environmental factors (Wong et al., 2019). Most CRC cases are sporadic and not hereditary. Obesity and metabolism dysregulation are considered among the top-ranking risk factors for CRC (Karahalios et al., 2015). Mineral and vitamin supplements have shown remarkable advances in preventing CRC (Takeshige et al., 2015). Vitamin D has other functions beyond the regulation of Ca and P metabolism. It has been shown that vitamin D has anti-neoplastic activity in many cancers, including colorectal cancer (Dou et al., 2016). Most of the conducted research thus far studied the relationship between the vitamin D level and VDR mutations in the carcinogenesis of CRC (Slattery et al., 2010; Alkhayal et al., 2016; Budhathoki et al., 2016; Ferrer-Mayorga et al., 2017). However, less research has focused on the application of circulating the VDR level as a diagnostic marker for CRC. Therefore, in the current study, the level of VDR in the serum of CRC patients was determined and related to the different clinical statuses of vitamin D and other bone profile tests that previously had shown an association with CRC carcinogenesis. The results of this study showed highly significant decreases in serum levels of VDR ($P < 0.0001$), vitamin D_3 ($P < 0.01$), and Ca ($P < 0.0001$) in CRC patients compared to controls. However, the ALP level was higher in CRC patients compared to controls ($P < 0.0001$). None of the significant blood markers showed a correlation with the clinical manifestation of CRC ($P > 0.05$). Conversely, other blood markers such as P, Mg, and PTH did not show any significant differences between the two groups ($P > 0.05$).

The serum level of total vitamin D showed a significant difference between CRC patients and controls, specifically in patients with insufficient level of vitamin D. This agreed with a

study conducted on 94 CRC patients in the San Francisco Bay area, which showed that vitamin D insufficiency was seen in more than half of the patients, and with vitamin D supplementation, serum levels did not decrease significantly after six months of chemotherapy (Savoie et al., 2017). Most studies on human cancer cells showed that expression of VDR and CYP27B1 increases initially at the transformation stage of cancer, but when the tumor becomes more malignant and aggressive, the expression of VDR and CYP27B1 decreases, and the expression of CYP24A1 strongly increases (Matusiak and Benya, 2007; Lopes et al., 2010). The explanation for such a change in levels is due to changes in physiological conditions. In the first steps of tumorigenesis, the synthesis and signaling of $1,25(OH)_2D_3$ are upregulated as a physiological defense system against epithelial tumor progression. Later, when tumors dedifferentiate (high-grade tumors), VDR and CYP27B1 levels drop while CYP24A1 expression increases, implicating that total vitamin D $(1,25(OH)_2D_3)$ concentrations decrease as shown in the current study.

Since there is a proportional relation between vitamin D and serum levels of Ca, the level of Ca in most studies performed on CRC patients with deficient or insufficient vitamin D levels decreased. In a study by Fuszek et al. (2004), blood analysis showed a lower level of ionized Ca in the serum of CRC patients compared with normal vitamin D levels. However, the ionized Ca concentration was inversely correlated with the serum level of CA 19-9, which is considered a prognostic marker for CRC. Moreover, the results showed that there was no difference in the distribution of Ca sensing receptor (CaSR) genotypes between CRC patients and controls (Fuszek et al., 2004). Further, in a Swedish cohort study, high serum Ca levels were associated with lower breast cancer mortality, while serum 25 hydroxyvitamin D_3 ($25D_3$) levels and breast

cancer mortality showed a u-shaped correlation (Luo et al., 2013; Huss et al., 2014). Interestingly, a study by Ahearn et al. (2016) showed that high expression of CaSR in prostate cancer cells was associated with lethal progression of the disease if the tumors expressed low VDR levels, but not if the tumors had high VDR levels (Ahearn et al., 2016). Serum ALP levels are frequently elevated in patients with metastatic CRC. However, in the meantime, the significance of ALP in terms of detecting hepatic metastasis in CRC patients or other cancers is not well-established. Saif et al. (2005) showed that ALP levels were elevated in 74 percent of patients with liver metastases and in 33 percent of patients without liver metastases in a time-dependent manner (Saif et al., 2005). A recent study found that an elevated level of ALP is associated with poor survival rates of CRC patients. They found that significant elevations in ALP levels were correlated with carcinoembryonic antigen (CEA) $\geq$ 5 ng/ml, aspartate aminotransferase (AST) $\geq$ 43 U/L, total bilirubin $\geq$ 1.5 U/L, albumin < 3.5 g/dL, and stage IV disease (Hung et al., 2017).

Conclusion

The results showed that the levels of VDR can be used as a diagnostic marker for CRC, as its concentration decreases in the patients' serum compared to healthy controls. The relationship of VDR was assessed with other blood markers that are usually affected by the level of vitamin D. Notably, the level of VDR was not related to the clinical progression of CRC, as it showed a close serum level in different stages.

However, this study has a number of limitations—most importantly the lower number of CRC patients and the mismatched alignment with controls. More investigation is needed to

elucidate different physiological processes that can be affected by these blood biomarkers, therefore contributing to CRC pathogenesis.

Availability of data materials

The datasets are used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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