The clinical significance of vitamin D levels and vitamin D receptor mRNA expression in colorectal neoplasms

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Abstract

Background/Aim: This study aimed to investigate the clinical significance of changes in vitamin D [25(OH)D] levels and vitamin D receptor (VDR) mRNA expression in colorectal adenoma development.

Methods: Plasma concentrations of 25(OH)D and mRNA expression of VDR in tissues were determined by enzyme-linked immunosorbent assay (ELISA) and real-time fluorescence quantitative polymerase chain reaction (RT-qPCR), respectively. In addition, the concentration of plasma 25(OH)D and levels of VDR mRNA in tissues were compared among healthy individuals and adenoma and adenocarcinoma patients.

Results: Vitamin D receptor expression in colorectal adenocarcinoma tissues was significantly lower than that in para-cancerous tissues that were >5 cm away from malignant tumor sites (p < 0.01). The level of VDR expression in normal colorectal tissues from healthy individuals was significantly higher than that in colorectal adenomas (p < 0.01) and colorectal adenocarcinomas (p < 0.01); however, the VDR expression was not significantly different between colorectal adenomas and colorectal adenocarcinomas (p = 0.106). The concentration of 25(OH)D in healthy individuals was significantly higher than that in patients with colorectal adenomas (p < 0.01) and colorectal adenocarcinomas (p < 0.01); however, the concentration of 25(OH)D was not significantly different between colorectal adenomas and colorectal adenocarcinomas (p = 0.489). A low concentration of 25(OH)D was considered a risk factor for colorectal adenoma and colorectal adenocarcinoma, with odds ratios of 4.875 and 2.925, respectively.

Conclusions: The 25(OH)D levels and VDR mRNA expression might be associated with the development of colorectal adenoma and its progression to adenocarcinoma.

KEYWORDS
colorectal adenocarcinoma, colorectal adenoma, vitamin D, vitamin D receptor
INTRODUCTION

Colorectal cancer is a common malignancy, with global incidence ranks of 3 and 2 among men and women, respectively. Currently, the incidence and mortality rates of colorectal cancer in developing and low-income countries have exhibited a continuously increasing trend. This trend is especially evident in China, where lifestyle changes have led to a significantly increased incidence of colorectal cancer. The risk of colorectal cancer decreases when the detection of colorectal adenomas increases. The entire process of the development and progression of colorectal cancer involves multiple stages, from abnormal hyperplasia of the intestinal mucosal epithelia to the formation of the final neoplasm. Vitamin D \([25(OH)D]\) is a special fat-soluble vitamin that was previously considered to be associated with the development and progression of colorectal cancer. \(^2\) \(25(OH)D\) functions by binding to the vitamin D receptor (VDR). The binding product mediates the regulation of proliferation, metaplasia, apoptosis, and the colonic epithelial cell cycle, which influences the development and progression of colorectal cancer. \(^2\) Two studies have investigated the role of \(25(OH)D\) in the prevention and treatment of precancerous lesions (adenoma) in colorectal cancer. \(^3\) The inclusion criteria for the group with colorectal adenocarcinoma were as follows: (1) patients diagnosed with colorectal adenoma by colonoscopy and with a biopsy specimen confirmed to be colorectal adenoma after pathological examination; (2) patients without any recent acute inflammation and liver or kidney dysfunction; and (3) patients without history of tumors, immune diseases, cardiovascular diseases, various acute and chronic inflammations, liver and kidney diseases, severe skin diseases, other major diseases, or surgery. Patients with familial polyposis or black spot polyp syndrome were excluded.

The inclusion criteria for the group with colorectal adenocarcinoma were as follows: (1) patients diagnosed with colorectal cancer by colonoscopy and with a biopsy specimen confirmed to be colorectal adenoma by pathological examination; (2) patients without any recent acute inflammation and normal liver and kidney functions; and (3) patients without history of tumors other than colorectal cancer, immune diseases, cardiovascular diseases, liver or kidney diseases, serious skin diseases, other major diseases, or surgery. Patients with colorectal cancer that was aggravated by familial polyposis and black-associated polyp syndrome were excluded.

The inclusion criteria for the normal control group were as follows: (1) participants with good health status, normal colonoscopy results, and no recent inflammation or liver and kidney dysfunction; participants who had recently completed physical examinations were preferred; and (2) participants without history of tumors, immune diseases, cardiovascular diseases, various acute and chronic inflammations, liver and kidney diseases, severe skin diseases, other major diseases, or surgery.

MATERIALS AND METHODS

Patient selection

The current study used the case-control method to randomly enroll 49 patients with colorectal adenomas and 55 patients with colorectal adenocarcinomas who were admitted to the Ningbo First Hospital in Zhejiang, China between 2013 and 2015. For analysis and comparison, 59 healthy individuals were randomly recruited as the normal control group. Healthy individuals were determined when they presented with good physical results after routine medical examinations at our hospital. Participants from the same province who were admitted to the hospital during the sunny seasons and who did not take calcium tablets and dietary \(25(OH)D\) were included in this study. The institutional review boards of the Ningbo First Hospital approved the study, and all 163 participants provided written informed consent.

Biopsy specimens were obtained from patients who underwent colonoscopy. Colorectal adenocarcinoma tissues were collected directly from the tumor site by colonoscopy, while the para-cancerous tissues were collected >5 cm away from the malignant tumor site. Patients were first diagnosed with colorectal adenomas or colorectal adenocarcinomas; thus, they did not receive any type of treatment modality at the time of sample collection. Normal colorectal tissues were collected from the colorectum of healthy individuals using colonoscopy.

Detection of plasma \(25(OH)D\) concentration by enzyme-linked immunosorbent assay

Blood samples from all participants were collected. Briefly, 3-5 mL of blood was collected in standard EDTA tubes from each participant. Plasma samples were then extracted by centrifugation and stored at \(-80^\circ\text{C}\). Plasma concentrations of \(25(OH)D\) were determined by enzyme-linked immunosorbent assay (ELISA). The human \(25(OH)D\) ELISA kit was purchased from Shanghai Qiaodu Biotechnology Co. Ltd., China (Cat#: DRE10185), and the \(25(OH)D\) concentration was measured according to the manufacturer’s protocol. The \(25(OH)D\) concentrations in healthy individuals ranged between 20 and 150 ng/mL. \(25(OH)D\) concentrations between 20 and 30 ng/mL indicates \(25(OH)D\) insufficiency, concentrations between 10 and 20 ng/mL indicates \(25(OH)D\) deficiency, and concentrations <10 ng/mL indicates severe \(25(OH)D\) deficiency. \(^1\)
2.3 | Detection of VDR mRNA levels in tissues by reverse-transcription quantitative PCR

Twenty-three cases of normal intestinal mucosa, 41 adenomas, and 45 adenocarcinomas and para-cancerous tissues were used to detect VDR mRNA levels. The collected colorectal tissues were stored in 2 mL centrifuge tubes containing 1 mL RNAlater® Stabilization Solution (Thermo Fisher Scientific, Waltham, MA, USA) at −80°C. RNA was then extracted using the TRizol method and reverse-transcribed into cDNA using a reagent kit purchased from Takara (Cat#: RR047A; Shiga-ken, Japan). The primer sequences for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and VDR genes are listed in Table 1. Primer sequences were obtained from the NCBI database (http://www.ncbi.nlm.nih.gov/), and the primers were synthesized by Shanghai Invitrogen Biological Engineering Co., Ltd. (Shanghai, China). GAPDH was used as the reference gene for the quantification of the VDR gene in tissues. A Takara reagent kit was used for reverse-transcription quantitative PCR (RT-qPCR). The PCR mixture included 10 µL SYBR Premix Ex Taq II, 0.8 µL of each primer (10 µM), 0.4 µL of ROX Reference Dye II, 2 µL of the cDNA solution, and 6 µL of RNA-free H2O. After the RT-qPCR reaction system was established, amplification was performed by qPCR in an Applied Biosystems Step One Plus Real-Time PCR System (Chicago, Illinois, USA). Each sample was tested in triplicate. The thermocycling program was set as follows: (1) 30 s at 95°C for initial denaturation; (2) 40 cycles of 5 s at 95°C for denaturation, 15 s at 55°C for annealing, and 19 s at 72°C for extension; and (3) 15 s at 95°C, 1 min at 60°C, and 15 s at 95°C for final dissociation. The Ct values were recorded, and the relative mRNA expression levels were analyzed using the 2−ΔΔCt method.13

2.4 | Statistical analysis

The normality of the data was tested when the sample size was ≤40. SPSS version 19.0 was used for the statistical analyses of data using universal descriptions. All data are expressed as the mean ± standard deviation (mean ± SD). For sample size >40, data were analyzed using the t test between the two groups and one-way analysis of variance (ANOVA) among more than two groups. For sample size ≤40, the data were analyzed using the Wilcoxon rank-sum test. Correlation analysis was performed using Spearman’s rank correlation analysis. Statistical significance was set at p < 0.05.

| Primer name     | Sequence                                      |
|-----------------|-----------------------------------------------|
| GAPDH forward   | GGAAGGTGAAAGTGCTGGACGC                      |
| GAPDH reverse    | AATGAAAGGCTTACATGG                         |
| VDR forward     | CTGACCTTGAGACTTGTG                          |
| VDR reverse     | TTCTCTGACACTTCTCATC                         |

Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; VDR, vitamin D receptor.

3 | RESULTS

3.1 | Distribution of plasma 25(OH)D in the three groups

Among the 163 participants, 24, 75, 12, and 52 had 25(OH)D insufficiency (14.6%), 25(OH)D deficiency (46%), severe 25(OH)D deficiency (7.4%), and normal 25(OH)D levels (31.9%), respectively. In the normal control group, 25(OH)D insufficiency, 25(OH)D deficiency, severe 25(OH)D deficiency, and normal 25(OH)D levels accounted for 1.37%, 30.5%, 3.4%, and 53.1%, respectively. In the adenoma group, 25(OH)D insufficiency, 25(OH)D deficiency, severe 25(OH)D deficiency, and normal 25(OH)D levels accounted for 10.2%, 57.1%, 14.3%, and 18.4%, respectively. In the adenocarcinoma group, 25(OH)D insufficiency, 25(OH)D deficiency, severe 25(OH)D deficiency, and normal 25(OH)D levels accounted for 18.2%, 54.5%, 5.5%, and 21.8%, respectively (Figure 1). A correlation analysis between the 25(OH)D levels (concentration <20 ng/ml) and the presence of neoplasms in the adenoma group showed that the odds ratio (OR) was 4.875 and the 95% confidence interval (CI) was 2.144–11.084 (p < 0.01). Between the 25(OH)D levels (concentration <20 ng/ml) and the presence of neoplasms in the adenocarcinoma group, the OR was 2.925 and the 95% CI was 1.364–6.271 (p < 0.01). These results suggest that 25(OH)D deficiency is a risk factor for the development of colorectal adenoma and colorectal adenocarcinoma.

3.2 | Comparison of plasma 25(OH)D concentrations among the three groups

The plasma 25(OH)D concentration in the normal control group (41.35 ng/mL ± 4.570 ng/mL) was significantly higher than that in the colorectal adenoma group (22.28 ng/mL ± 2.445 ng/mL, p < 0.01) and the colorectal adenocarcinoma group (26.01 ng/mL ± 2.927 ng/mL, p < 0.01); however, no significant difference was observed between the adenoma and adenocarcinoma groups (p = 0.47 and p > 0.05, respectively; Figure 2). Furthermore, the plasma 25(OH) D concentrations in the colorectal adenoma and colorectal adenocarcinoma groups did not correlate with patients’ age, sex, smoking history, alcohol consumption history, location, tumor size, tumor differentiation level, lymph node metastasis, or neoplasm pathologic type (Table 2).

3.3 | Level of VDR expression in tissues

In the 45 paired colorectal adenocarcinoma and para-cancerous tissues, we observed that the level of VDR mRNA expression in the adenocarcinoma group was 0.305 ± 0.06-fold greater than that in the para-cancerous group (p < 0.01; Figure 3). When normal tissues were used as the control, the level of VDR mRNA expression in normal tissues (1.00 ± 0.22) was significantly higher than that in
adenoma (0.52 ± 0.08, p < 0.01) and adenocarcinoma (0.37 ± 0.07, p < 0.05) tissues; however, the differences between the adenoma and adenocarcinoma groups were not statistically significant (p > 0.05; Figure 4). The results revealed no correlation between VDR mRNA expression and patients’ age, sex, smoking history, alcohol consumption history, tumor size, tumor differentiation level, lymph node metastasis, or neoplasm pathologic type in the colorectal adenocarcinoma and adenoma groups (Table 3).

4 | DISCUSSION

In the current study, the expression of VDR in colorectal adenocarcinoma tissues was significantly lower than that in para-cancerous tissues, and the level of VDR expression in normal colorectal tissues was significantly higher than that in colorectal adenomas and colorectal adenocarcinomas. The concentration of 25(OH)D in healthy individuals was significantly higher than that in patients with colorectal adenomas and colorectal adenocarcinomas; however, the 25(OH)D concentration and VDR expression were not significantly different between colorectal adenomas and colorectal adenocarcinomas.

The development and progression of colorectal neoplasms involve a process that is associated with multiple steps and factors. The incidence of colorectal neoplasms is rising, and advanced adenomas tend to be found predominantly in the distal colorectum in patients aged 45–49 years. In recent years, the function of 25(OH)D in the development and prevention of colorectal neoplasms has received considerable attention. The nested control study conducted by Jenab et al. supports the notion that 25(OH)D levels in circulating blood are negatively correlated with the risk of developing colorectal cancer. A meta-analysis also concluded that increased 25(OH)D uptake helps in reducing the development of colorectal cancer. The results of our study confirmed that the 25(OH)D levels in patients with colorectal adenoma and colorectal adenocarcinoma were significantly lower than those in healthy individuals, and the 25(OH)D concentrations between the two were not significantly different. Furthermore, 25(OH)D deficiency was shown to be a risk factor for the development of colorectal adenomas (OR, 4.875) and colorectal adenocarcinoma (OR, 2.925). Colorectal adenomas are precancerous lesions of colorectal cancer. Therefore, colorectal cancer patients might already be 25(OH)D deficient at the time when colorectal adenomas are present, and 25(OH)D deficiency would not worsen as the disease progresses.

25(OH)D has been reported for years to be associated with the regulation of calcium phosphate homeostasis. It is also involved in the regulation of immune response and brain development, such as...
| Classification                  | Colorectal adenoma | Colorectal adenocarcinoma |
|--------------------------------|--------------------|---------------------------|
|                                | N  | 25(OH)D (ng/mL) | P   | N  | 25(OH)D (ng/mL) | P   |
| Age (years)                    |    |                |     |    |                |     |
| <60                            | 22 | 20.23 ± 2.84   | 0.101 | 25 | 24.41 ± 3.88   | 0.372 |
| ≥60                            | 33 | 29.87 ± 4.41   | 0.015 | 24 | 20.06 ± 2.95   | 0.372 |
| Sex                            |    |                |     |    |                |     |
| Male                           | 33 | 24.14 ± 3.21   | 0.203 | 34 | 23.09 ± 3.19   | 0.494 |
| Female                         | 22 | 28.82 ± 5.56   | 0.203 | 15 | 20.45 ± 3.48   | 0.494 |
| Alcohol consumption            |    |                |     |    |                |     |
| Yes                            | 11 | 20.84 ± 3.14   | 0.318 | 13 | 24.94 ± 5.27   | 0.569 |
| No                             | 44 | 27.31 ± 3.56   | 0.318 | 36 | 21.31 ± 2.76   | 0.569 |
| Smoking                        |    |                |     |    |                |     |
| Yes                            | 15 | 25.48 ± 3.77   | 0.865 | 15 | 23.6 ± 2.96    | 0.527 |
| No                             | 40 | 26.21 ± 3.91   | 0.865 | 34 | 19.28 ± 4.37   | 0.527 |
| CEA                            |    |                |     |    |                |     |
| Negative                       | 29 | 25.97 ± 3.81   | 0.867 |    |                |     |
| Positive                       | 26 | 26.06 ± 4.58   | 0.867 |    |                |     |
| CA-199                         |    |                |     |    |                |     |
| Negative                       | 39 | 27.41 ± 3.22   | 0.392 |    |                |     |
| Positive                       | 16 | 22.61 ± 6.39   | 0.392 |    |                |     |
| Adenocarcinoma differentiation |    |                |     |    |                |     |
| High                           |  8 | 24.42 ± 3.15   | 0.409 |    |                |     |
| Medium                         | 38 | 28.10 ± 4.01   | 0.409 |    |                |     |
| Low                            |  9 | 18.71 ± 1.14   | 0.409 |    |                |     |
| Lymph node metastasis          |    |                |     |    |                |     |
| N0                             | 36 | 26.99 ± 3.86   | 0.653 | 69 | 22.83 ± 2.91   | 0.803 |
| N1-3                           | 19 | 24.16 ± 4.27   | 0.653 | 69 | 22.83 ± 2.91   | 0.803 |
| Invasion level                 |    |                |     |    |                |     |
| T1                             |  3 | 16.48 ± 3.50   | 0.464 |    |                |     |
| T2                             |  8 | 28.15 ± 6.12   | 0.464 |    |                |     |
| T3                             | 26 | 27.61 ± 4.85   | 0.464 |    |                |     |
| T4                             | 18 | 24.3 ± 3.56    | 0.464 |    |                |     |
| TNM stage                      |    |                |     |    |                |     |
| I, II                          | 33 | 24.02 ± 3.57   | 0.535 |    |                |     |
| III, IV                        | 22 | 26.00 ± 4.31   | 0.535 |    |                |     |
| Location                       |    |                |     |    |                |     |
| Colon                          | 28 | 25.88 ± 3.93   | 0.872 | 34 | 22.83 ± 2.91   | 0.803 |
| Rectum                         | 27 | 26.15 ± 4.42   | 0.872 | 15 | 21.01 ± 4.66   | 0.803 |
| Adenocarcinoma size            |    |                |     |    |                |     |
| ≥5 cm                          |  20| 27.2 ± 4.28    | 0.375 |    |                |     |
| <5 cm                          | 35 | 23.92 ± 3.93   | 0.375 |    |                |     |
| Neoplastic grade               |    |                |     |    |                |     |
| Low grade                      | 33 | 22.05 ± 3.09   | 0.893 |    |                |     |
| High grade                     | 16 | 22.74 ± 4.07   | 0.893 |    |                |     |
the immunomodulation of Th1 and Th2 cell proliferation and inflammatory cytokine secretion.\textsuperscript{17-19} 25(OH)D is also highly associated with cancer, although 25(OH)D supplementation alone does not reduce the incidence of cancer and cancer mortality, even after long-term follow-up.\textsuperscript{20} The effect of 25(OH)D on colorectal adenoma prevention cannot be estimated because of the different results shown.
### TABLE 3  Correlation between the level of VDR expression and the clinicopathologic characteristics of patients in the colorectal adenoma and adenocarcinoma groups

| Classification                         | Colorectal adenoma |                        |                        | Colorectal adenocarcinoma |                        |                        |
|---------------------------------------|--------------------|------------------------|------------------------|---------------------------|------------------------|------------------------|
|                                       | N                  | Fold change            | P                      | N                         | Fold change            | P                      |
| Age (years)                           |                    |                        |                        |                           |                        |                        |
| <60                                   | 15                 | 0.31 ± 0.14            | 0.294                  | 17                        | 0.33 ± 0.07            | 0.117                  |
| ≥60                                   | 30                 | 0.41 ± 0.08            |                        | 24                        | 0.65 ± 0.12            |                        |
| Sex                                   |                    |                        |                        |                           |                        |                        |
| Male                                  | 22                 | 0.41 ± 0.08            | 0.386                  | 21                        | 0.41 ± 0.10            | 0.183                  |
| Female                                | 23                 | 0.33 ± 0.14            |                        | 20                        | 0.64 ± 0.13            |                        |
| Alcohol consumption                   |                    |                        |                        |                           |                        |                        |
| Yes                                   | 9                  | 0.27 ± 0.08            | 0.193                  | 11                        | 0.47 ± 0.15            | 0.428                  |
| No                                    | 36                 | 0.40 ± 0.08            |                        | 30                        | 0.54 ± 0.10            |                        |
| Smoking                               |                    |                        |                        |                           |                        |                        |
| Yes                                   | 11                 | 0.43 ± 0.18            | 0.205                  | 15                        | 0.58 ± 0.14            | 0.336                  |
| No                                    | 34                 | 0.35 ± 0.07            |                        | 26                        | 0.49 ± 0.10            |                        |
| 25(OH)D level a                       |                    |                        |                        |                           |                        |                        |
| <20 ng/mL                             | 13                 | 0.32 ± 0.24            | 0.249                  | 13                        | 0.54 ± 0.15            | 0.103                  |
| ≥20 ng/mL                             | 8                  | 0.50 ± 0.12            |                        | 6                         | 0.92 ± 0.24            |                        |
| CEA                                   |                    |                        |                        |                           |                        |                        |
| Negative                              | 21                 | 0.27 ± 0.05            | 0.193                  |                           |                        |                        |
| Positive                              | 24                 | 0.46 ± 0.12            |                        |                           |                        |                        |
| CA-199                                |                    |                        |                        |                           |                        |                        |
| Negative                              | 28                 | 0.38 ± 0.09            | 0.810                  |                           |                        |                        |
| Positive                              | 17                 | 0.36 ± 0.11            |                        |                           |                        |                        |
| Adenocarcinoma differentiation        |                    |                        |                        |                           |                        |                        |
| High                                  | 3                  | 0.16 ± 0.10            | 0.208                  |                           |                        |                        |
| Medium                                | 37                 | 0.34 ± 0.07            |                        |                           |                        |                        |
| Low                                   | 5                  | 0.75 ± 0.30            |                        |                           |                        |                        |
| Lymph node metastasis                 |                    |                        |                        |                           |                        |                        |
| N0                                    | 31                 | 0.28 ± 0.08            | 0.127                  |                           |                        |                        |
| N1–3                                  | 14                 | 0.41 ± 0.09            |                        |                           |                        |                        |
| Invasion level                        |                    |                        |                        |                           |                        |                        |
| T1                                    | 3                  | 0.25 ± 0.14            | 0.749                  |                           |                        |                        |
| T2                                    | 5                  | 0.29 ± 0.10            |                        |                           |                        |                        |
| T3                                    | 25                 | 0.45 ± 0.12            |                        |                           |                        |                        |
| T4                                    | 12                 | 0.28 ± 0.08            |                        |                           |                        |                        |
| TNM stage                             |                    |                        |                        |                           |                        |                        |
| I, II                                 | 29                 | 0.44 ± 0.10            | 0.175                  |                           |                        |                        |
| III, IV                               | 14                 | 0.25 ± 0.07            |                        |                           |                        |                        |
| Location                              |                    |                        |                        |                           |                        |                        |
| Colon                                 | 26                 | 0.48 ± 0.11            | 0.122                  | 37                        | 0.56 ± 0.09            | 0.103                  |
| Rectum                                | 19                 | 0.22 ± 0.05            | 0.122                  | 4                         | 0.2 ± 0.16             |                        |
| Adenocarcinoma size                   |                    |                        |                        |                           |                        |                        |
| ≥5 cm                                 | 13                 | 0.43 ± 0.20            | 0.730                  |                           |                        |                        |
| <5 cm                                 | 32                 | 0.36 ± 0.06            |                        |                           |                        |                        |

(Continues)
TABLE 3 (Continued)

| Classification          | Colorectal adenoma | Colorectal adenocarcinoma |
|-------------------------|-------------------|---------------------------|
|                         | N     | Fold change | P    | N     | Fold change | P    |
| Neoplastic grade        |       |            |     |       |            |     |
| Low grade               | 34    | 0.49 ± 0.09 | 0.147 |       |            |     |
| High grade              | 7     | 0.72 ± 0.23 | 0.21  |       |            |     |
| Adenoma size            |       |            |     |       |            |     |
| <1 cm                   | 18    | 0.56 ± 0.13 | 0.693 |       |            |     |
| ≥1 cm                   | 23    | 0.50 ± 0.10 | 0.51  |       |            |     |
| Solitary/multiple       |       |            |     |       |            |     |
| Solitary                | 8     | 0.63 ± 0.22 | 0.621 |       |            |     |
| Multiple                | 33    | 0.50 ± 0.09 | 0.802 |       |            |     |
| Pathologic classification|      |            |     |       |            |     |
| Tubular                 | 33    | 0.52 ± 0.09 | 0.802 |       |            |     |
| Tubulovillous           | 6     | 0.49 ± 0.23 | 0.66  |       |            |     |
| Villous                 | 2     | 0.67 ± 0.66 | 0.66  |       |            |     |

Data are expressed as numbers or means ± standard deviations (SDs). 25(OH)D, vitamin D; CEA, carcinoembryonic antigen; CA-199, carbohydrate antigen 199. TNM stage, tumor, lymph node, and metastasis stage. The fold change was determined by calculating the ratio of VDR mRNA levels in the adenoma or adenocarcinoma to that in normal colorectal tissues. Correlation analysis was performed using Spearman's rank correlation analysis. Statistical significance was set at \( p < 0.05 \).

*Both the 25(OH)D concentration and the level of VDR mRNA expression in 21 patients in the colorectal adenoma group and in 19 patients in the colorectal adenocarcinoma group were detected.

in clinical trials. An investigation demonstrated that vitamin D3 (1000 IU daily) did not cause changes in intestinal barrier function-related biomarker expression in a subset of 105 participants from a large colorectal adenoma recurrence chemoprevention clinical trial.9

Other clinical trials have shown that no association exists between daily vitamin D3 supplementation and the risk of colorectal adenomas over a period of 3–5 years.10,21 Another study suggested that vitamin D3 supplementation prevents colorectal adenoma, which may vary according to the VDR genotype.22 The mechanism underlying the effect of 25(OH)D on human colorectal adenomas warrants further study.

The VDR is necessary for 25(OH)D to exert its biological effects. 25(OH)D regulates downstream target genes by binding to VDR.23 Previous studies comparing VDR expression between colorectal adenocarcinoma and normal tissues have yielded conflicting results. Barry et al.22 reported that the effect of vitamin D3 supplementation on colorectal adenoma prevention was a function of the VDR genotype. Castellano-Castillo et al.24 showed that VDR mRNA is expressed at high levels in normal colon tissues and low levels in colorectal cancer tissues; however, Cross et al.25 and Kure et al.26 reported contradicting results. The results of the present study showed that the VDR mRNA expression level in adenocarcinoma tissues was lower than that in para-cancerous tissues. In addition, the VDR mRNA expression level in adenoma tissues was lower than that in normal tissues. The tissue levels of VDR mRNA expression in para-cancerous and normal tissues were significantly higher than those in adenoma and adenocarcinoma tissues. The amount of VDR that can bind to 25(OH)D decreases when VDR expression in colorectal adenomas and adenocarcinomas decreases. Consequently, the biological effects of 25(OH)D are affected, and the anti-neoplastic and apoptosis-promoting functions of 25(OH)D significantly decrease, suggesting that VDR deficiency also participates in the development and progression of colorectal neoplasms. This finding is consistent with the opinion of Merchan et al.22 Therefore, low VDR expression in adenocarcinoma tissues and reduced plasma 25(OH)D concentrations in patients with colorectal adenocarcinoma promotes the development and progression of colorectal neoplasms. This pathway may persist through the progression of colorectal adenoma to adenocarcinoma, or even colorectal cancer. Šutalo et al.27 showed that 25(OH)D prevents tumor progression in normal colorectal mucosa and hyperplastic polyps, while the antitumor and chemopreventive effects are progressively weakened and ultimately absent in colorectal carcinoma. Nonetheless, studies are needed to investigate the role of 25(OH)D in different stages of colorectal neoplasms.

One limitation of our study was that the plasma 25(OH)D concentration may be affected by the body status; however, screen-detected adenomas and adenocarcinomas were selected using a standardized procedure, and the results were reliable. Another limitation was the small study population; multicenter and large-scale population trials are needed to confirm our study findings. Moreover, because colorectal adenoma and adenocarcinoma tissues were obtained from patients and the VDR expression and 25(OH)D concentration were not significantly different between colorectal adenoma and colorectal adenocarcinoma, the mechanism underlying the progression of colorectal adenoma to adenocarcinoma in the same patient has not been investigated. Further studies on this topic will facilitate a better understanding of the role of 25(OH)D in colorectal cancer.
5 | CONCLUSION

The present study elucidated the correlation between the levels of plasma 25(OH)D and the development and progression of colorectal neoplasms. The findings showed that low plasma 25(OH)D level is a risk factor for both colorectal adenomas and colorectal adenocarcinomas. In addition, the tissue levels of VDR mRNA expression in patients with colorectal adenomas and adenocarcinomas were significantly lower than in the normal population. The reduction in VDR mRNA expression might prevent 25(OH)D from exerting the proliferation-suppressing, apoptosis-promoting, and differentiation-promoting functions of colorectal neoplasms. Furthermore, 25(OH)D might affect the development and progression of colorectal adenoma to adenocarcinoma in parallel with VDR mRNA expression.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

Ying Fang, Haojun Song involved in conceptualization of the study. All authors involved in experiments and data acquisition. Jian Huang involved in data analysis and interpretation, and writing of first draft. Jianbo Zhou, Xiaoyun Ding involved in interpretation and critical All authors have approved the final draft of the article and have agreed to the submission.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424.
2. Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. Gut. 2017;66(4):683-691.
3. Zhu J, Tan Z, Hollis-Hansen K, Zhang Y, Yu C, Li Y. Epidemiological trends in colorectal cancer in China: an ecological study. Dig Dis Sci. 2017;62(1):235-243.
4. Corley DA, Jensen CD, Marks AR, et al. Adenoma detection rate and risk of colorectal cancer and death. N Engl J Med. 2014;370(14):1298-1306.
5. Meeker S, Seamons A, Maggio-Price L, Paik J. Protective links between vitamin D, inflammatory bowel disease and colon cancer. World J Gastroenterol. 2016;22(3):933-948.
6. Meyer MB, Goetsch PD, Pike JW. VDR/RXR and TCF4/beta-catenin cistromes in colonic cells of colorectal tumor origin: Impact on c-FOS and c-MYC gene expression. Mol Endocrinol. 2012;26(1):37-51.
7. Kaler P, Galea V, Augenlicht L, Klampfer L. Tumor associated macrophages protect colon cancer cells from TRAIL-induced apoptosis through IL-1beta-dependent stabilization of Snail in tumor cells. PLoS One. 2010;5(7):e11700.
8. Horváth HC, Lakatos P, Kösa JP, et al. The candidate oncogene CYP24A1: A potential biomarker for colorectal tumorigenesis. J Histochem Cytochem. 2010;58(3):277-285.
9. Mandle HJ, Jahan FA, Bostick RM, et al. Effects of supplemental calcium and vitamin D on tight-junction proteins and mucin-12 expression in the normal rectal mucosa of colorectal adenoma patients. Mol Carcinog. 2019;58(7):1279-1290.
10. Calderwood AH, Baron JA, Mott LA, et al. No evidence for post-treatment effects of vitamin D and calcium supplementation on risk of colorectal adenomas in a randomized trial. Cancer Prev Res (Phila). 2019;12(5):295-304.
11. Hamilton SR, Bosman FT, Boffetta P, et al. Carcinoma of the colon and rectum. In: Bosman FT, Carneiro F, Hruban RH, Theise ND, eds. WHO classification of tumors of the digestive system. IARC Press; 2010:134-146.
12. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011;96(7):1911-1930.
13. Rao X, Huang X, Zhou Z, Lin X. An improvement of the Z’-delta delta CT method for quantitative real-time polymerase chain reaction data analysis. Biostat Bioinforma Biomath. 2013;3(3):71-85.
14. Chen Z, Hu J, Zheng Z, et al. Location of colorectal adenomas and serrated polyps in patients under age 50. Int J Colorectal Dis. 2019;34(12):2201-2204.
15. Jenab M, Bueno-de-Mesquita HB, Ferrari P, et al. Association between pre-diagnostic circulating vitamin D concentration and risk of colorectal cancer in European populations: a nested case-control study. BMJ. 2010;340: b5500.
16. Touvier M, Chan DS, Lau R, et al. Meta-analyses of vitamin D intake, 25-hydroxyvitamin D status, vitamin D receptor polymorphisms, and colorectal cancer risk. Cancer Epidemiol Biomarkers Prev. 2011;20(5):1003-1016.
17. Bivona G, Agnello L, Bellia C, et al. Non-skeletal activities of vitamin D: from physiology to brain pathology. Medicina (Kaunas). 2019;55(7):341.
18. Bivona G, Agnello L, Ciaccio M. The immunological implications of the new vitamin D metabolism. Cent Eur J Immunol. 2018;43(3):331-334.
19. Bivona G, Agnello L, Ciaccio M. Vitamin D and immunomodulation: Is it time to change the reference values? Ann Clin Lab Sci. 2017;47(4):508-510.
20. Goulão B, Stewart F, Ford JA, MacLennan G, Avenell A. Cancer and vitamin D supplementation: A systematic review and meta-analysis. Am J Clin Nutr. 2018;107(4):652-663.
21. Song M, Lee IM, Manson JE, et al. No association between vitamin D supplementation and risk of colorectal adenomas or serrated polyps in a randomized trial. Clin Gastroenterol Hepatol. 2021;19(1):128-135.e6.
22. Barry EL, Peacock JL, Rees JR, et al. Vitamin D receptor genotype, vitamin D3 supplementation, and risk of colorectal adenomas: a randomized clinical trial. JAMA Oncol. 2017;3(5):628-635.
23. Bandera Merchan B, Morcillo S, Martin-Nuñez G, Tinahones FJ, Macias-Gonzalez M. The role of vitamin D and VDR in carcinogenesis: Through epidemiology and basic sciences. J Steroid Biochem Mol Biol. 2017;167:203-218.
24. Castellano-Castillo D, Morcillo S, Clemente-Postigo M, et al. Adipose tissue inflammation and VDR expression and methylation in colorectal cancer. Clin Epigenetics. 2018;10:60.
25. Cross HS, Bareis P, Hofer H, et al. 25-Hydroxyvitamin D(3)-1alpha-hydroxylase and vitamin D receptor gene expression in human colonic mucosa is elevated during early carcinogenesis. Steroids. 2001;66(3-5):287-292.
26. Kure S, Nosho K, Baba Y, et al. Vitamin D receptor expression is associated with PIK3CA and KRAS mutations in colorectal cancer. Cancer Epidemiol Biomarkers Prev. 2009;18(10):2765-2772.

27. Šutalo N, Tomić S, Bevanda M, et al. Immunohistochemical expression of vitamin D receptor in development stages of colorectal carcinoma. Psychiatr Danub 2017;29(Suppl 4):855-858. PMID: 29278636.

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