The association of serum vitamin D–binding protein and 25-hydroxyvitamin D in pre-operative and post-operative colorectal cancer

Yuanzi Liang¹ | Liejun Jiang² | Xiaowei Chi³ | Steven Hochwald⁴ | Feng Qiu¹ | Yanfang Luo² | Qiuwei Lu² | Xiafang Yang² | Huayi Huang²,⁴ | Junfa Xu⁵

¹Department of Laboratory Medicine, Nanhai Hospital, Southern Medical University, Foshan, Guangdong, China
²Department of Laboratory Medicine, The People’s Hospital of Guangxi Zhuang Autonomous Region, Nanning, Guangxi, China
³Department of Laboratory Medicine, The Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Jinan, Shandong, China
⁴Department of Surgical Oncology, Roswell Park Comprehensive Cancer Center, Buffalo, New York, NY, USA
⁵Department of Clinical Immunology, Institute of Clinical Laboratory Medicine, Guangdong Provincial Key Laboratory of Medical Molecular Diagnostics, Guangdong Medical University, Dongguan, Guangdong, China

Correspondence
Huayi Huang, Department of Surgical Oncology, Roswell Park Comprehensive Cancer Center, Buffalo, NY 14263, USA.
Email: Huayi.Huang@Roswellpark.org

Junfa Xu, Department of Clinical Immunology, Institute of Clinical Laboratory Medicine, Guangdong Provincial Key Laboratory of Medical Molecular Diagnostics, Guangdong Medical University, No. 1 Xincheng Road, Dongguan 523808, China.
Email: timxujunfa@163.com

Abstract

Background: The association between vitamin D–binding protein (VDBP) and 25-hydroxyvitamin D (25(OH)D) with colorectal cancer (CRC) is still ambiguous. This study was to further investigate the relationship between serum VDBP, 25(OH)D levels and the clinical and pathological features of patients with CRC.

Methods: Enzyme-linked immunosorbent assay (ELISA) and chemiluminescence immunoassay were used to analyze the VDBP and 25(OH)D concentrations in serum. Pearson's correlation analysis was applied to evaluate the association between serum VDBP and 25(OH)D levels in CRC. Conditional logistic regression was performed to analyze the prediction value of serum VDBP or 25(OH)D as a risk factor for CRC.

Results: The serological levels of 25(OH)D in patients were significantly lower than in healthy individuals, while VDBP levels were significantly higher than in healthy controls. The serum VDBP in pre-operative was significantly lower than in post-operative samples, while the serum 25(OH)D from pre-operative patients was significantly higher than post-operative patients. Patients with tumors with higher stage and increased lymph node involvement had lower serum post-operative VDBP levels. In addition, our results showed that the pre-operative VDBP level is a risk factor of CRC.

Conclusions: The levels of serum 25(OH)D and VDBP were both associated with CRC. Thus, serum 25(OH)D and VDBP levels might be of value in evaluating the pathogenesis and risk of CRC in the future. Moreover, serum VDBP or 25(OH)D levels were associated with patient's clinical and pathological features providing data for risk and prognostic prediction.
1 | INTRODUCTION

Colorectal cancer (CRC) is a common type of cancer worldwide.\textsuperscript{1} In China, the estimated new cases and deaths of CRC were 376 300 and 191 000, respectively, in 2015.\textsuperscript{2} Benefited by the development of screening tools for early detection, more and more patients could be treated at an early stage; however, the incidence and mortality of CRC among adults aged under 50 years is increasing in the United States.\textsuperscript{3} Accumulating evidence indicates that high levels of circulating vitamin D is an anti-cancer factor, which may reduce the risk of CRC.\textsuperscript{4, 6}

Vitamin D (VD) is photochemically synthesized in the skin when exposed to ultraviolet B (UVB) radiation or ingested from food and/or supplements. Once getting into blood circulation, VD is hydroxylated and forms 25-hydroxyvitamin D [25(OH)D] in the liver, the well-known biomarker of VD status. Then, 25(OH)D is hydroxylated and forms 1,25-dihydroxyvitamin D [1,25(OH)2D] in the kidney and other organs, the bioactive form of VD.\textsuperscript{7} Recent studies found that free 25(OH)D may be an independent CRC prognostic biomarker.\textsuperscript{9}

Vitamin D-binding protein (VDBP), primarily vitamin D carrier protein, is mainly synthesized and secreted by the liver, which belongs to the albumin gene family. Moreover, VDBP is the precursor to the Gc protein-derived macrophage-activating factor (Gc-MAF) that can activate macrophages against cancer cell.\textsuperscript{9, 10} Recently, several studies have reported that serum VDBP was associated with prostate, pancreatic, and bladder cancers as well as acute leukemia.\textsuperscript{11-14} Several papers have pointed that VDBP was not associated with CRC.\textsuperscript{15, 16} However, it has been reported that VDBP played a role in the modulation of serum 25(OH)D levels although it was not directly corrected with the risk of CRC.\textsuperscript{7} So far, the expression levels of 25(OH)D and VDBP in CRC, and their association with CRC pathogenesis and risk are still ambiguous. In this study, we analyzed the serum 25(OH)D and VDBP levels in pre- and post-operative CRC patients and correlated them with clinical features and the risk of CRC.

2 | MATERIALS AND METHODS

2.1 | Patients

Seventy-eight patients diagnosed with colorectal cancer were enrolled consecutively in this study in a period of time from May 2017 to July 2018. The diagnosis was made by pathologic examination. During hospitalization, all patients had no outdoor activity and were provided hospital diet without calcium or vitamin D supplements by the department of food and nutrition. Ninety healthy individuals, ranging from 45 to 87 years old, who had regular diet without calcium or vitamin D supplements before blood drawing, were selected as controls with matched ages and genders to the patient group. The study was approved by the Institutional Review Board of the People’s Hospital of Guangxi Zhuang Autonomous Region for the use of human materials.

2.2 | Blood sample collection

Fasting blood samples were obtained either the day before surgical operation or other treatments (baseline) and on day 5 after surgery, respectively, using the serum separation tube (BD healthcare). All patients with CRC had paired pre-operative and post-operative blood samples. The blood samples were transported to the Department of Laboratory Medicine within 30 minutes of collection. The samples were then centrifuged at 1500 \( g \) at 4°C for 10 minutes; the serum was collected into a 2 mL of centrifuge tube and stored at −80°C until analysis.

2.3 | Enzyme-linked immunosorbent assay (ELISA)

Serum vitamin D-binding protein (VDBP) was measured by using the Quantikine ELISA Human Vitamin D BP Immunoassay (Catalog number DVDBP0) kit from R & D Systems, Inc following the manufacturer’s instructions. In brief, 100 µL of Assay Diluent RD1-19 was added to each well in the microplate; then, 50 µL of standard, control, or sample were added per well and covered with the adhesive strip to incubate for 1 hour at room temperature on a horizontal orbital microplate shaker; all the liquid was aspirated and washed by adding 400 µL Wash Buffer for 4 times; after the last wash, inverted and blotted against the plate through clean paper towels to drain the remaining wash buffer; added 200 µL of Human Vitamin D BP Conjugate to each well and covered with a new adhesive strip to incubate for 2 hours at room temperature on the shaker; repeated the aspiration and wash; then added 200 µL of Substrate Solution to each well to incubate for 30 minutes at room temperature on the benchtop by protecting from light; following this step, 50 µL of stop solution was added to stop the color development; and finally, measured the plate immediately on a microplate reader at OD 450 nm.

2.4 | Chemiluminescence immunoassay

In clinical laboratory, chemiluminescence immunoassay is widely used to measure serum vitamin D (VD) because of its automatic capability with clinical satisfaction. In this study, serum VD was quantitatively analyzed on a Roche cobas E601 electrochemiluminescence
immunoassay platform using a Roche cobas Elecsys Vitamin D total (25 Hydroxyvitamin D, REF 05 894 913 190, Roche Diagnostics GmbH) assay kit. All the tests abovementioned were performed following our laboratory standard operating procedures described previously.\textsuperscript{14}

2.5 Statistical analysis

IBM SPSS Statistical 20.0 (SPSS Inc Chicago) was performed for statistical analysis. Differences in basic characteristics of patients were evaluated by using independent samples t test. Paired samples t test was performed to compare the difference in each parameter of paired pre- and post-operative serum VDBP or 25(OH)D levels. Moreover, Pearson's correlation analysis was applied to evaluate the association between serum VDBP and 25(OH)D levels in CRC. The pre- and post-operative 25(OH)D and pre- and post-operative VDBP (≥median and <median) levels were stratified by using conditional logistic regression (multivariate analysis) to evaluate whether serum VDBP or 25(OH)D is a risk factor for CRC, which estimated odds ratios (OR) and 95% confidence intervals (CI). A P-value of <.05 was considered as statistically significant.

Serum 25(OH)D and VDBP levels were correlated with clinical and pathological features including age, gender, lesion site, tumor stage, tumor size, lymph node involvement, vascular and nerve invasion, and cell differentiation.

2.6 Ethics statement

The study was approved by the Institutional Review Board of the People's Hospital of Guangxi Zhuang Autonomous Region for the use of human materials.

3 RESULTS

3.1 Correlation of serum VDBP and 25(OH)D levels with clinical-pathological features in CRC patients

The correlation of serum VDBP and 25(OH)D levels with clinical-pathological features from 78 patients with CRC were summarized as shown in Table 1. Of 78 patients with CRC, there were 33 females and 45 males with ages ranging from 32 to 86 years (a median age of 64 years). In this study, colon was the main lesion site of the tumor (49/78), and 63 cases were found to be moderately differentiated according to histopathologic grading.

According to the median or feature of the parameters we collected, we divided each parameter into two or three groups, which was analyzed through univariate analysis (displayed in Table 1). In independent samples test, our data showed that the groups, tumor stage III-IV and lymph node involvement, had distinctly lower serum post-operative VDBP levels than the groups, stage I-II and no lymph node involvement, with \( P = .006 \) and \( P = .004 \), respectively. As for the levels of pre-operative serum 25(OH)D, males and rectal tumor groups were significantly higher (\( P = .003 \), \( P = .007 \), respectively). While with post-operative 25(OH)D levels, males, rectal tumors, and tumor size <5.0 cm had significantly higher levels (\( P = .007 \), \( P = .032 \), and \( P = .040 \), respectively). In paired samples test, the pre-operative VDBP concentration of patients with age <64, males, rectal tumors, tumors stage I-II, moderate cell differentiation, tumor size ≥5.0 cm, no lymph node involvement, no vascular invasion, and no nerve invasion were significantly lower than the post-operative VDBP levels, all with \( P < .05 \). And as shown in Table 1, except for the high cell differentiation and low cell differentiation groups, the levels of pre-operative 25(OH)D were distinctly higher than post-operative 25(OH)D (\( P < .05 \)).

3.2 Serum VDBP and 25(OH)D levels in pre- and post-operative CRC patients

Table 2 shows that the serum VDBP in pre- and post-operative CRC patients were significantly higher than the controls (\( P < .001 \) for both). While serum 25(OH)D in both pre- and post-operative CRC patients were significantly lower than the controls (\( P = .001 \) and \( P < .001 \), respectively). In addition, the VDBP levels in pre-operative CRC patients was significantly lower than the post-operative levels (\( P = .005 \)). However, the level of pre-operative 25(OH)D was significantly higher than post-operative 25(OH)D (\( P < .001 \)).

3.3 Serum VDBP and 25(OH)D levels and the risk of CRC

In order to understand whether serum VDBP and 25(OH)D is a risk factor for CRC, multivariate analysis and conditional logistic regression were performed and the results are displayed in Table 3. The results showed that the pre-operative VDBP level is a risk factor for CRC (OR = 13.264, CI = 1.72-102.28, \( P = .013 \)), while the post-operative 25(OH)D level is a protective factor (OR = 0.099, CI = 0.01-0.90, \( P = .040 \)).

3.4 Correlation between serum VDBP and 25(OH)D levels

Statistical analysis results showed that there was a weak correlation between both pre-operative VDBP and post-operative VDBP with 25(OH)D levels (Table 4, Pearson \( r = .127 \) and \( .156, P = .267 \) and \( .172, \) respectively).

4 DISCUSSION

The association between serum VD and cancer occurrence is still unclear to date. The function of VDBP is to bind and carry VD to
organs and tissues, and its role and relationship with VD are not fully understood either.

In this study, we found that both serologic levels of pre- and post-operative 25(OH)D in patients with CRC were significantly lower than in healthy individuals ($P = .001$ and $P < .001$, respectively, Table 2). Many studies found that there is an inverse association between serum 25(OH)D and the risk of CRC.$^{15,18,19}$ VD is associated with enhancing cellular differentiation and apoptosis, reducing cellular proliferation and inflammation in animal and cancer cells.$^{20-22}$ Furthermore, VD activates cytochrome P27B1 (CYP27B1) and

| Parameters | Total (N = 78) | Pre-OP | Post-OP | $P^2$ | Pre-OP | Post-OP | $P^2$ |
|------------|---------------|--------|---------|-------|--------|---------|-------|
| Age (y)    |               |        |         |       |        |         |       |
| ≥64        | 40            | 330.98 ± 17.99 | 376.10 ± 24.90 | .074 | 55.22 ± 5.02 | 41.08 ± 2.59 | .002* |
| <64        | 38            | 329.31 ± 19.35 | 391.47 ± 24.07 | .035* | 48.38 ± 4.30 | 43.12 ± 3.38 | .009* |
| $P^1$      | .950          | .659   | .306    | .630  |         |         |       |
| Gender     |               |        |         |       |        |         |       |
| Female     | 33            | 343.50 ± 18.84 | 390.43 ± 25.78 | .161 | 40.61 ± 3.33 | 35.55 ± 2.74 | .002* |
| Male       | 45            | 320.39 ± 18.07 | 378.57 ± 23.38 | .011* | 60.16 ± 4.88 | 46.85 ± 2.86 | .001* |
| $P^1$      | .387          | .736   | .003*   | .007* |         |         |       |
| Lesion site|               |        |         |       |        |         |       |
| Colon      | 49            | 342.64 ± 17.50 | 384.59 ± 24.44 | .102 | 45.11 ± 3.55 | 38.61 ± 2.61 | .001* |
| Rectum     | 29            | 309.09 ± 18.90 | 381.89 ± 21.68 | .010* | 63.35 ± 6.31 | 47.92 ± 3.33 | .013* |
| $P^1$      | .218          | .934   | .007*   | .032* |         |         |       |
| TNM stage  |               |        |         |       |        |         |       |
| I-II       | 40            | 330.27 ± 17.42 | 429.33 ± 25.15 | .001* | 50.22 ± 4.91 | 40.05 ± 2.85 | .017* |
| III-IV     | 38            | 330.06 ± 19.92 | 335.44 ± 21.13 | .796 | 53.65 ± 4.49 | 44.20 ± 3.11 | <.001* |
| $P^1$      | .994          | .006*  | .608    | .328  |         |         |       |
| Cell differentiation | | | | | | | |
| High       | 6             | 351.69 ± 36.15 | 361.42 ± 57.11 | .912 | 69.12 ± 22.6 | 34.49 ± 6.88 | .214 |
| Moderate   | 63            | 327.64 ± 14.77 | 386.69 ± 18.60 | .006* | 50.67 ± 3.16 | 43.17 ± 2.36 | <.001* |
| Low        | 9             | 333.54 ± 43.50 | 376.68 ± 60.09 | .404 | 48.05 ± 11.7 | 39.41 ± 6.40 | .199 |
| $P^1$      | .887          | .920   | .325    | .501  |         |         |       |
| Tumor size |               |        |         |       |        |         |       |
| ≥5.0 cm    | 42            | 337.75 ± 17.71 | 403.62 ± 22.04 | .007* | 49.42 ± 4.59 | 37.97 ± 2.24 | .007* |
| <5.0 cm    | 36            | 321.33 ± 19.64 | 360.22 ± 26.93 | .207 | 54.78 ± 4.83 | 46.86 ± 3.60 | .001* |
| $P^1$      | .536          | .212   | .424    | .040* |         |         |       |
| Lymph node invasion | | | | | | | |
| Yes        | 38            | 328.02 ± 20.68 | 332.96 ± 21.00 | .892 | 53.52 ± 4.50 | 43.93 ± 3.12 | <.001* |
| No         | 40            | 332.21 ± 16.60 | 431.68 ± 25.02 | .001* | 50.35 ± 4.90 | 40.30 ± 2.85 | .019* |
| $P^1$      | .874          | .004*  | .636    | .392  |         |         |       |
| Vascular invasion | | | | | | | |
| Yes        | 32            | 337.79 ± 22.48 | 352.72 ± 23.34 | .577 | 50.52 ± 4.66 | 41.23 ± 3.27 | .001* |
| No         | 46            | 324.87 ± 15.95 | 405.06 ± 24.02 | .003* | 52.84 ± 4.64 | 42.65 ± 2.77 | .008* |
| $P^1$      | .631          | .136   | .734    | .740  |         |         |       |
| Nerve invasion | | | | | | | |
| Yes        | 19            | 375.17 ± 32.28 | 389.61 ± 31.76 | .679 | 58.24 ± 7.00 | 46.54 ± 5.11 | .006* |
| No         | 59            | 315.67 ± 13.50 | 381.65 ± 20.52 | .004* | 49.85 ± 3.76 | 40.63 ± 2.24 | .003* |
| $P^1$      | .051          | .844   | .281    | .231  |         |         |       |

Note: $P^1$ represents the comparison of intra-parameter. $P^2$ represents the comparison of VDBP and 25(OH)D in pre- and post-operative CRC. Abbreviations: Post-OP, post-operative; Pre-OP, pre-operative. *Means $P < .05$.

$^{1}$Liang et al. 2021
cytochrome P24A1 (CYP24A1), as well as vitamin D receptor (VDR) which are expressed in normal colorectal cells, suggesting that VD plays an important biological function in colorectal cells. The expression of CYP27B1 in moderately and well-differentiated CRC samples is increased, which indicates a possible response to VD in these patients. While in poorly differentiated CRC, the expression of CYP27B1 is repressed, indicating that cancer cells may be resistant to calcitriol therapy. CYP27B1 is abundantly present in colorectal epithelial cells which plays an important role in mediating the biological effects of calcitriol. There is a hypothesis that alteration of VRD may change transcriptional control of 1,25(OH)2D3 and impact anti-tumor effect of VD. Therefore, these results suggest that VD plays a role in inhibiting tumorigenesis of CRC.

Several studies have reported that serum VDBP was associated with prostate, pancreatic, bladder cancer, and acute leukemia. As mentioned above, several studies found that there was no association between VDBP and CRC. However, our study results showed that both serologic levels of pre- and post-operative VDBP in CRC patients were distinctly higher than the healthy controls (\(P < .001\) for both, Table 2), suggesting that serum VDBP may be associated with the pathogenesis of CRC. We also found that pre-operative VDBP levels was a risk factor for CRC (OR = 13.264, CI = 1.72-102.28, \(P = .013\), Table 3, respectively). VDBP plays an important role in macrophage activation, serving as an actin scavenger, anti-neoplastic, anti-angiogenic, pro-apoptotic, and immunomodulatory factors. Following tissue injury and cell death, actin is released into circulation and can lead to vascular obstruction and organ dysfunction. The ability of VDBP to rapidly sequester actin protects against these complications which may account for its elevation in CRC. At this point, serum VDBP might be a potential biomarker to evaluate the risk and pathogenesis of CRC.

The serum concentration of pre-operative VDBP was much lower than the post-operative level (\(P = .005\), Table 2), while the concentration of pre-operative 25(OH)D was much higher than the post-operative level (\(P < .001\), Table 2). It seems that VDBP and 25(OH)D had an inverse association with the disease progression. Anic et al and Ying et al have pointed that VDBP may change the 25(OH)D concentration in CRC. However, there was no significant correlation between these two markers in the serum by Pearson's correlation analysis (\(r = -.127\) and .156, \(P = .267\) and .172, Table 4) in our study. Maybe the small sample volumes would reduce statistical power to define the relationship between VDBP and 25(OH)D. The surgical procedure may change the serum level of 25(OH)D and VDBP such as plasma loss and loss of proteins which may influence the results.
due to the limitation of access to medical records and difficulty in observation of patients’ daily performance and manifestation, we were unable to exclude the stress factors mentioned above. Thus, it is necessary to consider these parameters and pay close attention on their effect on serum VD and VDBP in the future studies.

Results from univariate analysis showed that the serum concentration of pre- and post-operative 25(OH)D in male patients and rectal tumors were significantly higher ($P = .003$, $P = .007$, $P = .007$, and $P = .032$, respectively). Andersen et al found that the inverse association between serum 25(OH)D and CRC was more prominent in females than males. A meta-analysis including 1248 cases of CRC suggested that 25(OH)D had stronger inverse associations with colon than with rectal tumors. In other words, serum 25(OH)D shows higher levels in males than females, rectal than colon tumors, consistent with our study. In addition, higher stage (III-IV) tumors and patients with lymph node involvement had distinctly lower post-operative serum VDBP levels ($P = .006$ and $P = .004$, respectively). A report suggests that low VD levels were found in most cases of breast cancer with advanced tumor stage, large tumors, and positive lymph nodes. To our knowledge, the relationship of clinical-pathological features and serum VDBP or 25(OH)D in CRC has been rarely reported and merits further investigation in the future.

In conclusion, our study found that there was a relationship between serum 25(OH)D and VDBP in CRC. Thus, serum 25(OH)D and VDBP levels might be of value in determining the pathogenesis and risk of CRC and requires further study.

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AUTHOR CONTRIBUTIONS

Yuanzi Liang carried out the serological studies, participated in the experiment design and statistical analysis, and drafted the manuscript. Liejun Jiang participated in the experiment design, serum sample collection, and the statistical analysis and helped to draft the manuscript. Xiaowei Chi and Feng Qiu carried out the patients’ clinical and pathological information collection and participated in serum sample collection. Steven Hochwald carried out the English editing and participated in the manuscript revise. Yanfang Luo, Qiwei Lu and Xiafang Yang performed the immunoassays. Huayi Huang and Junfa Xu conceived the study and participated in its design and coordination and manuscript revise. All authors read and approved the final manuscript.

ORCID

Huayi Huang https://orcid.org/0000-0002-7272-0838

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