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Clinical Impact of Postoperative Vitamin D Deficiency on the Recurrence of Colon Cancer After Curative Surgical Resection

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Abstract. *Background/Aim: There are no clinically significant cutoff values of serum vitamin D levels and time points to predict the prognosis of colon cancer, particularly in patients who underwent curative surgical resection. Patients and Methods: We retrospectively analyzed serum vitamin D levels in 795 patients with stages I to III colon cancer who underwent curative surgical resection. Results: Patients with vitamin D levels below 12 ng/ml at one year after surgical resection demonstrated a significantly reduced disease-free survival (DFS) than those who did not have vitamin D deficiency (p=0.01). In the multivariate analysis, an age of 70 years or older [hazard ratio (HR)=1.992; p=0.001], pathologic stage (HR=3.739; p<0.001), and vitamin D deficiency (less than 12 ng/ml) at one year after surgery (HR=0.563; p=0.020) were factors unfavorably influencing DFS. Conclusion: In patients with stages I to III of colon cancer, vitamin D deficiency at one year after surgical resection was associated with increased disease relapse.*

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Since vitamin D was reported to be one of the key regulatory factors in various cancers, a considerable amount of literature has been published (1, 2). To date, it has been suggested that vitamin D plays a pivotal role in the tumor microenvironment, involved in cell differentiation and angiogenesis as well as gene regulation affecting cancer metabolism (3-5).

Although the relationship between serum vitamin D level and colon cancer remains inadequately understood, evidence from previous studies suggests vitamin D has an effect in the development and progression of colon cancer (6-9). Epidemiological studies have reported that patients with low vitamin D levels had higher risk of colon cancer incidence, while increased sun (ultraviolet B) exposure or residence at lower geographic latitudes led to a lower incidence of colon cancer (1, 10). In a meta-analysis of five studies, patients with higher vitamin D levels showed better overall survival and disease-specific mortality rates (11, 12). In a prospective study including 541 patients with colorectal cancer (CRC), higher pre-diagnostic serum 25-hydroxyvitamin D (25-OHD) levels were associated with improved survival, while Zgaga *et al.* suggested that postoperative serum 25-OHD was correlated with clinically important differences in survival outcomes in patients with stages I to III CRC (13, 14). Meanwhile, the issue of the therapeutic role of vitamin D has remained somewhat controversial; one major contention in the literature is that vitamin D supplementation could reduce the incidence of CRC and mortality (15). Some research has reported that the higher levels of serum vitamin D caused by vitamin D supplementation had no beneficial effect on survival (16).

At this time, there is no clinically significant cutoff value of serum vitamin D level and time point to predict the prognosis of colon cancer, particularly for patients who have undergone curative surgical resection. In this regard, we retrospectively analyzed consecutive serum vitamin D levels from patients with colon cancer who had undergone surgery to identify the prognostic role of the serum vitamin D level.

Patients and Methods

Patients. In this retrospective longitudinal study, we analyzed the serum vitamin D levels of 795 patients with stages I to III colon cancer who underwent curative surgical resection at Kyungpook National University Chilgok Hospital (KNUCH) between March 2014 and March 2019. Detailed clinical and pathological data of the enrolled patients were also reviewed from the hospital records. The present study was approved by the Institutional Review Board of KNUCH (KNUCH 2020-08-019) and informed consent was obtained from all study participants.

Serum vitamin D level. Available serum samples were collected at the time of diagnosis and at 6, 12, and 18 months after surgery. All collected serum samples were assayed in the same laboratory in KNUCH to minimize technical variability. Serum 25-OHD has been suggested to be the most adequate marker for assessing an individual's vitamin D status. Therefore, total serum 25-OHD levels were measured by liquid chromatography-tandem mass spectrometry according to a standardized protocol (17). A serum 25-OHD level below 12 ng/ml was considered to suggest a vitamin D deficiency with reference to previous research (18, 19).

Statistical analysis. Categorical variables were summarized by counts with proportions and continuous variables were described by median and range. Disease-free survival (DFS) was calculated from the time of surgery to the point of initial tumor relapse or death as a result of any cause, while overall survival (OS) was measured from the time of surgery to death or the last date of follow-up. The time to event outcome (*i.e.*, DFS and OS) was calculated using the Kaplan-Meier method, with curves compared using the log-rank test. Cox's regression model was used for identifying factors for long-term survival. In the univariate analysis, age, sex, primary tumor location, T stage, N stage, pathologic stage, neoadjuvant chemotherapy, adjuvant chemotherapy, CA 19-9 and CEA were included as variables. Factors with a *p*-Value of less than 0.1 in the univariate analysis were then included in the multivariate analysis. The hazard ratio (HR) and 95% confidence interval (CI) were estimated for each factor. A *p*-Value of less than 0.05 was considered to be statistically significant. The data of this retrospective study were analyzed using the R version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Patient characteristics. The serum vitamin D levels of a total of 795 patients newly diagnosed with colon cancer who underwent curative surgical resection were collected. As summarized in Table I, the median age of study participants was 65 years (range=15-89 years) at the time of diagnosis and 429 (54.0%) patients were male. The predominant histology finding was adenocarcinoma, and more than half of the included patients (63.4%) had descending colon cancer. In this study, 220 patients (27.7%) had stage I, 275 (34.6%) had stage II and 300 (37.7%) had stage III disease At the time of diagnosis, the median serum 25-OHD level was 15.9 ng/ml.

Table I. *Patient characteristics (N=795).*

Variable	Value
Age, median (range)	65 (15-89)
Gender, n (%)	
Male	429 (54.0%)
Female	366 (46.0%)
Primary tumor location, n (%)	
Ascending colon	269 (33.8%)
Transverse colon	22 (2.8%)
Descending colon	504 (63.4%)
Histology, n (%)	
Adenocarcinoma	771 (97.0%)
Mucinous adenocarcinoma	18 (2.3%)
Signet ring-cell carcinoma	1 (0.1%)
Other	5 (0.6%)
T stage, n (%)	
T4	81 (10.2%)
T3	455 (57.2%)
T2	93 (11.7%)
T1	166 (20.9%)
N stage, n (%)	
N2	86 (10.8%)
N1	213 (26.8%)
N0	496 (62.4%)
Pathological stage, n (%)	
III	300 (37.7%)
II	275 (34.6%)
I	220 (27.7%)
CA19-9, median (range)	10.6 (0.01-629.8)
CEA, median (range)	2.11 (0.09-313)
25-OHD at diagnosis, median (range)	15.9 (3-171.5)
25-OHD, at one year after surgery, median (range)	18.75 (4.4-95.8)
Neoadjuvant chemotherapy, n (%)	40 (5.3%)
Adjuvant chemotherapy, n (%)	398 (50.1%)
Relapse, n (%)	86 (10.8%)
Death, n (%)	10 (1.3%)

25-OHD: 25-hydroxyvitamin D.

Vitamin D deficiency and clinical outcomes. Serum 25-OHD levels at diagnosis and six months after surgery were not statistically associated with disease relapse or survival. The median serum 25-OHD level one year after surgery was 18.75 ng/ml and 136 patients (17.1%) showed a vitamin D deficiency. The median serum 25-OHD level one year after surgery was equal to 19.5 ng/ml in stage I, 18.5 ng/ml in stage II and 18.1 ng/ml in stage III. Patients with vitamin D deficiency one year after surgical resection demonstrated significantly inferior DFS when compared with those who did not have such a deficiency (p=0.01) (Figure 1A). The DFS rate at three years in patients with vitamin D deficiency was 84%, while those patients with 25-OHD levels of 12 ng/ml or greater totaled 89.8% of the study population. Patients who maintained a serum 25-OHD level of greater than 12 ng/ml from diagnosis to six months after surgery but developed vitamin D deficiency at one year after surgery also showed

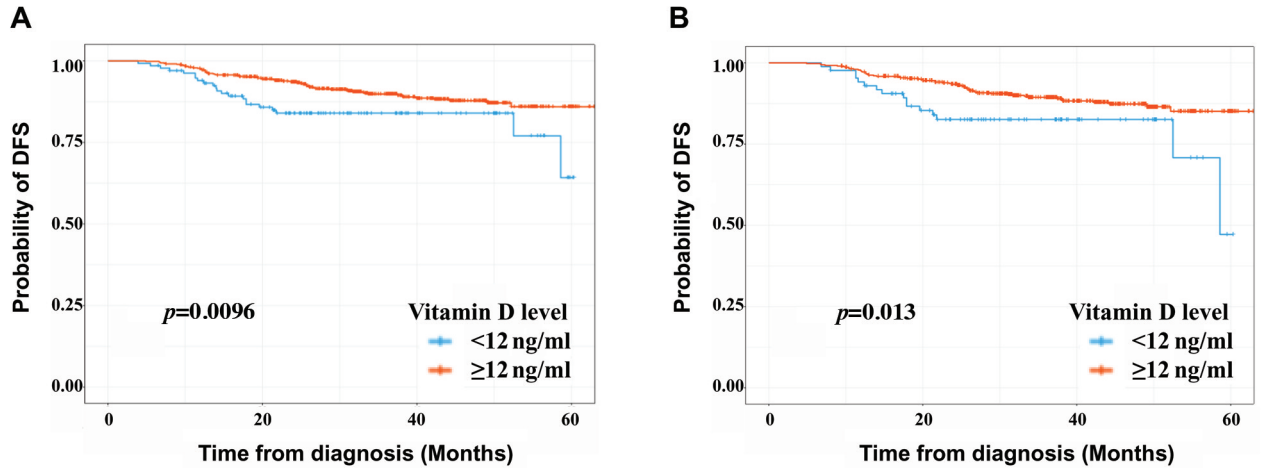

Figure 1. *Kaplan–Meier curves showing long-term survival outcomes. (A) Patients with vitamin D deficiency one year after surgical resection showed significantly lower disease-free survival (DFS) than those with no deficiency. (B) Patients who maintained 25-OHD levels higher than 12 ng/ml from diagnosis to six months after surgery but developed vitamin D deficiency at one year after surgery also showed poor DFS.*

poor DFS (p=0.013) (Figure 1B). In the subgroup analysis, patients with vitamin D deficiency at one year were older (p=0.029) than those without and showed a greater tendency to have ascending colon cancer (p=0.016) and a more advanced T stage (p=0.034). There was no statistical relevance between pathological stage and 25-OHD status (Table II). However, the serum 25-OHD levels were not statistically associated with OS at any time.

Survival and factors affecting long-term outcome. With a median follow-up duration of 36.7 months (range=6.5-64.7 months), the cumulative incidence rates of disease relapse and death were 10.8% (n=86 patients) and 1.3% (n=10 patients), respectively. Meanwhile, the DFS and OS rates at three years in the total study population were estimated to be 88.8% and 98.7%, respectively. In the multivariate analysis, an age of 70 years or older (HR=1.992; p=0.001), pathologic stage (HR=3.739; p<0.001), and vitamin D deficiency at one year after surgery (HR=0.563; p=0.020) were factors leading to an unfavorable DFS outcome (Table III).

Discussion

Previous studies have suggested that a lower level of serum 25-OHD could lead to poor survival outcomes in colon cancer (20-22). However, to our knowledge, there has not yet been a meaningful investigation of the relationship between the postoperative level of serum 25-OHD and DFS after colon cancer surgery. In the current study, we serially examined the postoperative serum 25-OHD levels of colon cancer patients to identify the clinical impact on treatment outcome after curative resection. We determined that low serum 25-OHD levels after surgery were strongly correlated with increased postoperative disease relapse rates. Furthermore, we determined a significant cutoff value of serum 25-OHD and key time points at which to conduct follow-up evaluations. Although some previous studies showed that higher serum vitamin D levels were associated with better clinical outcomes, the findings in this study do not support this (7, 14). In the previous studies, postoperative vitamin D level was measured during the relatively earlier follow-up period whereas we analyzed serum vitamin D levels serially up to 18 months after curative surgical resection (7, 14). Lower level of postoperative vitamin D in late follow-up period might increase the risk of clonal evolution of colon cancer.

A vitamin D deficiency could promote tumorigenesis and the self-renewal of colon cancer stem cells, which may contribute to the relapse of colon cancer after curative resection (23-25). Wang *et al*. found that vitamin D deficiency impedes the differentiation of colon cancer cells, which supports the maintenance of colon cancer stem cells (26). Elsewhere, Pendas-Franco *et al*. demonstrated that vitamin D could suppress colon cancer stem cells with negative regulation of the Wnt signaling pathway (27). Research concerning the impact of 25-OHD deficiency on the survival outcome among patients with postoperative colon cancer began emerging in the mid to late 2010s with the studies of Zgaga *et al*. (7, 14). It was suggested that a higher postoperative serum 25-OHD level is correlated with a better survival outcome. However, serum 25-OHD levels were checked within six months after surgery and there was no extended follow-up investigation with sequential sampling performed after six months. Still, the critical time points and serum 25-OHD cutoff level for the assessment of treatment response after curative surgery have not yet determined.

Table II. *Clinicopathological characteristics according to 25-hydroxyvitamin D leve1s (12 ng/ml).*

Factors	25-OHD status		*p*-Value
	<12 ng/ml (N=136, 17.1%)	≥12 ng/ml (N=659, 82.9%)	
Age, median (range)	66 (32-83)	63 (15-89)	0.029
Gender, n (%)			0.462
Female	67 (49.3%)	299 (45.4%)	
Male	69 (50.7%)	360 (54.6%)	
Primary tumor location, n (%)			0.016
Ascending colon	59 (43.4%)	210 (31.9%)	
Transverse colon	1 (0.7%)	21 (3.2%)	
Descending colon	76 (55.9%)	428 (64.9%)	
T stage, n (%)			0.034
T4	12 (8.8%)	69 (10.5%)	
T3	87 (64.0%)	368 (55.8%)	
T2	20 (14.7%)	72 (10.9%)	
T1	17 (12.5%)	150 (22.8%)	
N stage, n (%)			0.354
N2	19 (14.9%)	67 (10.2%)	
N1	38 (27.9%)	175 (26.6%)	
N0	79 (58.1%)	417 (63.3%)	
Pathological stage, n (%)			0.119
III	57 (41.9%)	243 (36.9%)	
II	51 (37.5%)	223 (33.8%)	
I	28 (20.6%)	193 (29.3%)	
Neoadjuvant chemotherapy, n (%)	15 (11.0%)	25 (3.8%)	0.873
Adjuvant chemotherapy, n (%)	111 (81.6%)	319 (48.4%)	0.115
CA19-9, median (range)	22.7 (0.01-629.8)	16.1 (0.01-321.9)	0.179
CEA, median (range)	6.2 (0.09-120.7)	4.3 (0.1-100.8)	0.096

25-OHD: 25-hydroxyvitamin D; CA19-9: carbohydrate antigen 19-9; CEA: carcinoembryonic antigen.

Table III. *Univariate and multivariate analyses for disease-free survival.*

Variables	Category	Disease free survival			
		Univariate analysis		Multivariate analysis	
		HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Age	<70 *vs.* ≥70 years	1.919 (1.252-2.942)	0.003	1.992 (1.309-3.031)	0.001
Pathologic stage	I *vs.* II, III	3.375 (2.003-5.688)	<0.001	3.739 (2.411-5.799)	<0.001
Adjuvant chemotherapy	none *vs.* conducted	1.056 (0.626-1.781)	0.838		
CA19-9	normal *vs.* elevated	1.744 (0.930-3.270)	0.082		
25-OHD, after 1 year from surgery	<12 *vs.* ≥12 ng/ml	0.546 (0.332-0.898)	0.017	0.563 (0.347-0.914)	0.020

HR: Hazard ratio; CI: confidence interval; CA19-9: carbohydrate antigen 19-9; 25-OHD: 25-hydroxyvitamin D.

It was previously demonstrated that cancer stem cells have the capacity to enter a quiescent state, allowing them to survive conventional treatment (28). Furthermore, only a small number of cancer stem cells, representing 0.1% to 10% of overall tumor cells, may be responsible for disease recurrence (29). In this study, patients with low serum 25-OHD levels below 12 ng/ml at 12 months after surgery showed a higher relapse rate as compared with those without this characteristic. It can thus be suggested that residual colon cancer stem cells might exist in a dormant state due to vitamin D deficiency during the early period after curative treatment.

The current study has highlighted the association between vitamin D deficiency and recurrence after curative surgical resection in patients with colon cancer, although it is a

retrospective analysis and there is a lack of information about possible epidemiologic confounding factors. Meanwhile, it is still controversial as to whether postoperative vitamin D supplementation could improve survival outcomes. Interestingly, Markowicz *et al*. determined that treatment with low-calcemic vitamin D analogs down-regulated cancer stem cell markers such as NANOG, OCT3/4, and ALDHA1 in chemo-resistant human colon cancer cells (24, 25). In addition, supplementation with vitamin D significantly reduced cancer mortality in other studies (30, 31). Conversely, however, it did not lower the incidence of cancer in a randomized controlled trial with a high dose of 2,000 IU/day given for the prevention of cancer (16). Therefore, well-designed further clinical trials are still necessary to evaluate the effect of vitamin D level and supplementation on the survival outcomes of patients with vitamin D deficiency.

In conclusion, our results suggest that serum vitamin D deficiency is a meaningful prognostic marker for disease relapse during the postoperative period. We also herein provide a specific serum 25-OHD cutoff value and follow-up time points for evaluation to help predict prognosis and design appropriate treatment strategies.

Conflicts of Interest

The Authors have no conflicts of interest or financial ties to disclose.

Authors' Contributions

All Authors contributed to the collection of clinical data. JGK, GSC: design and supervision of the work. JHK, DWB: data analysis, interpretation; manuscript drafting/revision. JHB, BWK: manuscript revision; agreement to accountability. SHS, HJK, SYP, JSP: data acquisition; agreement to accountability.

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