

Frequency of Combined Deficiencies of Vitamin D and Holotranscobalamin in Cancer Patients

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Abstract: *Vitamin D and holotranscobalamin (HTCII) deficiencies have been seen to demonstrate an association with various types of cancers. The objective of this study is to determine the frequency of vitamin D and HTCII deficiency in cancer patients. Our study investigated vitamin D, total B12, and HTCII levels in 70 cancer patients. Vitamin D status was measured as serum 25-hydroxyvitamin D [25(OH)D, Nichols Advantage assay], and serum B12 was measured as both total B12 and as the metabolically active HTCII (Immulate B12 assay followed by glass adsorption). Insufficiency of serum 25(OH)D levels for this study is defined based on differing literature standards of insufficiency and was selected to be either <50 or <75 nmol/l. When 25(OH)D insufficiency is defined as serum level of <75 nmol/l, 43 of 60 (72%) of cancer patients were found to be insufficient. At the lower definition of insufficiency, <50 nmol/l, 24 of 60 patients (40%) were insufficient. Of 52 patients, only 3 (6%) were found to have insufficient serum levels of total B12 (normal = >300 pg/ml), whereas 17 of 52 (34%) were found to be HTCII insufficient (normal = >69 pg/ml). Of these 17 patients, 14 (84.4%) had normal total B12 levels. Low serum levels of 25(OH)D strongly correlated with low serum HTCII. All 12 HTCII-deficient patients were vitamin D insufficient at the <75-nmol/l standard. Six of 12 HTCII-deficient patients (50%) were vitamin D deficient at the <50-nmol/l cutoff. The standard measurement of total serum B12 alone is inadequate for identifying patients with insufficient levels of metabolically active B12. Deficiency of vitamin D (72%) and HTCII (34%) is prevalent among newly diagnosed patients with cancer and could play a role in cancer development and host response to tumor and therapy. Possible explanations for combined HTCII and 25(OH)D deficiencies include patient age, presence of atrophic gastritis, and lack of sun exposure.*

Introduction

Low serum levels of 25-hydroxyvitamin D [25(OH)D] are associated with a higher frequency of at least 17 different malignancies, including breast, colon, prostate, ovarian, and other cancers (1–6). Vitamin B12 deficiency has been associated with an increased risk of breast cancer and theoretically

may contribute to other malignancies by allowing DNA hypomethylation of oncogenes suppressor genes (8,9). A previous study has reported that postmenopausal women had a 2.5–4.0 times greater likelihood of breast cancer when sustaining low levels of vitamin B12 compared with those with normal levels (10).

Preliminary work in our laboratory established a simple assay for the metabolically active form of vitamin B12 in serum holotranscobalamin (HTCII) (11). In earlier studies using this assay, we demonstrated B12 deficiency and hyperhomocysteinemia in some patients with cancer (11). This study further explores the frequency of low levels of HTCII in cancer patients and evaluates the frequency of serum 25(OH)D deficiency as well. Vitamin D status was measured with the Nichols Advantage assay, which measures both 25-hydroxyvitamin D₂ and 25-hydroxyvitamin D₃ [25(OH)D]. Study patients consisted of oncology patients seeking adjuvant or definitive chemo- or hormonal therapy in sunny Whittier, CA.

Methods

The study involved 70 patients ages 37–90 (Table 1). Patients were not required to fast before blood was obtained (12). Blood was collected in standard serum separator vacutainer tubes with gel and clot activator and allowed to clot at room temperature (BD, Franklin Lakes, NJ). After 1 h the tubes were centrifuged, and serum was separated and stored frozen at –20°C until assay (usually 7–14 days). Assay of serum 25(OH)D was performed with the Nichols Advantage assay kit (Nichols Institute Diagnostic, San Clemente, CA).

Serum total B12 and HTCII levels were determined by the technique previously described (11). The serum total B12 was first determined by enzyme-linked Immulate vitamin B12 assay (Diagnostic Products, Flanders, NJ). A second sample was preadsorbed with washed, microfine glass, and the supernatant was subjected to Immulate B12 assay. The difference (total B12 – adsorbed supernatant B12) represents the serum HTCII level. The assay technique was determined to have <10% coefficient of variation between samples and is rapid and reproducible.

Table 1. Study Patients

Patient Type	Patients (n)	Total (%)
Breast cancer	12	17.14
Colon cancer	6	8.57
Lymphoma	4	5.71
Prostate cancer	26	37.14
Lung cancer	3	4.29
Other	19	27.14

Table 2. Percentage of Patients With Vitamin D3 Insufficiency

Patient Type	<75 nmol/l, n (%)	<50 nmol/l, n (%)
Total (60 patients)	43 (72)	24 (40)
Breast cancer (10 patients)	6 (60)	3 (30)
Colon cancer (5 patients)	4 (80)	2 (40)
Lymphoma (3 patients)	3 (100)	1 (33.3)
Prostate cancer (24 patients)	17 (70.83)	7 (29.17)
Lung cancer (2 patients)	1 (50)	0 (0)
Other (16 patients)	12 (75)	11 (68.75)

Results

The lower level of normal serum concentration for 25(OH)D has been previously defined and varies between 50 and 75 nmol/l (7,13). Until there is universal agreement, we interpret our results using both low cutoff levels to define insufficiency. Based on a higher limit of 25(OH)D, 75 nmol/l, 43 of 60 patients (72%) were found to be vitamin D insuffi-

cient, whereas, with a lower limit of 50 nmol/l, 24 of 60 patients (40%) were vitamin D insufficient (Table 2).

A total of 50 patients aged 39–90 yr were studied for serum B12 content. A significant number of cancer patients were found to have normal total B12 levels (normal = ≥ 300 pg/ml) but had suboptimal HTCII levels (normal = ≥ 70 pg/ml) (Table 3).

Table 3 displays the average B12 level and the percentage of patients with HTCII levels lower than the normal value of 70 pg/ml. The next to the last column lists the percentage of patients with low HTCII levels that were found to have normal or high total serum B12 (normal range = 300–900 pg/ml). The lowest average B12 level was found in breast cancer patients. However, statistical analysis using a *t*-test for comparing means at 95% confidence revealed no significant difference between the average vitamin B12 level of breast cancer patients and other cancer types possibly due to an inadequate sample size.

The correlation of serum 25(OH)D deficiency and vitamin B12 deficiency was investigated. Comparisons were drawn between both standards of 25(OH)D deficiency and both total B12 levels and HTCII levels (Table 4). Results revealed little association between the incidence of vitamin D and total B12 deficiency. However, at both <75-nmol/l and <50-nmol/l vitamin D levels, there was a correlation between vitamin D and HTCII deficiency. χ^2 calculated = 11.28 was greater than χ^2 table = 2.71, allowing a statistical conclusion of association.

Of the 43 patients found to be serum 25(OH)D insufficient based on the <75-nmol/l standard, 27 patients (62.8%) also had B12 measurements available. Of these 27 none had insufficient total B12 levels, but 16 of 27 patients (59.3%)

Table 3. Percentage of Patients With HTCII Insufficiency^a

Patient Type	Vitamin B12 (pg/ml)	Low Total B12, n (%)	Low HCTII, n (%)	Low HTCII with Normal Total B12, n (%)
Total: all patients (52 patients)	Average: 723.11 Median: 679.5 Range: 225–1,200	3 (6)	17 (34)	14 (82.4)
Breast cancer (7 patients)	Average: 568.9 Median: 514 Range: 225–948	1 (14.3)	4 (57.1)	3 (75)
Colon cancer (6 patients)	Average: 672.83 Median: 645 Range: 304–1,200	0 (0)	3 (50)	3 (100)
Lymphoma (3 patients)	Average: 893.3 Median: 1,200 Range: 280–1,200	1 (33.3)	1 (33.3)	0 (0)
Prostate cancer (15 patients)	Average: 787.67 Median: 796 Range: 331–1,200	0 (0)	5 (35.71)	5 (100)
Lung cancer (3 patients)	Average: 686 Median: 709 Range: 335–1,014	0 (0)	1 (33.3)	1 (100)
Other (18 patients)	Average: 723.84 Median: 666.5 Range: 259–1,200	1 (5.88)	3 (17.65)	2 (66.7)

^a: Abbreviation is as follows: HTCII, holotranscobalamin.

Table 4. Combined Deficiencies^a

Patient No.	Age	Diagnosis	25(OH)D nmol/l	Total B12 pg/ml	HTCII pg/ml
1	66	Breast cancer	50	225	2
2	71	Breast cancer	57.5	576	82
3	81	Breast cancer	72	948	217
7	57	Breast cancer	42.5	344	24
8	51	Breast cancer	37.5	—	—
9	45	Breast cancer	67.5	470	18
11	82	Colon cancer	22	631	85
12	78	Colon cancer	62.5	>1,200	—
14	73	Colon cancer	22.5	675	175
15	74	Colon cancer	62.5	568	23
16	86	Lymphoma	35	280	36
17	51	Lymphoma	60	—	—
18	84	Lymphoma	55	>1,200	—
19	65	Prostate cancer	67	1,078	13
20	54	Prostate cancer	45	>1,200	—
22	71	Prostate cancer	57	—	—
23	74	Prostate cancer	70.25	1,008	240
25	63	Prostate cancer	40	696	211
27	82	Prostate cancer	36	—	—
28	75	Prostate cancer	59	749	82
30	77	Prostate cancer	74	—	—
31	76	Prostate cancer	57.5	624	46
32	90	Prostate cancer	67.5	796	0
33	72	Prostate cancer	74.5	—	—
34	60	Prostate cancer	31	—	—
36	68	Prostate cancer	32.5	—	—
37	73	Prostate cancer	40	—	—
38	68	Prostate cancer	54	—	—
40	79	Prostate cancer	72.5	364	12
42	70	Prostate cancer	47	—	—
44	66	Prostate cancer	70	1,014	99
47	53	Other	67	—	—
48	66	Other	22.5	1,064	182
49	77	Other	<17.5	259	21
50	78	Other	<17.5	392	33
52	67	Other	42.5	563	83
53	88	Other	45	588	—
55	73	Other	37.5	>1,200	—
56	66	Other	46	425	133
57	76	Other	49	522	115
58	85	Other	40	>1,200	—
59	65	Other	37.5	684	26
60	58	Other	47.5	725	—

a: Abbreviations are as follows: 25(OH)D, 25-hydroxyvitamin D; HTCII, holotranscobalamin. —, supraphysiological serum concentration of total B12 of >1,200 pg/ml or absent data.

were HTCII insufficient. Of the 24 patients found to be serum 25(OH)D insufficient based on the <50-nmol/l standard, 16 patients also had B12 measurements available. Of these 2 of 16 (12.5%) had insufficient total B12 levels, but 8 of 16 (50%) were HTCII deficient. Of the total 12 patients with HTCII deficiency and corresponding 25(OH)D data, 100% had serum 25(OH)D levels of <75 nmol/l and 50% had levels of <50 nmol/l.

Discussion

Relatively newly defined standards for vitamin D insufficiency, that is, <50 nmol/l and <75 nmol/l, determined the se-

rum cutoff levels in this study (7,13–16). Each standard corresponds to a minimal level at which serum 25(OH)D insufficiency is presumed to not contribute to the increased incidence of malignancies, diabetes mellitus, and hypertension while at the same time maintaining normal bone density, calcium homeostasis, and normal parathyroid function (14).

The frequency of serum 25(OH)D insufficiency is highest among elderly patients. The incidence depends on the definition of vitamin D deficiency employed but approaches 50% in the elderly (17–19). The ability of the skin to synthesize vitamin D when exposed to UV radiation decreases with age and is compounded by a lifestyle of decreased sun exposure (20–29). This study supports previous studies on the high

prevalence of vitamin D insufficiency in cancer patients (10,30–46). Vitamin D precursor synthesized within the skin is normally processed by both the liver and kidneys to its biologically active form, 1,25-dihydroxyvitamin D₃, calcitriol (27,47–52). More recently, the 25(OH)D form produced by the liver has been shown to be metabolized directly by benign and malignant colon, breast, and prostate tissue (apocrine regulation) to form the active *calcitriol* (51). Thus, forms of vitamin D other than calcitriol may be important by virtue of direct cellular metabolism. Calcitriol and other synthetic derivatives (paricalcitol, Zemplar) have been demonstrated to increase the clinical response to chemotherapy and irradiation (52,53). Most notable is the work of Beer et al. in hormone refractory prostate cancer patients. They demonstrated an almost doubling of response rate and median survival when large doses of calcitriol were added to taxotere chemotherapy (54). Vitamin D receptors on individual cells have been associated with its antiproliferative and possibly antineoplastic function (30,55–58). Vitamin D is an immune modulator (7). The receptors are found regardless of the cell having a role in calcium or phosphate homeostasis, and vitamin D has been shown to promote cell differentiation and apoptosis while inhibiting proliferation (59). Vitamin D insufficiency can result in increased risk of bone fracture by decreasing bone density and inducing hyperparathyroidism. Secondary hyperparathyroidism may worsen damage mediated by metastatic bone disease and has been suggested to inhibit the function of erythropoietin on red blood cell precursors. Thus, addressing vitamin D insufficiency in the form of serum 25(OH)D levels may be important for prevention and treatment of various cancers as well as for maintenance of bone health (8).

This study reveals low levels of vitamin B12 delivery in cancer patients. Thirty-four percent of our patients were found to have low levels of HTCII. Vitamin B12 can bind to transcobalamin I to form a metabolically inert storage glycoprotein (11). Vitamin B12 becomes metabolically active only after binding to transcobalamin forming HTCII. HTCII is a polypeptide and preferentially adheres to glass, allowing it to be assayed. It is the only metabolically active form of vitamin B12 and binds to HTCII receptors found on all dividing cells (60). It has been suggested that vitamin B12 is involved in the methylation regulation system of gene expression (9). Due to vitamin B12's role in the metabolism of methionine and *S*-adenosylmethionine, a deficiency of biologically active vitamin B12 may lead to hypomethylation of genes. This may allow for the expression of malignant oncogenes (9).

There are various possible causes of HTCII insufficiency found in cancer patients. The age of most cancer patients introduces factors known to be causative of B12 malabsorption, such as gastritis, hypochlorhydria, and helicobacter infection (60). Thirty to 40% of elderly people suffer from gastritis, an inability to secrete stomach acid. This inhibits the liberation of ingested food-bound B12, causing malabsorption of the vitamin, which is usually followed by hyperhomocysteinemia. Another cause may lie in the treat-

ment of cancer. Chemotherapy and radiation both disrupt mucosal surfaces of the gastrointestinal tract and can lead to decreased absorption of vitamin B12. Radiation to the terminal ileum is especially affective in lowering HTCII levels as it is the location of vitamin B12 absorption. Low HTCII in these patients may compound an already weakened myeloid and immune system. B12 plays an important role in maintaining normal numbers of white blood cells, red blood cells, and platelets (61). It also ensures the integrity of the mucosal cells in the gastrointestinal lining. Mucositis and diarrhea from chemotherapy may be worsened by concomitant B12 and/or folate deficiency, for example. Alimta (pemetrexed chemotherapy for non-small cell lung cancer and mesothelioma) toxicity is dramatically reduced by coadministration of B12 and folate (61). HTCII deficiency could delay regeneration of bone marrow after cytotoxic treatment, which may subject patients to infection.

This study gives evidence of an apparent association between vitamin D and HTCII deficiencies. This can be speculated to be a result of common factors found in the elderly. Elderly cancer patients have increased risk of gastritis and are also susceptible to low vitamin D synthesis due to inadequate sun exposure and reduced synthesis by the skin.

It should also be noted that previous studies have revealed a correlation between prediagnostic serum 25(OH)D levels and the risk of cancer (2,62–65). These studies show that the low serum 25(OH)D level precedes the diagnosis of cancer and, therefore, makes it unlikely that cancer was the cause of low 25(OH)D in these patients. Our study supports previous studies claiming a high incidence of serum 25(OH)D insufficiency in cancer patients (1–6,30,59,66). We found a deficiency rate of 72% at the <75-nmol/l cutoff and 50% at the <50-nmol/l cutoff. Previous studies also suggest a high incidence of vitamin B12 insufficiency in cancer patients. It has been found that postmenopausal women with low serum vitamin B12 levels had a 2.5–4.0 times greater likelihood of having breast cancer (10). Our results are consistent with these findings, showing an increased incidence of HTCII insufficiency among cancer patients. Thirty-four percent of all patients had insufficient levels of HTCII, 84% of which had normal serum vitamin B12 levels. Although our study supports this claim, it specifies B12 insufficiency as HTCII insufficiency. This study goes further to suggest an association between the deficiencies of both vitamin D and B12 delivery.

Our reported increased prevalence of insufficiencies of both serum 25(OH)D and HTCII in cancer patients can be addressed with clinical supplementation. We recommend supplementation of appropriate patients, especially those undergoing chemotherapy, with 500–1,000 mcg of vitamin B12 by mouth daily to maintain HTCII levels between 70 and 130 pg/ml. We also recommend 1,000–4,000 U of vitamin D₃ by mouth daily to maintain serum 25(OH)D levels between 80 and 130 nmol/l (14,20). We currently supplement our patients with oral vitamin D₃ and do not allow serum levels of 25(OH)D to exceed 200 nmol/l (14,16). All supplementation should be accompanied by monitoring levels of both vitamins during and posttherapy (14).

Acknowledgments and Notes

Glenn Tisman is the primary care physician of the patients studied and was responsible for the general scope and idea for the research. Dr. Tisman oversaw the research for and writing of the article for submission. Ashley Plant was responsible for writing the article and conducting the research under the guidance of Glenn Tisman. Address correspondence to G. Tisman MD, 13025 Bailey Street, Whittier, CA 90601. Phone: 562-789-8822. FAX: 562-698-4582. E-mail: gtisman@doctisman.com.

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