Menopause: The Journal of The North American Menopause Society
Vol. 18, No. 3, pp. 319-322
DOI: 10.1097/gme.0b013e3181f81ad5

Cancer prevalence in osteoporotic women with low serum vitamin D levels

Sophie Veldhuis, BS,[1] Floor Wolbers, PhD,[2] Olivier Brouckaert, MD,[1] Istvan Vermes, MD, PhD,[2] and Henk R. Franke, MD, PhD[1]

Abstract

Objective: The aim of this study was to assess the role of vitamin D in cancer development in postmenopausal osteoporotic women.

Methods: A cross-sectional and in vitro study was carried out, with statistical analysis with odds ratios and 95% CIs presented. Human estrogen receptor–positive breast cancer cells (MCF-7) were studied in vitro. The apoptosis-to-proliferation (A/P) ratio was also determined.

Results: A total of 885 women were included in this study. Any kind of cancer was found in 112 (12.7%) of all women. Breast cancer was the most prevalent malignancy, representing half of the cases (n = 56, 50%). The prevalence of any kind of cancer and breast cancer in women with low 25-hydroxyvitamin D_3 levels (25OHD; <50 nmol/L) was higher than in women with high 25OHD levels (≥50 nmol/L). The in vitro study demonstrated a statistically significant increased A/P ratio of 5.27 (95% CI, 4.054-6.493) with a high concentration of 1α,25-dihydroxyvitamin D (10 μM) after 96 hours.

Conclusions: Osteoporotic women with low serum levels of 25OHD (<50 nmol/L) have an increased prevalence of any kind of cancer and breast cancer; however, these differences are not statistically significant. 1α,25-dihydroxyvitamin D induced an increased A/P ratio in MCF-7 breast cancer cells in vitro.

Key Words: Osteoporosis – Vitamin D – Cancer – In vitro.

Breast cancer is the most frequent malignancy in women in Europe.[1] Osteoporosis is a skeletal condition characterized by low bone mass, resulting in reduced bone strength and increased fracture risk. Osteoporosis and breast cancer are both partially estrogen-dependent entities, and both disorders have the highest incidence in postmenopausal women.[2] Women with osteoporosis may have a lower risk for developing breast cancer because of lower estrogen concentrations during menopause.[3-5]

Vitamin D plays an important role in bone metabolism and osteoporosis, as well as in malignant disorders.[6] Women with osteoporosis are known to have lower concentrations of serum 25-hydroxyvitamin D_3 (25OHD).[7,8] Vitamin D deficiency is associated with increased breast cancer risk and decreased breast cancer survival.[9-14] A recent meta-analysis by Chen et al[15] offers strong evidence that vitamin D and calcium have a preventive effect against breast cancer. For this reason, we presume that osteoporotic women with low 25OHD levels might be at increased risk for developing breast cancer.

Received April 2, 2010; revised and accepted July 8, 2010.

From the [1]Department of Obstetrics and Gynecology, Medisch Spectrum Twente Hospital Group, Enschede, the Netherlands; and [2]University of Twente, Enschede, the Netherlands.

Financial disclosure/conflicts of interest: None reported.

Address correspondence to: Henk R. Franke, MD, PhD, Department of Obstetrics and Gynecology, Medisch Spectrum Twente Hospital Group, PO Box 50.000, 7500 KA Enschede, the Netherlands. E-mail: hrfranke@home.nl

The history of vitamin D goes far back. Phytoplankton and zooplankton have been producing vitamin D for more than 500 million years.[16] Vitamin D is a prohormone that plays an important role in calcium homeostasis and bone metabolism.[7,8] The vitamin D prohormone is incorporated in the body via dietary sources and sun exposure (UVB radiation).

The prohormone vitamin D undergoes two steps before it becomes active. The first conversion takes place in the liver, where it is converted into 25OHD, the major circulating metabolite. The second step takes place in the kidney, where 1,25 dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$), the active hormonal form, will be produced.[8,17] Vitamin D is known to have antitumor activity. It inhibits cell proliferation and stimulates cell differentiation and apoptosis in malignant cell populations. By forming a nuclear receptor ligand complex, vitamin D regulates the expression of target genes such as p21, p27, c-fos, and c-myc. These genes play an important role in the regulation of the cell cycle, cell proliferation, and apoptosis.[18-24] On the other hand, some of the vitamin D effects, for example, its antiproliferative effect, might be vitamin D receptor independent.[25]

Based on the hypothesis that women with osteoporosis may have a reduced prevalence of breast cancer because of low estrogenic concentration but might have an increased breast cancer prevalence because of the lower concentration of serum 25OHD, the objective of the present study was to assess breast cancer prevalence in relation to 25OHD levels in osteoporotic women. To answer the research questions,

TABLE 1. *Baseline characteristics*

	Normal	Osteopenia	Osteoporosis	$P_{\text{normal-osteopenia}}$	$P_{\text{normal-osteoporosis}}$	$P_{\text{osteopenia-osteoporosis}}$
n	119	263	503			
Age, y	62.4 (9.6)	64.5 (10.2)	67.6 (10.1)	0.063	<0.001	<0.001
Weight, kg	81.2 (14.0)	74.3 (12.7)	68.9 (11.4)	<0.001	<0.001	<0.001
Height, cm	167.2 (6.5)	164.6 (6.9)	162.8 (6.7)	0.001	<0.001	0.001
Lost height, cm	1.9 (2.1)	2.5 (2.5)	3.1 (3.2)	0.031	<0.001	0.002
Menopause age, y	50.1 (5.6)	48.6 (5.5)	48.5 (5.4)	0.037	0.019	0.696
25-OHD, nmol/L	54.7 (17.3)	54.0 (23.8)	49.6 (22.0)	0.755	0.018	0.012

Values are presented as mean (SD).
25-OHD, 25(OH) vitamin D_3.

an epidemiological study and in vitro research were carried out.[26,27]

METHODS

Epidemiological study

This retrospective, cross-sectional study included all women who visited the osteoporosis and fracture policlinic of The Medical Spectrum Twente Hospital Group between September 2004 and September 2009. All dual-energy x-ray absorptiometry scans were performed with the Hologic Discovery device in all women. Osteoporosis and osteopenia were defined as a T score of the lumbar spine or femur below −2 or between −1 and −2 SDs, respectively. Serum 25OHD levels were measured in all women using the vitamin D_3 Elecsys test of Roche (Basel, Switzerland). The medical histories, including malignancies, weight, height, physical activity, and fall risk, of all women were recorded. Women with incomplete data were excluded.

Statistical methods

Vitamin D deficiency was defined as a serum 25OHD level less than 50 nmol/L.[28] The prevalences of osteoporosis, osteopenia, normal bone density, any kind of malignancy, and breast cancer were calculated. SPSS 16.0 for Windows was used for the statistical analysis. Baseline characteristics were calculated using descriptive statistics. The chi-square test was used to test for statistical significance between two groups. $P < 0.05$ was considered as statistically significant.

In vitro study

Human estrogen receptor–positive breast cancer cells (MCF-7; obtained from DSMZ) were grown in a 24-well plate for 24 hours. 1α,25$(OH)_2D_3$ (Roche) in a dose of 100 nM and 10 μM was added and cells were incubated for 24, 48, 72, or 96 hours at 37°C combined with 5% CO_2 in a RPMI 1640 medium (Cambrex), supplemented with 10% fetal bovine serum gold (Cambrex), 100 IU/mL penicillin (Lonza), 100 mg/mL streptomycin (Lonza), 2 mM L-glutamin (Lonza), and 0.4 μg/mL fungizon (Lonza). Individual proliferation and apoptosis (programmed cell death) values were measured, and the corresponding apoptosis-to-proliferation (A/P) ratio was calculated. The A/P ratio clearly indicates whether there is cell growth or cell death. An A/P ratio more than 1 means induction of apoptosis, whereas an A/P ratio less than 1 means stimulation of proliferation.

Proliferation

Proliferation of the MCF-7 cells was measured with the CellTiter 96 AQ_{ueous} One Solution Cell Proliferation Assay (Promega). After incubation, the cells were washed with phosphate-buffered saline, and RPMI medium with 2% fetal bovine serum was added. Then, 60 μL MTS reagents was added. After 2 hours of incubation, proliferation was measured with the Tecan Safire-2 plate reader (Tecan).

Apoptosis

Apoptosis of the MCF-7 cells was measured with a DNA fragmentation assay, according to the Nicoletti method.[29,30] Cells were washed with phosphate-buffered saline, detached with trypsin (Lonza), centrifuged, and fixated in ice-cold ethanol. Fixated cells were incubated for 10 minutes in the dark in 1 mL sodium nitrate solution (1 mg/mL) that contained 10 μg/mL propidium iodide (Sigma). DNA fragmentation was measured with flow cytometry (FACSCalibur BD).

TABLE 2. *ORs and 95% CIs and statistical significance for different entities by vitamin D stratum*

	25-OHD <50 nmol/L	25-OHD ≥50 nmol/L	OR	95% CI	*P*
All women, n (%)	449 (100)	436 (100)	1.03	[0.903-1.175]	0.662
Osteoporosis, n (%)	278 (61.9)	225 (51.6)	1.24	[1.039-1.479]	0.018
Osteopenia, n (%)	122 (27.2)	141 (32.3)	0.87	[0.682-1.109]	0.241
Normal, n (%)	49 (10.9)	70 (16.1)	0.7	[0.486-1.008]	0.054
Malignancy, n (%)	60 (13.4)	52 (11.9)	1.15	[0.794-1.666]	0.450
Osteoporosis and malignancy, n (%)	40 (8.9)	26 (6.0)	1.6	[0.976-2.622]	0.085
Breast cancer, n (%)	33 (7.3)	23 (5.3)	1.43	[0.839-2.437]	0.181
Osteoporosis and breast cancer, n (%)	19 (4.2)	11 (2.5)	1.7	[0.809-3.573]	0.144
Osteopenia and malignancy, n	12 (2.7)	18 (4.1)	0.7	[0.337-1.454]	0.273
Osteopenia and breast cancer, n (%)	9 (2.0)	9 (2.1)	1	–	1.000

The only significant difference is the increased prevalence of osteoporosis in women with low 25-OHD levels compared with women with high 25-OHD levels. Any malignancy and breast cancer in osteoporotic women demonstrated a trend toward an increased prevalence in women with a low level of 25-OHD.
OR, odds ratio; 25-OHD, 25(OH) vitamin D_3.

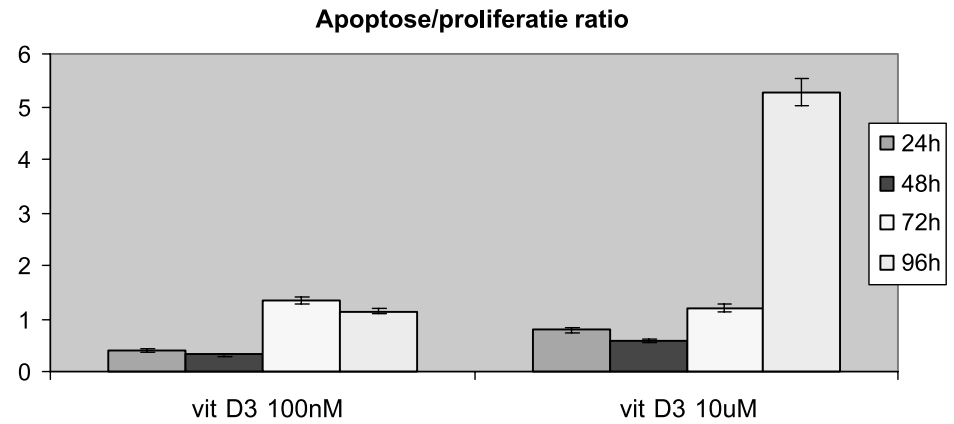

FIG. 1. Apoptosis-to-proliferation ratio of MCF-7 cells incubated with vitamin D for various time periods. Data are represented as mean ± SEM. vit D_3, $1\alpha,25[OH]_2D_3$.

Statistical analysis

All experiments with MCF-7 cells were carried out in duplicate. The results are expressed as absolute values and/or as a relative risk compared with untreated samples. From these results, the A/P ratio was calculated. Of these ratios, the standard error and 95% CI were calculated. *P* values were calculated using the one-sample *t* test, and statistical significance was defined as $P < 0.05$.

RESULTS

Epidemiological data

From September 2004 to September 2009, 1,045 postmenopausal women visited the osteoporosis and fracture polyclinic. All women (n = 885) with complete data concerning 25OHD levels and dual-energy x-ray absorptiometry scan were included. Table 1 shows the baseline characteristics of the participating women. Women were divided into three groups: women with normal bone density (13%), women with osteopenia (30%), and women with osteoporosis (57%). The mean (SD) age of the women in the osteoporosis group was 67.6 (10.1) years. This is significantly higher than that in the two other groups ($P < 0.001$). Weight and height were significantly lower and loss of height was significantly higher in women diagnosed with osteoporosis compared with the other two groups.

Of the 885 women included, 449 (51%) had a 25OHD serum concentration less than 50 nmol/L and 436 (49%) had a 25OHD serum concentration of 50 nmol/L or higher. Malignancy was found in 112 women, and 56 (50%) of these women had breast cancer. Other diagnosed malignancies were endometrial, cervical, colorectal, ovarian, and bladder cancer and melanomas (Table 2).

In vitro research

The results of the epidemiological study indicate that vitamin D plays a role in breast cancer. To further investigate this effect, in vitro experiments were performed. The results of these experiments are shown in Fig. 1. After 96 hours of incubation with a $1\alpha,25(OH)_2D_3$ concentration of 10 μM, the A/P ratio was 5.27 (95% CI, 4.054-6.493), which is statistically significant.

DISCUSSION

This cross-sectional study demonstrated that low 25OHD levels (<50 nmol/L), compared with levels greater than 50 nmol/L, are associated with a statistically significant increased prevalence of osteoporosis ($P = 0.018$). We found a trend supporting our hypothesis that cancer prevalence in women with low 25OHD levels might be increased ($P = 0.085$). These results are in accordance with the results of several ecological, hospital-based case-control and population-based case-control studies.[18] Interestingly, the previous hypothesis coincides exactly with the findings of our in vitro study. Our in vitro research shows that vitamin D possesses anticancer activity as well. A time- and concentration-dependent effect of vitamin D on apoptosis and proliferation was indeed found. In vivo studies are necessary to obtain information about the pharmacokinetic behavior, toxicity, and antitumor activity of high levels of 25OHD in women with breast cancer. At this time, there is already one such study being conducted in men with prostate cancer.[31]

CONCLUSIONS

The results of this cross-sectional and in vitro study are in accordance with the previously documented beneficial effects of vitamin D on cancer prevalence and tumor biology. We noticed that osteoporotic women with low 25OHD levels demonstrate a trend toward increased cancer prevalence.[32] However, we could not demonstrate a statistically significant effect possibly because the 25OHD levels should be much higher than 50 nmol/mL, for instance, on the order of 100 nmol/L.[13] Therefore, vitamin D supplements might not only be beneficial for bone health but also lower cancer prevalence. Further research is needed to investigate whether higher dose regimens (in contrast with current low-dose therapy regimens) of vitamin D could lower cancer prevalence in women with osteopenia or osteoporosis. Further elucidation of the exact mechanisms of vitamin D action on tumor biology is needed as well.

REFERENCES

1. Ferlay J, Autier P, Boniol M, Heanue M, Colombet M, Boyle P. Estimates of the cancer incidence and mortality in Europe in 2006. *Ann Oncol* 2006;18:581-592.
2. Johnston CC, Slemenda CW, Slemenda JR. Pathogenesis of osteoporosis. *Bone* 1995;17:19S-22S.
3. Orwoll ES, Nelson HD. Does estrogen adequately protect postmenopausal women against osteoporosis: an iconoclastic perspective. *J Clin Endocrinol Metab* 1999;84:1872-1874.
4. Wren BG. The origin of breast cancer. *Menopause* 2007;14:1060-1068.
5. Cauley JA, Gutai JP, Kuller LH, LeDonne D, Powell JG. The epidemiology of serum sex hormones in postmenopausal women. *Am J Epidemiol* 1989;129:1120-1131.
6. Garland CF, Garland FC, Gorham ED, et al. The role of vitamin D in cancer prevention. *Am J Public Health* 2006;96:252.
7. Dusso AS, Brown AJ, Slatopolsky E. Vitamin D. *Am J Physiol Renal Physiol* 2005;289:F8-F28.
8. Heaney RP. Vitamin D in health and disease. *Clin J Am Soc Nephrol* 2008; 3:1535-1541.
9. Garland CF, Gorhama ED, Mohra SB, et al. Vitamin D and prevention of breast cancer: pooled analysis. *J Steroid Biochem Mol Biol* 2007; 103:708-711.
10. Abbas S, Linseisen J, Slanger T, et al. Serum 25-hydroxyvitamin D and risk of post-menopausal breast cancer: results of a large case-control study. *Carcinogenesis* 2008;29:93-99.
11. Mehta RG, Moriarty RM, Mehta RR, et al. Prevention of preneoplastic mammary lesion development by a novel vitamin D analogue, 1α-hydroxyvitamin D5. *J Natl Cancer Inst* 1997;89:212-218.
12. Jacobson EA, James KA, Newmark HL, Carroll KK. Effects of dietary fat, calcium, and vitamin D on growth and mammary tumorigenesis induced by 7,12-dimethylbenz(a)anthracene in female Sprague-Dawley rats. *J Cancer Res* 1989;49:6300-6303.
13. Lappe JM, Travers-Gustafson D, Davies KM, Recker RR, Heaney RP. Vitamin D and calcium supplementation reduces cancer risk. *Am J Clin Nutr* 2007;85:1586-1591.
14. Crew KD, Shane E, Cremers S, McMahon DJ, Irani D, Hershman DC. High prevalence of vitamin D deficiency despite supplementation in premenopausal women with breast cancer undergoing adjuvant chemotherapy. *J Clin Oncol* 2009;27:2151-2156.
15. Chen P, Hu P, Xie D, Qin Y, Wang F, Wang H. Meta-analysis of vitamin D, calcium and the prevention of breast cancer. *Breast Cancer Res Treat* 2010;121:469-477.
16. Holick MF. Evolution and function of vitamin D. *Recent Results Cancer Res* 2003;164:3-28.
17. Colston KW, Mørk Hansen C. Mechanisms implicated in the growth regulatory effects of vitamin D in breast cancer. *Endocr Relat Cancer* 2001;9:45-59.
18. Crew KD, Gammon MD, Steck SE, et al. Association between plasma 25-hydroxyvitamin D and breast cancer risk. *Cancer Prev Res* 2009;2: 598-604.
19. Eisman JA, Sutherland RL, McMenemy ML, et al. Effects of 1,25-dihydroxyvitamin D3 on cell-cycle kinetics of T 47D human breast cancer cells. *J Cell Physiol* 1989;138:611-616.
20. Simboli-Campbell M, Narvaez CJ, Tenniswood M, et al. 1,25-Dihydroxyvitamin D3 induces morphological and biochemical markers of apoptosis in MCF-7 breast cancer cells. *J Steroid Biochem Mol Biol* 1996;58:367-376.
21. Simboli-Campbell M, Narvaez CJ, van Weelden K, et al. Comparative effects of 1,25(OH)2D3 and EB1089 on cell cycle kinetics and apoptosis in MCF-7 breast cancer cells. *Breast Cancer Res Treat* 1997;42:31-41.
22. Narvaez CJ, Welsh J. Role of mitochondria and caspases in vitamin D–mediated apoptosis of MCF-7 breast cancer cells. *J Biol Chem* 2001; 276:9101-9107.
23. Pendas-Franco N, Gonzalez-Sancho JM, Suarez Y, et al. Vitamin D regulates the phenotype of human breast cancer cells. *Differentiation* 2007;75:193-207.
24. Verlinden L, Verstuyf A, Convents R, et al. Action of 1,25(OH)2D3 on the cell cycle genes, cyclin D1, p21 and p27 in MCF-7 cells. *Mol Cell Endocrinol* 1998;142:57-65.
25. Costa JC, Eijk PP, van de Wiel MA, et al. Anti-proliferative action of vitamin D in MCF7 is still active after siRNA-VDR knock-down. *BMC Genomics* 2009;10:499.
26. Verdijk R, Franke HR, Wolbers F, Vermes I. Differential effects of bisphosphonates on breast cancer cell lines. *Cancer Lett* 2007;246:308-312.
27. Jansen GH, Franke HR, Wolbers F, Brinkhuis M, Vermes I. Effects of fulvestrant alone or combined with different steroids in human breast cancer cells in vitro. *Climacteric* 2008;11:315-321.
28. Holick M. The vitamin D pandemic and its health consequences. Paper presented at: the 34th European Symposium on Calcified Tissues; May 5, 2007; Copenhagen, Denmark.
29. Saito T, Okamoto R, Haritunians T, et al. Novel Gemini vitamin D3 analogs have potent antitumor activity. *J Steroid Biochem Mol Biol* 2008;112:151-156.
30. Verlinden L, Verstuyf A, Camp MV, et al. Two novel 14-epi-analogues of 1,25-dihydroxyvitamin D3 inhibit the growth of human breast cancer cells in vitro and in vivo. *Cancer Res* 2000;60:2673-2679.
31. Vitamin D effects on prostate pathology (DProstate). Clinical trial NCT00741364.
32. Sontag A. Estimated risk of invasive breast cancer in Caucasian postmenopausal women with osteoporosis using the Gail Model compared with US general population. *Menopause* 2009;16:59-60.

