SHORT REPORT

# Serum 25-hydroxyvitamin D levels in early and advanced breast cancer

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**Background:** Laboratory and epidemiological studies have implicated vitamin D deficiency in the pathogenesis of breast cancer. 1,25-Dihydroxyvitamin D ($1,25(OH)_2D$) promotes differentiation and apoptosis, and potently inhibits proliferation of malignant breast epithelial cells in culture. Serum levels of $1,25(OH)_2D$ are higher in normal women than in patients with primary breast cancer.
**Aim:** To clarify the role of vitamin D in breast cancer progression by comparing the levels of serum vitamin D in patients with early and in those with advanced breast cancer.
**Methods:** Circulating levels of 25-hydroxyvitamin D (25(OH)D), parathyroid hormone (PTH) and calcium were measured prospectively in 279 Caucasian women with invasive breast cancer, 204 women with early-stage disease and 75 women with locally advanced or metastatic disease.
**Results:** Patients with early-stage breast cancer had significantly higher circulating levels of 25(OH)D ($p<0.005$) and significantly lower PTH ($p<0.001$) levels than those with advanced disease. Calcium levels did not differ significantly ($p=0.74$).
**Conclusion:** Serum levels of 25(OH)D are significantly higher in patients with early-stage breast cancer than in those with locally advanced or metastatic disease.

Laboratory and epidemiological studies implicate vitamin D deficiency in the pathogenesis of breast cancer. In vitro studies show that 1,25-dihydroxyvitamin D ($1,25(OH)_2D3$) inhibits the proliferation of breast cancer cell lines,[1] promotes differentiation[2] and induces apoptosis.[3] Animal studies have shown that dietary vitamin D can abrogate the tumorigenic effects of a high-fat diet on mammary tissue[4] and that $1,25(OH)_2D3$ and its analogues can inhibit growth of breast cancer cell lines,[5] prevent the development of carcinogen-induced mammary tumours[6] and have a pro-apoptotic effect in nude mice bearing MCF-7 xenografts.[7]

Epidemiological studies have shown a higher incidence of breast cancer in countries at higher latitudes and in those living at higher latitudes in the same country,[8 9] and an association with an increased risk of dying from breast cancer for those living at such latitudes.[8] Vitamin D sufficiency and exposure to sunlight have been shown to reduce the risk of developing breast cancer in the National Health and Nutrition Survey Epidemiological Follow-up Study I.[10] A case–control study of normal women and those with breast cancer showed that blood levels of $1,25(OH)_2D3$ were higher in the cohort of healthy women.[11] In women with breast cancer, tumour DNA aneuploidy was associated with a low dietary vitamin D intake,[12] whereas increased skin pigmentation was associated with larger-sized breast cancers and increased frequency of nodal involvement.[13] In women with bone metastasis secondary to breast cancer, serum $1,25(OH)_2D3$ fell in those who developed disease progression.[14] To further investigate the potential role of vitamin D in breast cancer progression, we measured circulating levels of 25-hydroxyvitamin D (25(OH)D), the major circulating form of vitamin D and the best indicator of vitamin D status, as well as that of parathyroid hormone (PTH) and calcium in patients with early or advanced breast cancer.

## METHOD

Serum was obtained from 279 Caucasian women with invasive breast cancer: 204 with early-stage breast cancer (stage I or II) and 75 with locally advanced or metastatic disease (stage III or IV). Patients were staged according to the 6th edition of the American Joint Committee on Cancer staging system for invasive breast cancer.[15] Patients with renal impairment or those receiving bisphosphonate therapy or epilepsy drugs were excluded. Blood samples were obtained with informed consent at the time of the clinic appointment and analysed immediately after collection. Serum 25(OH)D was quantified by radioimmunoassay (ImmunoDiagnostic Systems, IDS, Boldon, UK) and PTH by immunoradiometric assay (Nicols Institute Diagnostics, San Clemente, California, USA). Serum calcium (corrected for albumin binding) was measured by the automated standard laboratory method. Normal laboratory reference ranges were as follows: 25(OH)D 15–100 nmol/l, PTH 1.06–5.3 pmol/l and corrected calcium 2.15–2.65 mmol/l.

### Statistical analysis

The unpaired t test was used for comparative statistical analysis of biochemical data on each group of patients. The study had 80% power to detect a difference between mean serum 25(OH)D concentrations of 10 nmol/l, with a significance level ($\alpha$) of 0.05 (two tailed).

## RESULTS

Patients with early-stage breast cancer were found to have circulating concentrations of 25(OH)D ranging from 15.0 to 184.0 nmol/l (mean 57 nmol/l, 95% confidence interval (CI) 52.7 to 60.5 nmol/l) as compared with levels of 16–146 nmol/l (mean 46.0 nmol/l, 95% CI 40.9 to 51.8 nmol/l) in patients with locally advanced or metastatic breast cancer. Comparison of the two groups yielded a significant difference in vitamin D levels ($p=0.048$). PTH levels ranged from 1.2 to 11.3 pmol/l (mean 3.91 pmol/l, 95% CI 3.7 to 4.2 pmol/l) in the early-stage breast cancer group as compared with a range of 1.05–14.1 pmol/l (mean 5.06 pmol/l, 95% CI 4.4 to 5.8 pmol/l) in the locally advanced/metastatic cancer group; there was a significant difference between these two groups with regard to PTH levels, ($p<0.001$). We found no significant difference between these two groups with regard to calcium levels ($p=0.74$). Table 1 summarises these results.

**Abbreviations:** $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone

**Table 1** Comparison of the serum levels of calcium, parathyroid hormone and vitamin D between patients with early-stage breast cancer and locally advanced or metastatic breast cancer

| | Early-stage breast cancer | Advanced-stage breast cancer |
|---|---|---|
| Total number of patients | 204 | 75 |
| Age range (years) | 28–90 | 32–92 |
| Mean | 55 | 59 |
| Calcium (mmol/l) | | |
| Range | 2.07–2.80 | 2.04–2.72 |
| Mean | 2.34 | 2.34 |
| SEM | 0.01 | 0.02 |
| 95% CI | 2.3–2.4 | 2.3–2.4 |
| p = 0.74 | | |
| Parathyroid hormone (pmol/l) | | |
| Range | 1.2–11.30 | 1.05–14.10 |
| Mean | 3.91 | 5.06 |
| SEM | 0.12 | 0.35 |
| 95% CI | 3.7–4.2 | 4.4–5.8 |
| p<0.001 | | |
| 25(OH)D (nmol/l) | | |
| Range | 15–184 | 16–146 |
| Mean | 57.0 | 46.0 |
| SEM | 2.0 | 3.0 |
| 95% CI | 52.7–60.5 | 40.9–51.8 |
| p = 0.005 | | |

25(OH)D, 25-hydroxyvitamin D.

## DISCUSSION

This study has shown that serum levels of 25(OH)D were markedly higher and that PTH levels were considerably lower in patients with early-stage breast cancer than in those with locally advanced or metastatic disease. The notably higher serum PTH in patients with metastatic disease than that in those with early-stage disease is presumably due to the lower vitamin D level, resulting in a lower serum calcium and therefore a rise in serum PTH. The raised PTH level can therefore account for the lack of any difference in serum calcium between these two groups. Epidemiological studies have previously shown that maintenance of adequate levels of vitamin D via exposure to sunlight is associated with a reduced incidence and mortality of breast cancer.[8 9 11] This may reflect the fundamental importance of vitamin D in regulating aspects of cellular behaviour such as cell growth. This is supported by in vitro data in breast cancer cell lines, as well as in vivo animal studies,[6 7] which have shown the ability of $1,25(OH)_2D3$ to inhibit proliferation and promote differentiation and apoptosis.[1–4] In addition, an observational study showed lower levels of $1,25(OH)_2D3$ in women diagnosed with primary breast cancer compared with a healthy cohort,[11] and a decrease in vitamin D in patients with breast cancer with bone metastasis that progressed.[14] The results of this study showing lower levels of 25(OH)D in women with advanced breast cancer lends weight to the hypothesis that the growth of breast cancer in vivo is inhibited by vitamin D. The exact reason for the deranged and low 25(OH)D levels in patients with advanced cancer as compared with those with early-stage breast cancer is unclear, and also whether the decrease in 25(OH)D is causative for the advanced disease or is a direct consequence of the advanced disease as a result of cancer-related reduced dietary intake or altered synthesis in the skin due to reduced sun exposure.

24-Hydroxylase inactivates $1,25(OH)_3$ and is involved in the homeostasis of serum levels of $1,25(OH)_3$, and it is suggested to be an oncogene.[16] The expression of 24-hydroxylase is shown to be higher in primary breast tumours than in normal breast tissue,[17] and the levels of $1,24,25(OH)_3$ were considerably higher in malignant breast tumours. It is also known that $1,24,25(OH)_3$ does not induce an anti-proliferative response in breast cancer cell lines. This can be reversed by antisense inhibition of 24-hydroxylase in vitro.[17] Therefore, a resistance mechanism to the potential effects of vitamin D—namely, via the dysregulation of 24-hydroxylase activity—seems to exist in breast tumours. How this dysregulation differs between early-stage and locally advanced or metastatic breast cancer is not known, given the previously normal tissue was compared with primary breast tumours.[17] Possibly, in advanced breast cancer, a further dysregulation in the metabolism of vitamin D may result from some paracrine tumour effect, or tumours that have high 24-hydroxylase levels may have a greater propensity to progress to advanced stage disease.

Vitamin D binds to the vitamin D receptor, which is a ligand-activated transcription factor that controls gene transcription via binding to vitamin D response elements in DNA. Microarray analysis has shown that several key genes are up regulated or down regulated as a result of vitamin D treatment. One such key gene that is up regulated is the cyclin-dependent kinase inhibitor p21, which has an important role in controlling cell cycle progression.[18] Whatever the cause for the change in vitamin D levels, it can potentially have a marked effect on gene transcription and therefore on cellular phenotype. Lower serum vitamin D levels might therefore have some causative role in the progression from early-stage to advanced disease as a result of altered gene transcription.

### Take-home messages

- Epidemiological studies, as well as in vitro and in vivo data, suggest a role for vitamin D in the pathogenesis of breast cancer.
- Vitamin D levels have been shown to be higher in normal women compared with those who have primary breast cancer, and decrease with the progression of bone metastases.
- This study shows a significantly higher level of 25-hydroxyvitamin D in women with early breast cancer compared with those with advanced disease. Further work is required to determine the precise mechanism for the dysregulation of vitamin D levels in advanced breast cancer.

In summary, these findings lend support to the hypothesis that vitamin D has a role in the pathogenesis and progression of breast cancer. This report, while being an observational study, clearly shows that circulating vitamin D levels are lower in patients with advanced breast cancer than in those with early breast cancer. However, several questions remain unanswered. These include the potential causes and mechanisms underlying this dysregulation of vitamin D regulation, their precise molecular consequences and the potential clinical implications of monitoring or maintaining high circulating vitamin D levels in patients diagnosed with breast cancer. Answering these questions offers the potential of improving the risk stratification, surveillance and treatment of women with breast cancer.

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