Serum 25-Hydroxyvitamin D Levels in Patients with Vulvar Cancer

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Abstract. *Background: The anticarcinogenic potential of vitamin D 25(OH)D has been attributed to the inhibition of proliferation of cells from different carcinomas. Reduced serum levels of 25(OH)D are associated with an increased incidence of various types of cancer. The influence of serum 25(OH)D on the incidence and outcome of patients with vulvar cancer is unknown. Patients and Methods: The serum 25(OH)D levels in 24 patients with vulvar cancer and 24 age-matched cancer-free patients was investigated. The blood samples were collected between October 2009 and September 2010 and time of blood collection of each patient and control was matched to avoid seasonal variations between the pairs. Results: The median 25(OH)D serum levels in the under 50 year old group of patients were significantly lower in the vulvar cancer group than the controls. The younger cancer group also had an age-related trend of lower median serum level than the older population. In the control population the trend was viceversa, yet this finding was not statistically significant. Conclusion: Serum 25(OH)D has a possible role in the pathogenesis and progression of vulvar cancer, but further investigations of the association of vitamin D and vulvar cancer as well as regarding its influence on patient survival and quality of life are warranted in the future.*

Squamous cell carcinoma of the vulvar is a rare disease with an annual incidence of 1.5-4 per 100,000 women in Germany (1). Vulvar cancer accounts for 3% to 5% of all genital carcinomas affecting women following endometrial, ovarian and cervical cancer (2). Since 1970 the incidence of carcinoma *in situ*, attributed to VIN III, has nearly doubled (3-5), while invasive carcinoma has shown a tendency to increase over the last few years (6, 7).

Vitamin D is a liposoluble vitamin, like vitamins A, E and K. It is obtained from food such as fish, liver, milk and eggs or is endogenically synthesized from cholesterol (8-10). The synthesis starts in the liver. Cholesterol is dehydrogenated to 7-dehydrocholecholesterol (7-DHC) (11) and bonded to vitamin-D-binding-protein (DBP) it is transported via blood circulation to the skin. Here cholecalciferol is synthesized by light in the 290-315 nm UV-B range (16), and then binds to DBP and circulates back to the liver. The cholecalciferol hydroxylated to 25-hydroxycholecalciferol (25(OH)D), which is the main form of circulating vitamin D (13, 14) is further hydroxylated in the kidney to the biologically active metabolite 1,25-dihydroxycholecalciferol (calcitriol) (15, 16). Its predominant role is to preserve skeletal calcium by ensuring that adequate absorption of dietary calcium and phosphorous takes place.

Regions north of the 40th degree of latitude only have the required wave length of 290-315 nm for cholecalciferol synthesis in the months from April to October. The months from November to March are known as the "vitamin-D-winter", a deficit situation (15-17). For this reason there are different ranges of standard of vitamin D plasma values in summer and winter.

Although the conversion of 25(OH)D primarily takes place in the kidney, several studies have shown that, 1α-hydroxylase is also present in other tissues such as the prostate (18), colon (19, 20), pancreas (21), parathyreoid (22) and breast (23).

25(OH)D is also found in carcinomas such as breast, colon and prostate cancer (18, 20, 23, 24-28), but in breast cancer its levels are significantly decreased in comparison to the normal tissue (24). The anticarcinogenic effect of vitamin D has been detected in breast, colon and prostate cancer (27, 28) and includes the acceleration of cell differentiation, the inhibition of cell growth and the regulation of apoptosis (29-32). Deficit of vitamin D correlates with these carcinomas (33-35).

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To our knowledge, no study has been published so far, examining the association between circulating 25(OH)D concentrations and vulvar cancer.

Patients and Methods

Study participants and settings. The study population consisted of patients with histologically proven squamous cell carcinoma of the vulvar of different stages. The patients, aged between 41 and 91 years, were treated from October 2009 to September 2010 at the Department of Gynecology and Obstetrics of the Helios Hospital of Krefeld, Germany. After the exclusion of patients with other than squamous cell carcinoma, vulvar intraepithelial neoplasia or *in situ* carcinomas, the final study population consisted of 24 vulvar cancer patients. The matched control group consisted of 24 cancer-free and healthy women, who were at the same time patients at the Department of Gynecology and Obstetrics of the Helios Hospital Krefeld, Germany. Women with a history of liver or kidney diseases, osteoporosis, diabetes mellitus, endometriosis or pregnancy were excluded from the study.

None of the patients were receiving a vitamin D supplement. One control was matched to each case (n=24) on age (±1,4 years) and time of blood collection (±3 weeks). The blood samples were stored at the Institute of Hygiene and Laboratory Medicine, Helios Hospital Krefeld, Germany, after the completion of routine preoperative serological diagnostics.

Exposure assessment. The venous blood samples collected for the biochemical analyses were immediately processed and separated. The serum samples were aliquoted and stored at -20°C until further analysis. Basal 25(OH)D serum levels were analyzed at the laboratory using radioimmunassay (RIA).

The patients' clinical and histopathological characteristics were abstracted from their hospital and pathology records. Because the 25(OH)D serum levels were normally distributed (demonstrated by Kolmogorov-Smirnov Test), differences in the quantitative variables between individual groups were analysed with *t*-Test and Anova analyses. All the statistical analyses were performed using the statistical package SPSS, version 18.0 (Statistical Package for Social Sciences, SPSS GmbH, München, Germany). A *p*-value of less than 0.05 was considered statistically significant.

Results

25(OH)D serum levels. The characteristics of the study population are summarized in Table I. A high prevalence of vitamin D deficiency, defined as serum 25(OH)D levels <20 ng/ml, was found both in the vulvar cancer patients (79.2%) and the controls (75.0%). The median 25(OH)D serum levels were slightly lower in the vulvar cancer patients (15.4 ng/ml) as compared to the controls (15.6 ng/ml), although this difference was not statistically significant (p=0.723).

Seasonal differences in 25(OH)D serum levels. Comparison of the 25(OH)D serum level of the nine observable months between October 2009 and September 2010 showed no significant difference between the vulvar cancer patients and the control group (Table II).

Table I. *Patient characteristics*.

Variable	Vulva cancer patients (n=24)	Control group (n=24)	Total (n=48)
Age group			
<50.9 years	16.7% (4)	20.8% (5)	18.8% (9)
51.0-60.9 years	20.8% (5)	20.8% (5)	20.8% (10)
61.0-70.9 years	41.7% (10)	33.3% (8)	37.5% (18)
>71.0 years	20.8% (5)	25.0% (6)	22.9% (11)
Serum 25(OH)D			
<20 ng/ml	79.2% (19)	75.0% (18)	77.1% (37)
<10 ng/ml	8.3% (2)	8.3% (2)	8.3% (4)
10-20 ng/ml	70.8% (17)	66.7% (16)	68.8% (33)
>20 ng/ml	20.8% (5)	25.0% (6)	22.9% (11)

The patients diagnosed in autumn (14.9±4.3 ng/ml) (standard deviation SEM) and winter (14.4±4.8 ng/ml) had lower 25(OH)D serum levels as compared to the patients diagnosed in spring (16.7±8.7 ng/ml) and summer (22.4±10.2 ng/ml), although this difference was not statistically significant (Figure 1).

Correlation between age and 25(OH)D serum levels. No statistically significant association was found when the 25(OH)D levels were compared with respect to age. Among the vulvar cancer patients, an age-related trend (p=0.119) was observed concerning the 25(OH)D levels: the group of under 50.9 years had a median 25(OH)D serum level of 10.8 ng/ml compared to 16.4 ng/ml observed in the older population (>71.0 years) (Figure 2). The opposite trend (p=0.128) was observed in the control group: the group of under 50.9 years had a median 25(OH)D serum level of 20.2 ng/ml compared to 13.7 ng/ml observed in the older population (>71.0 years).

The median 25(OH)D serum levels were significantly (p=0.017) lower in the vulvar cancer patients under 50.9 years (10.8±2.6 ng/ml) as compared to the control group under 50.9 years (20.2±5.5) (Table III).

Discussion

25(OH)D is an ideal parameter for categorizing the vitamin D content of an organism and for determining deficiency (36). This is due to the longer half-life of 25(OH)D approximately one month compared to three to eight hours for the active form of vitamin D, 1,25(OH)D. In addition, 25(OH)D is under strict homeostatic control by the renal 1α-hydroxylase.

A high prevalence of vitamin D deficiency was observed in this study. Although there is still no consensus on optimal levels of 25(OH)D (37). People who are exposed

Table II. *Season of diagnosis and 25(OH)D (ng/ml) serum levels in vulvar cancer patients and control group. Spring: March-May , summer: June-August, autumn: September-November and winter: December-February).*

	Vulvar cancer (n=24)		Control group (n=24)			Total (n=48)	
	n	mean±SEM	n	mean±SEM	*p*	n	
January	2	17.1±7.5	2	19.0±9.1	0.808	4	18.0±5.7
March	2	11.3±3.5	2	14.3±3.3	0.351	4	12.8±2.7
May	3	20.4±10.1	3	11.5±2.7	0.213	6	15.9±8.2
July	1	38.2	1	20.7		2	29.5±12.4
August	5	19.2±7.4	5	19.0±7.4	0.964	10	19.1±7.0
September	2	16.2±0.3	2	14.7±4.0	0.642	4	15.4±2.5
October	5	13.7±5.5	5	16.8±5.8	0.410	10	15.2±5.6
November	2	16.5±3.4	2	16.3±1.1	0.944	4	16.4±2.1
December	2	11.6±5.2	2	15.3±2.8	0.472	4	13.5±4.0
Spring	5	16.7±8.7	5	12.6±2.9	0.346	10	14.7±2.1
Summer	6	22.4±10.2	6	19.3±6.6	0.546	12	20.9±2.4
Autumn	9	14.9±4.3	9	16.2±4.4	0.527	18	15.5±1.0
Winter	4	14.4±4.8	4	17.2±5.9	0.490	8	15.8±1.8

Table III. *Comparison of 25(OH)D serum levels and age in vulvar cancer patients and control group.*

Age (years)	Vulvar cancer patients (n=24)		Control group (n=24)			Total (n=48)	
	n	mean±SEM	n	mean±SEM	*p*	n	
≤50.9	4	10.8±2.6	5	20.2±5.5	0.017	9	16.0±6.5
51.0-60.9	5	15.1±4.0	5	14.4±2.0	0.749	10	14.7±3.0
61.0-70.9	10	20.9±9.8	8	17.3±6.3	0.384	18	19.3±8.4
≥71.0	5	16.4±2.0	6	13.7±3.8	0.180	11	14.9±3.3

to sunlight frequently have serum concentrations of about 40-70 ng/ml 25(OH)D which are regarded as normal values. In a large study of the Robert-Koch-Institute, nationwide vitamin D deficiency was delineated in Germany (38). Thus Cannell *et al*. postulated that these normal values for people in nothern latitudes are not realistic (39). Although the range of 40-70 ng/ml in special patient groups is desirable, serum concentrations of 25 ng/ml are now regarded as the baseline for global normal values (40). So values below 25 ng/ml are already, or should be considered as vitamin D deficiency.

In the vulvar cancer patients and controls, 87.5% and 83.3%, respectively, showed 25(OH)D serum levels <25 ng/ml, which was not significantly different and was comparable with results of other recently published investigations among the German population (38). The patients diagnosed with vulvar cancer in winter compared to summer had lower 25(OH)D serum levels. For the control group there was a discrete opposite trend in the spring months. This result for the control group is unexpected, as one would expect a higher intensity of exposure to the sun. A higher 25(OH)D serum level should be expected in summer (41). However, the blood samples were collected on a single date before the operation therefore, no intra-individual variation between summer and winter could be assessed. It is assumed that several additional influencing factors concerning the 25(OH)D serum levels were involved. In general, it is estimated that one billion people worldwide are vitamin D deficient or insufficient (16,42). The skin normally supplies more than 50% of the vitamin D requirements which has clinical relevance particularly in older people, who are bedbound (43). In 80% of older people a vitamin D deficiency has been observed, which could explain the low 25(OH)D serum levels of the older patients. Due to limitations including the low number of cases, the present study did not allow a definite conclusion as to whether the season of diagnosis affected the prognosis of vulvar cancer.

This was the first study identifying young patients with vulvar cancer to be at high risk of having vitamin D deficiency, the median 25(OH)D serum level (10.8±2.6 ng/ml) of the younger patients with vulvar cancer (<50 years) was statistically significantly at lower values compared to younger controls (20.2±5.5 ng/ml). These results are consistent with the literature, where patients with breast cancer had lower 25(OH)D serum levels compared to healthy women (44, 45, 46), and significantly lower 25(OH)D serum levels were found in stage IV melanoma patients (37).

Populations in less sunny latitudes have a higher risk of breast, colon and prostate cancer than the population in sunny countries (47,48) and sun light exposure is inversely correlated with the breast cancer risk (49). Furthermore sun exposure, avoiding sunburn, can reduce the risk for cancer development, without increasing the risk of the emergence of skin tumors (50). In addition intake of vitamin D supplements was accompanied by a lower risk for breast cancer (34).

It can only be speculated whether reduced serum 25(OH)D levels affect vulvar cancer progression directly. However we suggest that it is of high importance to detect and to treat vitamin D deficiency in vulvar cancer, especially in younger women. Further investigations in larger studies of the association of vitamin D and vulvar cancer and the influence on patient survival and quality of life are warranted. Determining the effects of achieving and maintaining adequate 25(OH)D levels with supplementation on patient outcomes is also an important research avenue.

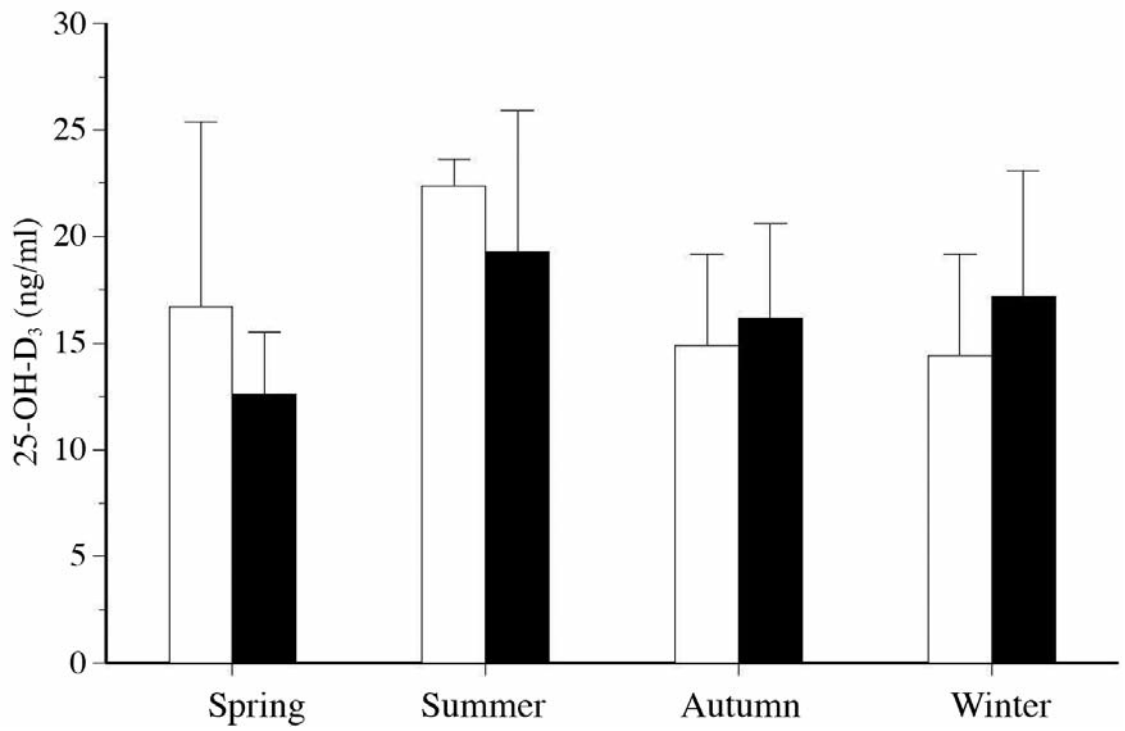

Figure 1. *Season of diagnosis and 25(OH)D (ng/ml) serum levels in vulvar cancer patients (white) and control group (black).*

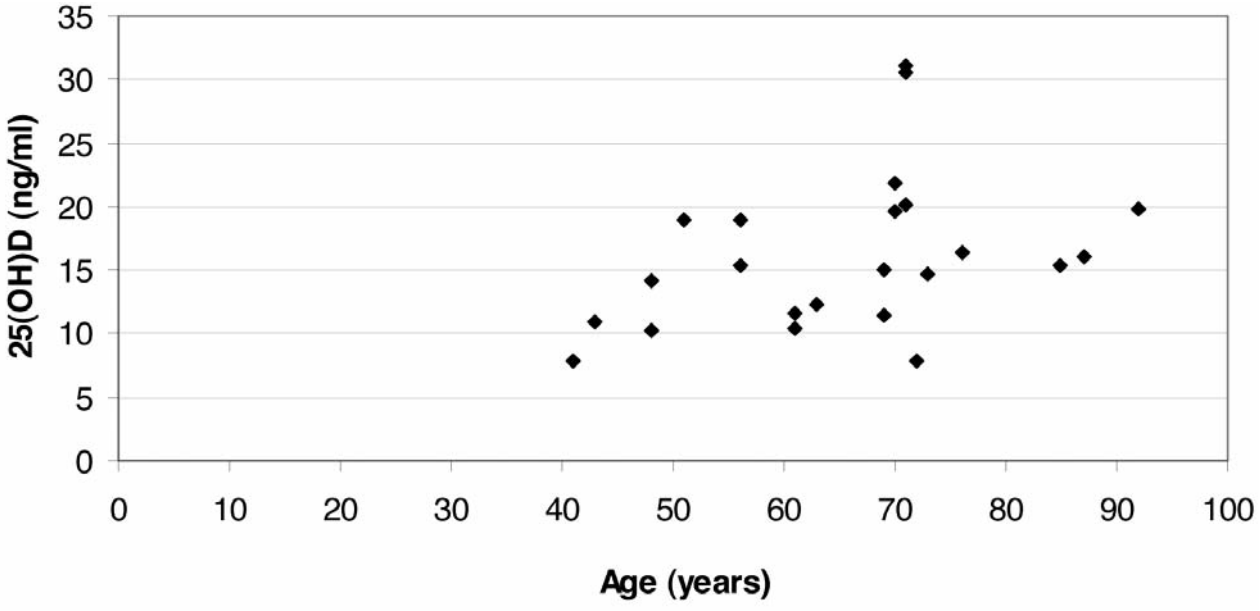

Figure 2. *Patient 25(OH)D serum levels in relation to age.*

Conclusion

Patients with vulvar cancer are also at high risk of vitamin D deficiency. Although a possible role of serum 25(OH)D in the pathogenesis and progression of vulvar cancer is indicated, due to the study limitations, definite conclusions cannot be drawn.

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