Insufficient Vitamin D Supply as a Possible Co-factor in Colorectal Carcinogenesis

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Abstract. Vitamin D, the "sunshine vitamin", may play a role in the development of colorectal cancer. In a prospective open study, the plasma levels of 25-hydroxyvitamin D [25(OH)D], the marker for sufficient or insufficient vitamin D supply, were determined in three groups of patients whose diagnoses were confirmed by colonoscopy: healthy individuals (n=239), patients with colorectal adenoma (n=203) and with carcinoma (n=98). In order to assess other factors such as nutrition, sunlight exposure and physical activity as co-variates for the risk of colorectal cancer, the individuals completed a questionnaire. Patients with colorectal cancer (CRC) had significantly decreased plasma 25(OH)D levels (p<0.001) compared to the controls in contrast to patients with adenomas, who had lower levels exclusively in the winter (p=0.01). When analyzed by the Kruskal-Wallis test, the groups of patients with adenomas (p=0.03) and colorectal carcinomas (p<0.0001) had significantly different mean plasma values compared to the controls. The plasma 25(OH)D levels showed an inverse correlation to the UICC stages of CRC; however, the differences were not significant. Patients with CRC were significantly older than the controls, but regression analysis showed no significant correlation between the plasma 25(OH)D levels and age, and the influence of age on the plasma levels of 25(OH)D was minimized in a group of individuals over 65 years of age, in which the patients with CRC had significantly decreased plasma 25(OH)D levels below 20 μg/l (the normal range) and one-third even had plasma levels below 20 μg/l. Regression analysis showed a significant influence of age on plasma 25(OH)D levels in healthy individuals, of physical activity in patients with adenomas and of season in patients with CRC. Other co-variates, such as nutrition or sunlight exposure, had no significant influence on plasma 25(OH)D. In conclusion, an insufficient vitamin D supply might act as a co-factor in colorectal carcinogenesis.

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Colorectal cancer (CRC) is a major cause of morbidity and mortality in Western industrialized countries. Up to 70% of CRC might be preventable by a moderate change in lifestyle and nutrition (1, 2). In 1980, it was suggested that calcium and vitamin D may reduce the risk of CRC (3). Vitamin D₃ (cholecalciferol) is mainly produced in the skin by ultraviolet light (UV-B), the normal diet containing only very low amounts (vitamin D₂=ergocalciferol is not used in Germany). Vitamin D₃ is then metabolized in the liver to 25-hydroxyvitamin D₃ [25-hydroxycholecalciferol, 25(OH)D₃, calcidiol], which is the main circulating form of the vitamin D metabolites and the best indicator of the vitamin D supply. The biologically most active form of vitamin D₃ is 1,25(OH)₂D₃ (calcitriol). It is released by the activity of 1α-hydroxylase present in human normal and malignant colorectal tissue (4, 5) and exerts its biological effects on growth control via the nuclear vitamin D receptor (VDR) in tumor cells (6). VDR expression increases during the transition from normal colonic mucosa to polyps and to the early stages of CRC. In later stages, however, the VDR levels are dramatically reduced (7). It was suggested to be a predictive marker of the biological behavior of CRC (8). Calcitriol inhibited proliferation and promoted differentiation of colon epithelial cells (9-13) and induced apoptosis (14), though epidemiological studies showed contradictory results. Some studies showed an inverse association between plasma 25(OH)D levels and the risk of CRC (2, 15-18), while others did not show any protective effects of vitamin D (19-21). Sunlight exposure was
associated with a reduced risk of a variety of cancers including CRC (3, 22, 23).

The purpose of the present study was to determine the plasma levels of 25(OH)D as a marker of the individual vitamin D status in subjects with normal colon, adenomas and colorectal carcinomas, and to assess other factors such as nutrition, sunlight exposure and physical activity as co-variates for the risk of CRC.

Materials and Methods

Study population. Cases and controls in this open prospective study were patients with large adenomas (>1 cm), with CRC and with normal colonic mucosa, respectively, who underwent total colonoscopy in ten gastroenterology practices in southwest Germany. After informed consent, blood samples were collected (routine testing before colonoscopy: coagulation testing, hemoglobin and complete blood count). The remaining EDTA plasma was used for 25(OH)D testing. These plasma samples were stored for up to one week in the deep freeze at –20°C for determination of 25(OH)D. Two hundred and seventy-five patients were examined in the winter (November 2002 to April 2003) and 264 in the summer (May 2003 to October 2003). Patients with chronic inflammatory bowel diseases, below the age of 38 years and after colonic surgery, were excluded from the study.

The patients answered a questionnaire about sunlight exposure, nutrition and physical activity. The questions are shown in Table I.

Vitamin D assays. The plasma 25(OH)D was determined by chemiluminescence protein-binding assay on the automated instrument Nichols Advantage (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA), as described by Roth et al. (24). This assay detects 25(OH)D$_3$ and 25(OH)D$_2$ in EDTA plasma and serum. However, since vitamin D$_3$ is not used in Germany, the reported plasma levels consist of 25(OH)D$_2$. According to the instructions of the test kit, the results are reported as 25(OH)D.

The 25-hydroxylase of vitamin D is not subject to strict homeostatic control in contrast to the 1α-hydroxylase. Therefore, 25(OH)D is influenced by dietary vitamin D uptake and cutaneous endogenous production after sunlight exposure. Its half-life in the plasma is about one month. Therefore, plasma 25(OH)D is the appropriate marker for studies on vitamin D supply. The optimum reference range of plasma or serum 25(OH)D is 25 - 70 µg/l, with values below 20 µg/l being considered low, indicating vitamin D deficiency.

Data analysis. Statistical analysis was performed on SAS, Version 8.2 and Medcalc software. The Kruskal-Wallis test was used for univariate variance analysis with plasma 25(OH)D as the variable. If the result was significant, the Wilcoxon test (npar1way procedure in SAS) was performed to compare independent groups. In an additional step, a multiple regression model was separately fitted for healthy individuals, for patients with adenomas and for patients with CRC. The SAS procedure reg was used with the backward elimination option. Variables were removed from the model at $p=0.05$. Different co-variates (age, physical activity, sunlight exposure, fish and milk consumption, and season winter/summer) were tested as possible confounding factors using this procedure. The significance level for all tests was 0.05. Adjustment for multiple testing was not done.

Results

About one-half of the subjects with normal colonic mucosa (controls, $n=239$) had plasma 25(OH)D levels below 25 µg/l and one-third even had plasma levels below 20 µg/l (Figure 1). Patients with CRC ($n=98$) had significantly decreased plasma 25(OH)D levels compared to controls in contrast to patients with adenomas ($n=203$), who had lower levels exclusively in the winter (Table II). When analyzed with the Kruskal-Wallis test, the groups of patients with adenomas ($p=0.03$) and CRC ($p<0.0001$) had significantly different mean values compared to the controls. To analyze different patient groups, the Wilcoxon test was used for statistical analysis. When analyzed separately, the plasma 25(OH)D levels of female and male patients with CRC showed significantly lower values in winter ($p=0.006$ and $p=0.03$, respectively) in contrast to patients with adenomas who showed no significant difference vs. controls, except for females in winter ($p=0.04$). The plasma 25(OH)D showed an inverse correlation to the CRC stage (Table III and Figure 2), but without significant difference (Table III). Patients with adenomas and CRC were significantly older than the healthy controls ($p<0.01$ for subjects tested in winter and summer). To minimize the influence of age on the plasma 25(OH)D levels, those above 65 years of age were analyzed separately. In winter, patients with CRC had significantly lower 25(OH)D levels than the controls, in contrast to patients with adenomas (Table IV). However, in the above 65 year age-group, no significant differences were observed in summer (Table IV).

Multiple regression analysis with backward elimination showed a significant influence of age on plasma 25(OH)D levels in healthy individuals, of physical activity in patients with adenomas, and of season in patients with CRC (Table V). Unexpectedly, however, patients with CRC had significantly higher plasma 25(OH)D levels in the winter than in the summer.
Germany suffers one of the highest incidence rates of and mortality from CRC, with approximately 57,000 new cases and 27,000 deaths annually (1). The most important risk factors for many types of cancer are environmental (25). Changes in diet and lifestyle have been proposed to strongly influence the risk of CRC. Sunlight exposure has been associated with a reduced risk of a variety of cancers, including CRC (3, 22, 23), probably acting through photo-initiation of vitamin D production.

Figure 1. Plasma 25(OH)D levels in controls in summer (rhombus) and winter (rectangles). The line indicates the lower limit of the normal range (25 µg/l).

Table II. Plasma 25(OH)D levels in controls and patients with CRC and adenoma in winter and summer.

<table>
<thead>
<tr>
<th></th>
<th>Winter</th>
<th></th>
<th></th>
<th>Winter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>Adenomas</td>
<td>CRC</td>
<td>Controls</td>
</tr>
<tr>
<td>n</td>
<td>127</td>
<td>95</td>
<td>53</td>
<td>112</td>
</tr>
<tr>
<td>25(OH)D µg/l (mean±S.D.)</td>
<td>29±14</td>
<td>24±11 p=0.01</td>
<td>21±11 p=0.0007</td>
<td>29±14</td>
</tr>
<tr>
<td>95% CI</td>
<td>26-31</td>
<td>22-27</td>
<td>18-24</td>
<td>27-31</td>
</tr>
<tr>
<td>Summer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>34</td>
<td>39</td>
<td>24</td>
<td>30</td>
</tr>
<tr>
<td>25(OH)D µg/l (median)</td>
<td>23</td>
<td>22 p=0.7</td>
<td>15 p=0.001</td>
<td>23</td>
</tr>
<tr>
<td>95% CI</td>
<td>20-25</td>
<td>20-25</td>
<td>13-17</td>
<td>20-25</td>
</tr>
</tbody>
</table>

95% CI=95% confidence interval.

Discussion

Much of the geographical variation in cancer mortality rates seen in the U.S. can be attributed to variations in solar UV-B radiation exposure (23). Studies in vitro have shown the antiproliferative effects of the active metabolite of vitamin D, 1,25(OH)2D3 (9, 12, 13), and the induction of apoptosis (14) in human colon cancer cells. 25-Hydroxyvitamin D-1α-hydroxylase converts vitamin D to its active metabolite and was found in normal and malignant colon

Table III. Plasma 25(OH)D levels in correlation to colorectal carcinoma stage.

<table>
<thead>
<tr>
<th>UICC stage</th>
<th>Mean</th>
<th>S.D.</th>
<th>Median</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>20.9</td>
<td>8.7</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>17.7</td>
<td>9.4</td>
<td>18 ns</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>19.8</td>
<td>12.5</td>
<td>16 ns</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>15.7</td>
<td>7.9</td>
<td>14 ns</td>
<td></td>
</tr>
</tbody>
</table>

*Wilcoxon test compared to UICC Stage I (ns=not significant).

Table IV. Plasma 25(OH)D levels in over 65-year-old controls and patients with CRC and adenoma in the winter and summer.

<table>
<thead>
<tr>
<th></th>
<th>Winter</th>
<th></th>
<th></th>
<th>Winter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls &gt;65y</td>
<td>Adenomas &gt;65y</td>
<td>CRC &gt;65y</td>
<td>Controls &gt;65y</td>
</tr>
<tr>
<td>n</td>
<td>34</td>
<td>39</td>
<td>24</td>
<td>30</td>
</tr>
<tr>
<td>25(OH)D µg/l (mean±S.D.)</td>
<td>25±9</td>
<td>21±9 p=0.08</td>
<td>17±9 p=0.003</td>
<td>25±9</td>
</tr>
<tr>
<td>95% CI</td>
<td>22-28</td>
<td>18-24</td>
<td>14-21</td>
<td>22-28</td>
</tr>
<tr>
<td>Summer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>30</td>
<td>52</td>
<td>19</td>
<td>30</td>
</tr>
<tr>
<td>25(OH)D µg/l (median)</td>
<td>18</td>
<td>23 p=0.1</td>
<td>15 p=0.2</td>
<td>18</td>
</tr>
<tr>
<td>95% CI</td>
<td>14-21</td>
<td>18-27</td>
<td>11-19</td>
<td>14-21</td>
</tr>
</tbody>
</table>

95% CI=95% confidence interval.

Table V. Influence of different co-variates on plasma 25(OH)D levels in controls and patients with adenomas and CRC. Analysis was performed by multiple linear regression. The results are expressed as p-values of the co-variates investigated.

<table>
<thead>
<tr>
<th>Age &gt;65 y</th>
<th>Physical activity</th>
<th>Sunlight exposure</th>
<th>Fish</th>
<th>Milk</th>
<th>Season (summer/winter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>0.005</td>
<td>0.4</td>
<td>0.3</td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>Adenomas</td>
<td>0.2</td>
<td>0.01</td>
<td>0.07</td>
<td>0.7</td>
<td>0.2</td>
</tr>
<tr>
<td>CRC</td>
<td>0.6</td>
<td>0.28</td>
<td>0.06</td>
<td>0.8</td>
<td>0.5</td>
</tr>
</tbody>
</table>
tissue (5, 10, 26). VDR was expressed in human CRC with high levels of expression being found in the early stages of cancer in contrast to the late stages or undifferentiated carcinomas, where the vitamin D system was severely compromised (7, 8, 10, 11).

Case-control studies have shown a protective effect of vitamin D intake on colorectal neoplasia (27, 28), but prospective cohort studies (15, 21, 29, 30) as well as interventional studies (31-33) came to contradictory results. In our study, the plasma 25(OH)D levels of patients with CRC and adenoma were significantly decreased in winter in agreement with previous studies (34, 35), but in contrast to another study where no differences were found (36). Different laboratory methods and different distribution of the CRC stages may account for these divergent results. The plasma 25(OH)D levels were inversely correlated with the UICC stages of CRC according to a previous study (37). However, the differences were below the level of significance.

A large number of the healthy controls showed plasma 25(OH)D levels below the normal range. This may be due to the low UV-B radiation exposure in Germany and may partly explain why Germany is among the countries with a high incidence of CRC (1). Vitamin D supplementation may be important in such countries.

The 25(OH)D levels decrease with age (38-40), probably because of a diet poor in vitamin D and lack of exposure to sunshine. The patients with adenomas and carcinomas were significantly older than the controls. Age was a significant co-variate for plasma 25(OH)D in normal individuals, as shown by the multiple regression analysis. However, the inverse correlation between the 25(OH)D levels and age was below significance. Low 25(OH)D plasma levels are not completely explained by increased age as the analysis of individuals over 65 years old showed significantly lower levels of 25(OH)D in carcinoma patients in the winter.

A significant influence of physical activity on plasma 25(OH)D in patients with adenomas was shown by logistic regression analysis, in agreement with a previous study (41). In patients with CRC, only season was a significant confounding variate for plasma 25(OH)D. Surprisingly, the plasma 25(OH)D levels were higher in winter. This may be due to the selection of seasonal periods (November to April and May to October). In May and June many people in Germany still have low levels, with a tendency to rise in July and August. In addition, the number of persons per month was quite variable. Unfortunately, only single 25(OH)D plasma levels were determined so that intra-individual comparison was not possible and, furthermore, the group of carcinoma patients was rather small (53 in winter and 46 in summer). Other confounding factors, such as ingestion of dairy foods and fish or sunshine exposure, were below the level of significance.

In conclusion, about one-half of the healthy controls showed plasma 25(OH)D levels below the normal range. Compared to normal individuals, the plasma 25(OH)D levels in patients with CRC were significantly decreased independently of age, and the levels showed an inverse correlation to the tumor stage. An insufficient vitamin D supply and suboptimal levels of plasma 25(OH)D might, therefore, act as a co-factor in colorectal carcinogenesis.

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