Vitamin D influences the prevalence of non-cutaneous carcinomas after kidney transplantation?

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**ABSTRACT**

Malignancy is a key factor that significantly reduces the graft and patient survival after kidney transplantation. Vitamin D (VD) is gaining attention for its pleiotropy, including neoplasia prevention. The aim of our study was to assess the possible association between de novo non-cutaneous carcinomas (non-cuCa) and the VD status in kidney transplant recipients (KTRs). All patients followed up in our transplant center were included in the study from May 2012 until May 2016. We compared KTRs with non-cuCa to those without carcinomas. The demographic characteristics, immunosuppression protocols and 25-hydroxyvitamin D levels were evaluated. Patients with unstable kidney function, renal transplant duration less than 5 years, other malignancies, cholecalciferol supplementation and outliers for VD were not included in the study. KTRs with virus-associated carcinomas were also excluded. The total 25-hydroxyvitamin D was measured by a validated liquid chromatography–tandem mass spectrometry (LC-MS/MS) method. Two hundred fifty-six patients met the selection criteria. Of these, 11 were detected with non-cuCa with different organ localisation. The VD deficient patients had higher non-cuCa prevalence compared to the rest of the cohort (16.7\% vs. 3.4\%, \(p = 0.034\)). The VD status was significantly lower in the patients with malignancy (39.27 ± 18.16 vs. 59.87 ± 22.82 nmol, \(p = 0.005\)). No other significant differences between the two groups were detected. Poorer VD status may be an independent risk factor for post-transplant non-cutaneous cancer. VD supplementation may be considered as an option to reduce non-cuCa prevalence after kidney transplantation.

**Introduction**

Malignancy is the third most common cause for patient loss after kidney transplantation (KT), following infection and cardiovascular disease. Furthermore, its incidence is expected to increase in the future and possibly to surpass that of cardiovascular disease [1]. There are two groups of factors associated with increased risk for neoplasia after KT: factors typical for the general population, such as age, sun exposure and smoking; and transplant-specific factors, such as immunosuppression and infections (Ebstein–Barr virus, hepatitis B and C viruses, human papilloma virus, etc). Malignancies after KT have different characteristics including presentation time and prognosis. The post-transplant lymphoproliferative disorder (PTLD) has earlier onset, whereas the cancers of the skin and the internal organs present later in the post-transplant period. Non-cutaneous cancers (non-cuCa) have the poorest prognosis, compared to skin cancers and PTLD [1]. Currently, a modification of immunosuppression and screening protocols has been suggested by guidelines [2]. Alternative options have been considered, too. One of these is vitamin D (VD). There is an increasing amount of data indicating that the benefits from vitamin D span beyond the calcium–phosphorus metabolism. VD is associated with hypertension and diabetes control, renal protection and immunomodulation. Antineoplastic properties are detected as well in in vitro studies, animal models and in the general population [3–5]. However, the data for the association between VD and cancer disease after KT are insufficient and conflicting [6–8]. Therefore, the aim of our study was to assess the association between VD status and the prevalence of non-cuCa in kidney transplant recipients (KTRs).
Subjects and methods

Patients’ selection

The study was approved by the Institutional Ethics Committee and was in accordance with the Helsinki Declaration of 1975 (as revised in 2000). All participants gave their informed consent prior to inclusion in the study.

The patients that were followed up in the Transplant Center at University Hospital ‘Alexandrovska’ from May 2012 until May 2016 were included in the study. The KTRs were divided into two groups: patients with non-cuCa (diagnosed within the above-mentioned time interval) and patients without carcinoma. The two groups were compared according to their demographic characteristics (age, gender, transplant duration, body mass index and kidney function), the immunosuppressive agents used and the VD status. The VD status was assessed by the serum levels of 25-hydroxyvitamin D [25(OH)D], as generally accepted [9]. For the patients with non-cuCa, we took into consideration any 25(OH)D tests within 12 months prior to the neoplasia diagnosis. Patients with unstable kidney function, renal transplant duration of less than 5 years, vitamin D supplementation and outliers for vitamin D (absolute value for Z-score above 3.29) were not included in the study. Only patients with histologically detected carcinoma were included. We excluded patients that developed cutaneous carcinomas under the influence of extreme sun exposure, as well as carcinomas associated with infections (Hepatitis B, Hepatitis C, Human papilloma virus and Helicobacter pylori).

Routine laboratory tests

Routine laboratory tests, including enzymatic serum creatinine assay, were performed on a standard clinical chemistry analyser. We used the Modification of Diet in Renal Disease (MDRD) Study equation to calculate the estimated glomerular filtration rate (eGFR).

Testing for 25-hydroxyvitamin D

Determination of 25(OH)D was performed by a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method developed in-house, utilising extraction with hexane. The method was calibrated with commutable commercial materials traceable to the US National Institute of Standards and Technology Standard Reference Material 972, and was validated according to the current guidance requirements, with documented selectivity and matrix effects within 11%, accuracy and precision within 7.5%, extraction recoveries averaging from 57% to 73%, and a linearity range of 3.0 to 300.0 nmol/L ($R^2 > 0.99$). The method was certified by the UK Vitamin D External Quality Assessment Scheme, with certification valid since 2012.

Statistics

Statistical analysis included descriptive statistics, Mann–Whitney U test and Fisher exact test. The level of significance adopted was $p < 0.05$. SPSS 22.0 Software (SPSS Inc., Chicago, IL, USA) was used. In order to avoid distortions of parameter and statistic estimates, we screened the data for 25(OH)D for outliers, using the Z-score method, with cut-off values lower than $-3.29/$ and higher than $+3.29/$.

Results and discussion

Two hundred fifty-six KTRs met our selection criteria. Of these, 11 received a non-cuCa diagnosis within the period of observation. The location of the primary cancer is shown in Table 1.

We evaluated the prevalence of non-cuCa in the different groups according to the vitamin D status. Non-cuCa was most prevalent in the VD deficient patients [25(OH)D < 25 nmol/L, n = 18] – 16.67%, whereas in the VD sufficient KTRs (n = 61), no cases of neoplasia were found (Figure 1). The VD deficient subjects had statistically higher prevalence of non-cuCa compared to the rest of the cohort ($p = 0.034$, Figure 2) and compared to the VD sufficient group ($p = 0.010$, Figure 1).

We compared the demographic parameters (age, transplant duration, gender and body mass index), laboratory indicators (kidney function evaluated by eGFR), immunosuppressive agents and 25(OH)D levels across the two groups. The KTRs with neoplasia had significantly lower vitamin D levels (39.27 ± 18.16 nmol/L) vs. the KTRs without malignancy (59.87 ± 22.82 nmol/L), $p = 0.005$. There were no other significant associations (Table 2). In our previous study, several risk factors for lower 25(OH)D in KTRs were detected [10]. However, no significant differences in the distribution of these factors across the groups with and without non-cuCa were established (Table 2).

| Location          | n  |
|-------------------|----|
| Esophagus         | 1  |
| Colon             | 2  |
| Lungs             | 3  |
| Kidneys (native and transplant) | 3  |
| Urinary bladder   | 1  |
| Thyroid gland     | 1  |
Current evidence demonstrates the inverse association between the prevalence of different primary non-skin cancers and the 25(OH)D level in the general population [11,12]. In addition, VD supplementation may be beneficial in reducing the neoplasia incidence and improving the outcomes in malignancy [13]. Suboptimal 25(OH)D is highly prevalent after kidney transplantation [14]. However, the knowledge about the relationship between VD and cancer in KTRs is insufficient, as the reports are usually single-center studies, encompassing a relatively small number of patients and presenting conflicting results [6–8,15]. Our study demonstrated poorer VD status in KTRs with non-skin cancer, compared to transplant recipients without cancer, indicating a possible link between 25(OH)D and cancer prevalence. What is more, our findings show a significant rise in cancer prevalence in VD deficient KTRs, compared to non-deficient patients and VD sufficient ones, indicating that these patients are at highest risk of neoplasia. No cut-off values for VD pleiotropy have been recognised, though target 25(OH)D for patients with breast cancer has been suggested [16]. Therefore, we can speculate that avoiding 25(OH)D values below 25 nmol/L may play an important role in cancer prevention after kidney transplantation.

Several mechanisms could explain the antineoplastic effect of VD. Calcitriol inhibits the mitogenic signalling by suppressing growth factors (e.g. insulin growth

**Figure 1.** Prevalence of non-cutaneous carcinoma in different groups according to vitamin D status.

*Note: n = 256; 25(OH)D, 25-hydroxyvitamin D.*

**Figure 2.** Significantly higher cancer prevalence detected in vitamin D deficient kidney transplant recipients.

*Note: n = 256; 25(OH)D, 25-hydroxyvitamin D.*
factor-1, epidermal growth factor) and by increasing growth inhibitors such as transforming growth factor-β. VD inhibits cyclin-dependent kinase activity, thus suppressing the cell cycle. It induces apoptosis through suppression of apoptosis-specific genes, and induces cell-specific differentiation mechanisms via regulation of β catenin and nucleotide factor kB pathways. Calcitriol inhibits angiogenesis by down-regulation of vascular endothelial growth factor through suppression of hypoxia-inducible factor 1 alpha and Interleukin-8 [17,18].

The above-mentioned anticancer mechanisms could explain the VD-associated antineoplastic effects in organ-specific cancers. Our data demonstrate three major groups of organ localisation in KTRs: gastro-intestinal tract (esophagus and colon), lungs, kidneys (native and transplant). Better VD status was associated with lower risk of lung cancer, due to suppressing cell proliferation, induction of apoptosis and inhibition of angiogenesis [19]. The relationship between esophageal cancer and VD status is not clearly defined, as there have been conflicting reports. Several studies indicate increased risk with better VD status, whereas others demonstrate reduced incidence after ultraviolet exposure [20]. One of the key pathogenic mechanisms in colorectal cancer is the disruption of the β catenin signalling pathway, which is inhibited by calcitriol [21]. The association between VD and renal carcinoma has been studied extensively, too. VD influences cancer cells not only by inhibiting their proliferation and angiogenesis, but also by modifying risk factors for renal carcinoma, such as diabetes, obesity and hypertension [20]. In addition, studies demonstrate inverse relationship between VD and renal carcinoma and bladder carcinoma [22]. Finally, some studies link low 25(OH)D to increased risk for thyroid cancer, with similar oncogenic mechanisms suggested [23]. However, this relationship is still under debate and further trials are needed.

Our study has some limitations. A prospective trial with cholecalciferol supplementation may better demonstrate the relationship between the VD status and supplementation on the one hand, and cancer prevalence and prognosis after KT on the other hand, including evaluation of metastatic disease and patient survival.

Conclusions

Our results suggest that poorer VD status is associated with significantly increased risk for non-skin cancer in KTRs. VD deficient patients are at particular risk. A randomised controlled multicenter trial is needed to further evaluate the association between VD and cancer after renal transplantation.

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Disclosure statement

The authors declare no conflict of interest.

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