SHORT COMMUNICATION

Vitamin D Status from Dried Capillary Blood Samples

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SUMMARY

Background: Given the huge impact of vitamin D deficiency on a broad spectrum of diseases such as rickets, osteoporosis, mineral bone disease-vascular calcification syndrome, infectious diseases, but also several types of cancer and CNS diseases, reliable and simple methods to analyze the vitamin D status are urgently needed.

Methods: We developed an easy technique to determine the 25-OH vitamin D status from dried blood samples on filter paper. This allows determination of the 25-OH vitamin D status independently of venous blood taking, since only sampling of capillary blood is required for this new method. We compared the results of vitamin D measurements from venous blood of 96 healthy blood donors with those from capillary blood taken from the same patients at the same time. The capillary blood was dried on filter paper using the D-Vital ID® dry-blood collection system.

Results: 25-OH vitamin D concentration data from extracted dried capillary blood filters correlated very well with data obtained after direct measurement of venous blood samples of the same blood donor (R: 0.7936; p<0.0001). The correlation was linear over the whole range of 25-OH vitamin D concentrations seen in this study. A Bland-Altman plot revealed good agreement between both tests.

Conclusions: The D-Vital ID® dry-blood collection system showed an excellent performance as compared to the classical way of 25-OH vitamin D measurement from venous blood. This new technique will facilitate easy and reliable measurement for vitamin D status, in particular, in rural or isolated areas, developing countries, and field studies.

KEY WORDS

25-OH vitamin D, filter paper, capillary blood, new analysis method

INTRODUCTION

Vitamin D is a generic name for a group of fat-soluble steroids of which the two major forms are vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Vitamin D3, or calciferol, without a subscript, refers to either D2, D3, or both. In humans, vitamin D is unique because it both functions as a prohormone and the body can synthesize it (as vitamin D3) when sun exposure is sufficient [1,2,3,4,5].

Vitamin D is involved in a variety of biological processes (see Table 1). There are two major, different forms of vitamin D, D2 and D3, which are very similar in structure. The structural difference between vitamin D2 and vitamin D3 is in their side chains. Vitamin D3 (cholecalciferol) is produced by ultraviolet irradiation (UV) from its precursor 7-dehydrocholesterol. This molecule occurs naturally in the skin of animals and in milk. Vitamin D2 is a derivative of ergosterol, which is produced by some organisms of phytoplankton, invertebrates, and fungi. The vitamin ergocalciferol (D2) is produced in these organisms from ergosterol in response to UV irradiation [11]. In the liver, both forms of vitamin D are hydroxylated to 25-hydroxyvitamin D (25-OH vitamin D), the major circulating metabolite of vitamin D. In the

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Calcitriol mediates its biological effects by binding to the vitamin D receptor (VDR), which is principally located in the nuclei of target cells. The binding of calcitriol to the VDR allows the VDR to act as a transcription factor that modulates the gene expression of transport proteins (such as TRPV6 and calbindin), which are involved in calcium absorption in the intestine. The vitamin D receptor belongs to the nuclear receptor superfamily of steroid/thyroid hormone receptors, and VDRs are expressed by cells in most organs, including immune cells like macrophages, brain, heart, skin, gonads, prostate, and breast. VDR activation in the intestine, bone, kidney, and parathyroid gland cells leads to the maintenance of calcium and phosphorus levels in the blood and to the maintenance of bone content [1,2,3,4,5]. Both excess as well as deficiency of 25-OH-vitamin D seem to cause abnormal vascular structure and function and hence premature aging. Published data suggest a u-shaped risk curve between serum 25-OH vitamin D level and all-cause mortality with an increased risk at low and high serum levels. The most detrimental effects appear at a lower level in people with black skin [6,7,8,9]. Vitamin D has pleiotropic effects on a variety of organ systems such as bones, blood vessels, heart, and immune system [1,2,3,4,5]. Hence, it is not surprising that the 25-OH-vitamin D status is associated with a variety of diseases such as rickets, osteoporosis, and mineral-bone disease-vascular calcification syndrome in uremic patients. Moreover, vitamin D3 affects both the innate as well as the adaptive immune responses. Epidemiological studies have established that vitamin D3 deficiency plays an important role in tuberculosis [10] and viral influenza prevalence as well as susceptibility to active disease in tuberculosis. Vitamin D3 status has been associated with the clinical course of HIV infection and drug interaction with anti-retroviral therapy [11]. The 25-OH-vitamin D status is also associated with the incidence of several types of cancer such as colorectal cancer [12], lung cancer, and breast cancer. CNS diseases such as multiple sclerosis are also associated with the vitamin D status. However, it remains to be demonstrated whether treatment with vitamin D may prevent the occurrence of malignancies and CNS diseases and/or decrease disease progression. More adequately powered and designed clinical trials are urgently needed. Given the huge impact of vitamin D deficiency on a broad spectrum of diseases such as outlined above, reliable, and simple methods to analyze the vitamin D status are urgently needed. We developed an easy technique to determine the 25-OH vitamin D status from dried blood samples on filter paper making it possible to determine the 25-OH vitamin D status independently of venous blood collection, since only sampling of capillary blood from fingertips or earlobes is required for this new method.

MATERIALS AND METHODS

Clinical Samples
Capillary blood was taken from 96 randomly selected healthy blood donors at the Transfusion Center, University Medical Center, Johannes Gutenberg-University Mainz, Germany. The study was approved by the local ethical board of the university.

Preparation of blood samples
Capillary blood for 25-OH vitamin D analysis was obtained with the D-Vital ID® dry-blood collection system (DZ9002; Immundiagnostik, Bensheim, Germany) according to the manufacturer’s instructions. Briefly, a finger of an apparently healthy blood donor was pricked with the supplied lancet and 50 µL of the emerging blood drop were collected using the supplied plastic pipet. The blood sample was dripped onto the filter of the sampling device and let dry for 30 minutes. At the same time, venous blood samples were obtained from the blood donors. Serum samples were stored at 2°C for 1 day before analysis.

Determination of 25-OH vitamin D
Dried capillary blood was eluted by moistening the filter of the sampling device with activating solution (K 2110ACTSOL; Immundiagnostik, Bensheim, Germany) and vortexing. 25-OH vitamin D was extracted by adding precipitation solution (K 2110PREC; Immundiagnostik, Bensheim, Germany), incubation at 37°C for 30 minutes and followed by centrifugation. 25-OH vitamin D was determined in serum and in the clear supernatant of extracted capillary blood using a commercially available immunoassay (K 2110; Immundiagnostik, Bensheim, Germany) according to the manufacturer’s instructions, described in detail elsewhere [13,14,15,16].

Statistical analyses
Data were analyzed using the SPSS 19.0 software package. We performed a linear regression analysis as well as a Bland Altman plot to investigate the association and agreement of 25-OH vitamin D measurements using either dried - on filter paper - capillary blood or venous blood samples. To determine the inter-assay variability of the D-Vital ID® dry-blood collection system in combination with the 25-OH vitamin D immunoassay, data were compared by paired measurements of 20 patients. The intra-assay variability was estimated using venous blood, since determination of intra-assay vari-
A)

Figure 1. A) Linear regression analysis demonstrating close agreement between the two methods (25-OH vitamin D in capillary blood dried on filter paper and 25-OH vitamin D from the corresponding venous blood samples). The solid line shows the calculated regression line. The slope of this line is 1.022 (close to the ideal line with a slope of 1.000). R: 0.7936 and p<0.0001 indicating an excellent performance of the D-Vital ID® dry-blood collection system as compared to the analysis of 25-OH vitamin D from venous blood. B) Bland-Altman plot (Difference plot) of 25-OH vitamin D measurements comparing 25-OH vitamin D in capillary blood dried on filter paper to 25-OH vitamin D from corresponding venous blood samples using ELISA [13,14,15,16]. The mean absolute differences in 25-OH vitamin D levels measured between whole venous blood and capillary blood dried on filter paper was 6.6 nmol/L (solid line); [mean + 2SD = 51.6 nmol/L (upper dotted line) and mean - 2SD = - 64.7 nmol/L (lower dotted line)].
ability from a single donor providing capillary blood for all tests is not feasible.

RESULTS AND DISCUSSION

We analyzed the 25-OH vitamin D status of healthy blood donors, whose health status had been checked by the physicians of the Transfusion Center, University Medical Center of the Johannes Gutenberg-University of Mainz according to the guidelines of the German Medical Association (Bundesärztekammer). The mean age of the 96 (30 female/66 male) blood donors was 36.99 +/-12.55 years (range 19 - 68 years). The inter-assay variability was 5.18% +/-3.71%. The intra-assay variability was 3.63% +/-2.52%. The concentrations of 25-OH vitamin D in the serum samples covered the whole range from extremely low concentrations to concentrations above the recommended 25-OH vitamin D target blood concentration for healthy individuals. Our study thus tested exactly the spectrum of concentrations seen in daily clinical practice. 25-OH vitamin D analysis using the D-Vital ID® dry-blood collection system showed an excellent performance as illustrated in Figure 1A. 25-OH vitamin D concentration data from extracted dried capillary blood correlated very well with data obtained from measurements of venous blood samples of the same blood donor (for details see Figure 1A). The correlation was linear over the whole range of 25-OH vitamin D concentrations seen in this study. We also tested the agreement in 25-OH vitamin D measurements using either venous blood samples or capillary blood dried on filter paper using Bland-Altman plots. The results are presented in Figure 1B. The mean absolute differences in 25-OH vitamin D measurements between whole venous blood and capillary blood dried on filter paper was only 6.6 nmol/L, for details see Figure 1B. Only three out of 96 points fall outside of the two standard deviation intervals indicating good agreement of both tests.

However, it is important to note that the sampling of capillary blood must be carried out properly. The test pipet must be filled completely so that exactly 50 µL of capillary blood are dried on the filter. If the pipet is not filled completely the vitamin D concentration will be underestimated. This potential limitation of the method, however, is negligible when the users comply with the instructions of the D-Vital ID® dry-blood collection system and do not use pipets other than the ones supplied.

Our test system is of major clinical impact, because it allows easy screening for vitamin D deficiency. This is of broad clinical impact given the fact that vitamin D deficiency can be treated easily and is involved in the pathogenesis of a very broad spectrum of diseases such as rickets, osteoporosis, and mineral bone disease-vascular calcification syndrome in uremic patients [17], infectious diseases, but also several types of cancer and CNS diseases. Our system will, for example, allow screening in patient populations where venous blood taking is sometimes difficult, like young children and elderly people. It also makes vitamin D screening easier from a logistic point of view (Table 1).

Table 1. Diseases/conditions where the vitamin D status is affected [1,2,3,4,10,11,12].

<table>
<thead>
<tr>
<th>Bone Diseases/Osteoporosis</th>
<th>Cardiovascular Disease/Diabetes</th>
<th>Infectious Diseases</th>
<th>Cancer</th>
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<tbody>
<tr>
<td>Autoimmundiseases</td>
<td>Mineral Bone Disease-Vascular Calcification Syndrome</td>
<td>Vascular Ageing</td>
<td>Dementia</td>
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Samples for vitamin D measurements can be prepared even by the patients themselves without the need to go to a special medical center. They can send the dried filters to their physicians simply by mail. The dried filter samples are stable for at least 3 months. This makes shipping and storage much easier. This might be of huge impact in rural areas, developing countries, field studies, and isolated areas (South Pole Stations, Amazon).

In conclusion, the D-Vital ID® dry-blood collection system showed an excellent performance and thus offers an easy and reliable screening for vitamin D status.

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Declaration of Interest:
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