

## Vitamin D Status in Diabetes Mellitus: Comparison between Outpatients and Inpatients

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### Abstract

**Objectives:** Vitamin D (25(OH)D) status has been extensively evaluated in different populations and care settings. A negative relationship between glycosylated hemoglobin (HbA1c) and serum 25(OH)D levels in outpatients with diabetes has been reported, while data about 25(OH)D status in inpatients with diabetes are inconsistent. The aim of the study was to evaluate 25(OH)D levels in a large series of inpatients with type 1 and type 2 diabetes and in an age-, sex-, serum creatinine-, and HbA1c-matched group of outpatients with diabetes.

**Design:** After the preliminary exclusion of patients with confounding factors, 540 subjects with diabetes were retrospectively evaluated in a 1:1 matched case-control study between inpatients and outpatients.

**Results:** 25(OH)D levels resulted significantly lower in inpatients versus outpatients with diabetes (37.9 nmol/L, median, 25.3 interquartile range, vs 44.9, 31.8 nmol/L, respectively), regardless of season. 25(OH)D levels were inversely correlated with HbA1c levels and BMI in outpatients, and with fibrinogen and erythrocyte sedimentation rate in inpatients.

**Conclusions:** Vitamin D deficiency is common in diabetic inpatients and more frequent than in diabetic outpatients. 25(OH)D status in diabetic inpatients is not related to glycemic control but is likely influenced by acute inflammatory condition.

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**Running Title:** Vitamin D status in diabetes mellitus

**Key Words:** diabetes mellitus, metabolic control, vitamin D, inpatients

**Received:** 05 May 2017 ; **Accepted:** 26 June 2017 ; **Published:** Jul 24, 2017

## Introduction

Vitamin D status has been extensively evaluated in different populations and care settings, with a vitamin D deficiency (VDD)<sup>1</sup> prevalence of 50–90%, depending on the used diagnostic criteria and the study population.<sup>2</sup>

Hypovitaminosis D is usually exacerbated in seasons with lower ultraviolet B (UVB) irradiation. Low UVB exposure in the modern lifestyle and atmospheric pollution partially blocking UVB are among the factors that contribute to the risk of VDD.<sup>3,4,5</sup>

Patients with diabetes have reportedly a higher prevalence of VDD in comparison to the general population,<sup>3,4</sup> and a role of vitamin D supplementation in improving insulin secretion and sensitivity has been suggested.<sup>6</sup> VDD, indeed, contributes to both the initial insulin resistance and the subsequent onset of diabetes caused by  $\beta$ -cell death. Vitamin D reduces inflammation, which is a major process in inducing insulin resistance. Vitamin D maintains the normal resting levels of both Ca<sup>2+</sup> and reacting oxygen species that are elevated in the  $\beta$ -cells during diabetes.<sup>7,8</sup>

Moreover, in outpatients with diabetes a negative relationship between glycosylated hemoglobin (HbA1c) and serum 25-OH-vitamin D (25(OH)D) levels has been reported,<sup>9,10</sup> while data about 25(OH)D status in inpatients with diabetes are inconsistent.

It is well-known that inpatients with diabetes have worse health outcomes than inpatients without diabetes, with increased risk of all-cause death.<sup>11,12</sup> VDD has also been related to worse outcomes in inpatients, such as length of stay (LOS), morbidity, and mortality.<sup>13-17</sup>

At present, however, no data are available about differences of 25(OH)D status between in- and outpatients with diabetes, nor for the impact of VDD on glycemic control in inpatients with diabetes.

This study was thus aimed to evaluate 25(OH)D levels in a large series of inpatients with type 1 and type 2 diabetes and in an age-, sex-, serum creatinine-, and HbA1c-matched group of outpatients with diabetes.

## Subjects and Methodology

The research was carried out in accordance with the ethical standards of the local Ethics Committee and with the Helsinki declaration of 1975 as revised in 2008.

No formal consent is required for this type of study.

## Design

This was a retrospective case-control study that enrolled 540 subjects with diabetes.

We performed a 1:1 matched case-control study on 270 inpatients with diabetes consecutively hospitalized in our Endocrine Unit from January 2011 to December 2013 as cases and 270 age-, sex-, serum creatinine-, and HbA1c-matched outpatients with diabetes as controls. Gender distribution was identical in the two groups and the maximal allowed differences regarding age, serum creatinine and HbA1c were  $\pm 2$  years,  $\pm 0.1$  mg/dL and  $\pm 0.2\%$ , respectively.

## Patients

All subjects lived in Piedmont, a Region in the North-Western part of Italy, were of Caucasian ancestry, and on the whole were exposed to the same UV spectrum of sunlight.

Type 2 Diabetes Mellitus (DM) had been diagnosed in 96% of patients and type 1 DM or other types of DM had been diagnosed in the remaining. The mean DM duration was  $13.6 \pm 12.4$  years before enrolment.

Among inpatients, 110 patients (40.7%) were on insulin treatment and 98 (36.3%) had at least one micro- or macroangiopathic diabetic complication. The respective figures for outpatients were 89 (33%) and 74 (27.4%).

None of the subjects in the study was on chronic dialysis or was affected by severe liver disease. Moreover, participants were excluded if affected by diseases or conditions associated with impaired vitamin D metabolism or if used medications known to affect calcium or vitamin D metabolism, including vitamin D supplements.

## Measurements

The collected data included age, sex, BMI, HbA1c, 25(OH)D, creatinine, and month of evaluation. In addition, for inpatients we evaluated LOS and admission diagnosis. All data were obtained from the letter of discharge and classified according to the diagnosis related group into the followings: diabetic foot infection, other infections, acute nephropathy, heart disease, cerebrovascular disease, and others.

In a subgroup of 89 inpatients, not statistically different from the whole group of inpatients for age, sex, glycemic control and LOS, we also evaluated the

correlation of 25(OH)D levels with fibrinogen, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), contextually assayed.

In agreement with Endocrine Society<sup>18</sup> patients were classified as VDD or severe VDD when serum 25(OH)D levels were less than 50 nmol/L (20 ng/mL) or 25 nmol/L (10 ng/mL), respectively, whereas sufficiency was defined as levels higher than 75 nmol/L (30 ng/mL).

### Assays

Biochemical parameters were assayed at the time of hospital admission in inpatients, and just before a routine visit in outpatients. All measurements were performed by standard procedures in the same laboratory.

HbA1c was assayed by high pressure liquid chromatography, with the upper limit of normality for our laboratory set at 5.7% (38.8 mmol/mol).

Serum 25(OH)D was assessed by direct competitive chemiluminescent immunoassay. The lowest detection limit of this assay is 12.5 nmol/L (5 ng/mL). Our laboratory performed periodically a quality control of every kit used with the quality control material provided by the manufacturer. Our laboratory is a member of External Quality Assessment scheme for the estimation of 25(OH)D conducted by the QualiMedLab-CNR (Pisa, Italy), as a means of determining accuracy of results.

We considered arbitrarily two different seasons for the assessment of vitamin D levels, taking into account different sun exposure: the "Summer" included the months from April to September, while the "Winter" included the months from October to March.

### Statistical analysis

Variables were tested for normal distribution (Wilk-Shapiro's test) and, where confirmed, results were expressed as mean  $\pm$  standard deviation (SD). Otherwise, they were given as median and interquartile range (IQR).

Statistical differences in continuous variables were assessed by t-test or Mann-Whitney for unpaired samples, when normally or not normally distributed, respectively. Dichotomous variables (percentages) were compared by  $\chi^2$  analysis. Correlations between continuous variables were assessed using Spearman correlation coefficients.

P values are two-sided and considered significant when  $<0.05$ . All analyses were performed using Statistica software (version 5.0 for Windows; StatSoft, Tulsa, OK).

### Results

Table 1 summarizes results in the study cohort: age, sex, BMI, creatinine and HbA1c levels were not different between inpatients and outpatients.

The most common cause of admission for inpatients was diabetic foot infection (45.1%). Infections as a whole accounted for near half of admissions, while other causes were evenly distributed (heart disease, renal failure, neurological disorders, etc). Mean LOS was  $12.18 \pm 9.2$  days (range 4 - 71).

VDD was present in 40.6% of the whole series (and severe VDD in 20.7%).

Table 2 shows that VDD and severe VDD were more

**Table 1. Patients characteristics.**

	Inpatients	Outpatients	P
<b>Patients (n)</b>	270	270	-
<b>Age (years) mean <math>\pm</math> SD</b>	65.1 $\pm$ 15.6	63.6 $\pm$ 15.7	ns
<b>Sex (F/M)</b>	134/136	134/136	ns
<b>BMI (kg/m<sup>2</sup>) mean <math>\pm</math> SD</b>	29.23 $\pm$ 6.11	29.32 $\pm$ 6.35	ns
<b>HbA1c (%) mean <math>\pm</math> SD</b>	9.01 $\pm$ 1.92	8.7 $\pm$ 2.1	ns
<b>Creatinine (mg/dL) mean <math>\pm</math> SD</b>	1.1 $\pm$ 0.67	1.1 $\pm$ 0.76	ns
<b>LOS (days) mean <math>\pm</math> SD</b>	12.2 $\pm$ 9.4	/	-
<b>25(OH)D (nmol/L) median, IQR</b>	37.9, 25.3	44.9, 31.8	$<0.01$

**Table 2. 25(OH)D status: values are expressed as percentage.**

	Inpatients (n = 270)	Outpatients (n = 270)	P
<b>Severe VDD (&lt;25</b>	24.8	16.6	0.0255
<b>VDD (&lt;50 nmol/L)</b>	67	55.5	0.008
<b>Vitamin D sufficiency</b>	15.9	22.9	0.05

prevalent in inpatients vs. outpatients (67% vs 55.5% and 24.8% vs 16.6%, respectively).

Median 25(OH)D levels were significantly different (p <0.05) according to season, both in the whole series (summer 49.9 nmol/L vs winter 35.4 nmol/L) and in the two study groups (inpatients: 46.2 nmol/L vs 32.1 nmol/L, outpatients: 58.6 nmol/L vs 39.2 nmol/L).

25(OH)D levels and age were not correlated, either in outpatients or in inpatients.

Table 3 shows the correlations between serum 25(OH)D and clinical and biochemical parameters: we observed a significant negative correlation with HbA1c and BMI only in outpatients. There was a negative correlation between serum 25(OH)D and inflammatory markers in the subgroup of evaluated inpatients.

### Discussion

Our study shows a remarkable prevalence of VDD in a large cohort of patients with diabetes. The figure was higher in inpatients compared to a matched group of outpatients. Moreover, the negative correlation between 25(OH)D levels and metabolic control observed in outpatients with diabetes was not confirmed in inpatients with diabetes; similarly, the expected negative correlation between vitamin D status and BMI was not

found in inpatients. Finally, in a subgroup of patients with diabetes admitted for acute illness, we found a negative correlation between 25(OH)D levels and inflammatory markers.

The prevalence of VDD in inpatients with diabetes is unknown. An association between VDD and the presence of DM in unselected inpatients admitted in medical wards has been reported,<sup>3,19</sup> but neither VDD prevalence in inpatients with diabetes, nor serum 25(OH)D levels were available.

Only Ducloux et al<sup>20</sup> reported a VDD prevalence of 69% in a large series of diabetic inpatients with planned hospitalization without acute illness, but severe obesity (median BMI > 40 kg/m<sup>2</sup>) was present in half of the studied population.

In contrast with that series, our patients were admitted consecutively for acute illness, mostly infections (a frequent cause of hospitalization in patients with diabetes in real practice<sup>21</sup>) and their BMI was consistent with overweight/first degree obesity (BMI 25÷35 kg/m<sup>2</sup>).

Our study shows a high prevalence of VDD, even severe, in comparison to data previously reported in healthy subjects living at the same latitude.<sup>22</sup> Moreover, our

**Table 3. 25(OH)D correlations with clinical parameters: in- and outpatients.**

	Inpatients	Outpatients
<b>vs HbA1c</b>	R = -0.03, p = 0.6	<b>R = -0.14, p = 0.03</b>
<b>vs BMI</b>	R = -0.06, p = 0.4	<b>R = -0.18, p = 0.02</b>
<b>vs LOS</b>	R = -0.08, p = 0.17	
<b>vs fibrinogen</b>	<b>R = -0.22; p = 0.04</b>	
<b>vs ESR</b>	<b>R = -0.27; p = 0.01</b>	
<b>vs CRP</b>	R = -0.27; p = 0.06	

inpatients with diabetes were found to be more 25(OH)D deficient than outpatients with diabetes, in agreement with previously reported data.<sup>3,4,23</sup>

The effect of hospitalization on vitamin D status is poorly known, even though it is likely that factors influencing vitamin D status in the general population, including sun exposure, nutrition and drug use, may also work during hospitalization. VDD prevalence increases in critically ill patients, especially in those with pre-existing comorbidities and in bedridden ones, where a profound depletion can occur.<sup>24,25</sup>

An inverse relationship between HbA1c and 25(OH)D levels in a large series of outpatients with diabetes, comparable to our patients for latitude and sun exposure, was recently reported<sup>9</sup>. Our study confirms this relationship in outpatients with diabetes, supporting the hypothesis that glycemic control can affect vitamin D status in these patients. On the other hand this correlation has not been confirmed in inpatients with diabetes, suggesting that vitamin D status is not influenced by metabolic control in hospitalized patients with diabetes.

We found the same pattern of correlation between BMI and 25(OH)D levels for inpatients and outpatients, suggesting that additional factors may affect 25(OH)D status of inpatients with diabetes. In this regard, inflammation has been reported to play a role.<sup>25,26</sup> Studies performed during a systemic inflammatory response pointed indeed to a severe decrease in serum 25(OH)D.<sup>27,28</sup> Waldron et al<sup>29</sup> found a quick decrease in serum 25(OH)D concentrations after an inflammatory reaction, persisting for at least 3 months. The decrease is accounted for by a loss of vitamin D-binding protein, which binds up to 90% of the total circulating 25(OH)D. In addition, renal wasting of 25(OH)D may play a role in vitamin D depletion during acute stress.<sup>30</sup> Near half of patients with diabetes in our series had been actually admitted for an acute infection, as commonly observed in real practice. In this regard, in a subgroup of inpatients, not different from the whole inpatients group, we observed a significant inverse relationship between 25(OH)D levels and inflammatory markers. This finding supports the hypothesis that acute inflammation may aggravate VDD in inpatients with diabetes.

Among the potentially interfering factors, our study confirms the well-known relationship between vitamin D

status and seasonality.<sup>3,4,31</sup>

In inpatients with diabetes we didn't find statistically significant correlations between 25(OH)D and gender, age, and creatinine. Moreover, 25(OH)D levels were not predictive of LOS, as recently observed in general inpatients.<sup>32</sup>

Strengths and limits of our study need to be taken into account. The 1:1 matched case-control design of the study strengthens the evidence and supports the results. In addition, all examinations and measurements were performed in the same laboratory, assuring a good quality of data. Moreover, this is the first study assessing vitamin D status in inpatients with diabetes. The greatest limit is the single-spot measurement of biomarkers.

In conclusion, VDD is common in diabetic inpatients and more frequent than in outpatients with diabetes. 25(OH)D status in inpatients seems to be influenced by the acute inflammatory condition; for this reason, we suggest that hospitalization may not be the appropriate setting for 25(OH)D evaluation in diabetic populations.

#### **Grants and Fellowship Supports:**

None

#### **Conflict of Interests:**

The authors declare that they have no conflict of interest.

#### **Abbreviations**

25(OH)D: serum vitamin D

BMI: body mass index

CRP: C-reactive protein

DM: Diabetes Mellitus

ESR: erythrocyte sedimentation rate

HbA1c: glycated hemoglobin

IQR: interquartile range

LOS: length of stay

SD: standard deviation

VDD: vitamin D deficiency

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