25(OH)D Deficiency and Metabolic Control of Diabetes: A Problematic Balance

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Abstract
Increasing evidence suggests that vitamin D plays a role in the development of chronic diseases including type 2 diabetes (DM). Aim of the study was to explore the association of vitamin D levels with prevalent DM and we will focus our attention in a Northern Italy population.

Methods
This cross-sectional study involved 614 not compensated diabetic patients and 446 compensated subjects. Clinical characteristics were available including blood samples to determine vitamin D levels and diabetes status by fasting plasma glucose (FPG) and glycosylated haemoglobin (HbA1c). Vitamin D was grouped in four categories (<10 ng/ml, 10-19.9 ng/ml, 20-29.9 ng/ml, ≥30 ng/ml). Bivariate associations between vitamin D categories and a composite indicator for DM (FPG ≥126 mg/dl or HbA1c ≥6.5% or self-reported diagnosis) were calculated; statistical models were used to test this association.

Results
(83.1% male, mean age 51.9±5.6 years). Severe vitamin D deficiency (<10 ng/ml) was associated with increasing FPG (β 3.13; 95% CI: 0.78, 5.47; p≤0.01) and HbA1c (β 0.15; 95% CI: 0.08, 0.23; p≤0.001) values in adjusted linear regression models.

Conclusions
Vitamin D deficiency is associated with prevalent DM in adults.

Keywords
Diabetes; Evaluation; Chronic Disease; Comparative Effectiveness

Introduction
The prevalence of Vitamin D (25-hydroxyvitamin D, 25(OH)D) deficiency is an important public health problem because of its great impact on bone metabolism and its possible implication in cardiovascular outcome, diabetes, cancer and mortality [1]. Deficiency of 25(OH) D has been characterized by a concentration < 20 ng/mL (50nmol/L) and has been well recognized as a cause of rickets and adult osteomalacia. The suggested relationship between type 1 diabetes mellitus and 25(OH)D deficiency has been extensively reported [2]. These effects have been mainly attributed to the immune-modulatory actions of vitamin D [3]. Lack of knowledge still exists concerning association between 25(OH)D and type 2 diabetes mellitus.
Vitamin D causes reduced insulin secretion in humans and its replenishment improves β-cell function and glucose tolerance [4]. Allelic variations in the vitamin D receptor and vitamin D binding protein might influence glucose tolerance and insulin secretion thus contributing to the genetic risk for type 2 diabetes [5]. Observational studies have related vitamin D deficiency with the prevalence of diabetes but only few prospective studies had linked levels of 25-hydroxyvitamin D to the incidence of type 2 diabetes [6]. Glycosylated haemoglobin (HbA1c) is a form of haemoglobin that it was considered as a marker for average blood glucose levels over the previous 3 months, and in diabetes mellitus, higher amounts of glycosylated haemoglobin, indicating poorer control of blood glucose levels.

Our study is aimed to examine the relationship between Vitamin D serum levels and plasma HbA1c levels in a cohort of people of North Italy during an observational study of one year.

Patients and Methods
Patients
We matched 614 not compensated diabetic patients with 446 compensated subjects for vitamin D levels, for a total of 1060 subjects. This patients cohort enrolled only outpatients underwent insulin therapy and with a follow-up period higher than 10 years.

Diagnosis of type 2 diabetes
Following the criteria of the American Diabetes Association [7] and consensus statements issued by the World Health Organization (WHO), International Diabetes Federation, and European Association for the Study of Diabetes and for ADA revision guidelines and, diabetes mellitus was considered present if at least one of the following criteria was met: (1) two FPG ≥126 mg/dl (7.0 mmol/l); (2) two HbA1c ≥6.5% (48 mmol/mol); [8] self-reported diabetes with confirmation of physician diagnosis.

Classification of vitamin D levels
We used widely accepted cut-off values for 25(OH)D to create four categories:

- Severe deficiency, <10 ng/ml (25 nmol/l);
- Moderate deficiency, 10-19.9 ng/ml (25-49.9 nmol/l);
- Insufficiency, 20-29.9 ng/ml (50-74.9 nmol/l);
- Sufficiency, ≥30 ng/ml (75 nmol/l) [9, 10].

Methods
25(OH)D concentration on serum samples was measured by a competitive assay (25(OH) vitamin D TOTAL, Liaison®, DiaSorin, USA) with the analytical performances of: sensitivity 1 ng/ml; linearity 4 – 150 ng/ml, CV intra-serie 5% and inter-series 10%. The normal range proposed by Manufacturer’s laboratory kit is 10 – 55 ng/ml. HbA1c concentration was determined by a chromatographic separation on a ion exchange high performance (HPLC, Bio-Rad Laboratories GmbH®, Munchen, Germany). The VARIANT II Clinical Data Manager (CDM) software is used for the analysis of the data.

The different areas of the chromatogram are calculated using an exponentially modified Gaussian (EMG) algorithm and the results are expressed both in % of areas and mmol/mol Hb. The normal range proposed is 4-6 % end 20-42 mmol/mol Hb.

Data Analysis
Demographic characteristics are showed as the mean value ± standard deviation (SD) and as frequencies and percentages for categorical variables. We tested linear trend for continuous variables and used the p for linear-by-linear test for categorical variables.

Results
Demographics
The mean age in not compensated diabetic patients was 69.5 +/- 9.04 (range 55-98) with 65% women and 35% men, in the compensated group a mean age was of 63.12 years +/- 12 years (range 51-99) with a 72% of women and 28% of men. 15% of patients were current smokers. The excluded subjects did not differ significantly from those included in the analysis with respect to age, gender, and smoking status.

Data Analysis
The mean 25(OH)D concentration were 20.89 ng/mL in compensated group and 18.89 ng/mL in non compensated patients with a median concentration of 5.9% ± 1.95 in the first (mmol/ ml) and 8.57% ± 3.9 (mmol/ml) in the second one. We focused our observation on the median levels of 25(OH)D between two groups of diabetic patients and we registered that in non compensated the levels of VIT D is lower than in compensated (Figure1).
We have classified the 25(OH)D concentration data on the basis of: a) normal range proposed by Manufacturer; b) by the set point 20 ng/mL as proposed by Ioh; c) by the set point 32 ng/mL as Bischoff-Ferrari HA and Binkley N [9, 11]. The evaluation of results by three different classifications is summarized in Table 1 and 2.

Table 1. Patients classification from different criteria of Vitamin D references divided for sex and different proposed cuts

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<tr>
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<th>Scompensed</th>
<th>Compensed</th>
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<tr>
<td>10 - 55 ng/mL</td>
<td>WOMEN 83 (57%)</td>
<td>155 (76%)</td>
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<td>MEN 44 (56%)</td>
<td>76 (73%)</td>
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<tr>
<td>&lt;20 ng/mL</td>
<td>WOMEN 92 (63%)</td>
<td>112 (55%)</td>
</tr>
<tr>
<td></td>
<td>MEN 50 (64%)</td>
<td>69 (66%)</td>
</tr>
<tr>
<td>&lt;32 ng/mL</td>
<td>WOMEN 115 (79%)</td>
<td>164 (80%)</td>
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<td>MEN 69 (88%)</td>
<td>85 (81%)</td>
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Table 2. Summary of patient’s classification based on the different set points proposed. Only 32% of patients are deficient for VitD if we adopt the “normal range” 10-55 ng/mL, in contrast 88% are deficient for VitD if we adopt the “normal range” <32 ng/mL

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<tr>
<td>10 - 55 ng/mL</td>
<td>127 (57%)</td>
<td>231 (75%)</td>
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<tr>
<td>&lt;20 ng/mL</td>
<td>142 (64%)</td>
<td>181 (59%)</td>
</tr>
<tr>
<td>&lt;32 ng/mL</td>
<td>184 (83%)</td>
<td>249 (81%)</td>
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Figure 1. We compared the median levels of VitD and glycosilated haemoglobin: group 1 are not compensated patients and group 2 are compensated patients. The blue columns are median levels of vitamin D the red columns are median levels of glycosilated haemoglobin.

Figure 2. Variation of the two metabolite analyzed during time for three different patients.

Discussion

The relationship between HbA1c and 25(OH)D levels show an inverse correlation which demonstrated strong and mutual relations. Low levels of 25(OH)D has been already reported in patients with type 2 diabetes [8, 12-15]. With the data available, it is not possible to speculate about the intrinsic biochemical mechanism to establishing a negative synergism between the levels of two different molecules. However we can observe that the diabetic patients in good metabolic control have higher values of 25(OH)D [16, 17].

This observation also underlined by data collected in monitoring of three patients from which it appears that improvement of metabolic control are correlated an increase in vitamin D as already reported by Zoppini G et...
al [18]. The definition of 25(OH)D status and serum levels could be very important in terms of therapeutic decisions. Defining normal/abnormal values is a critical step before the clinical use of a biomarker (2). At least three potential approaches exist for defining abnormal biomarker levels: (1) reference limits (2) discrimination limit (3) threshold defining risk or desirable level. The choice of a different approach for defining abnormal levels, can lead to inappropriate clinical evaluation of patients. Accordingly reference values should be defined for different pathology and subsets of. So for the assay used in the course of this study, the “normal” values are represented by the range 10 - 55 ng / ml. Currently the literature is oriented towards a cut-off that defines an optimal level, but unfortunately there is no consensus about the proposals range (> 20 ng / ml, > 25 ng / ml, > 32 ng / ml) [9].

According to these criteria “the clinical meaning” of the data, clearly shifts significantly the supplementation accordingly with the cut off chosen. The latest indications from the literature [9] tend to outline that “optimal levels” can vary for different “patients types” and different clinical purposes, and this lack of consensus make necessary to find an agreement between clinical laboratory and physicians on the “mode of reporting” of the analytical data.

On the basis of this consideration, remains to be determined what is the optimal levels to be achieved and validated, and it is the duty of the laboratory to give an help to the clinicians in defining them. As matured even from this limited experience, the value of 25 ng / ml could be considered “applicable” as the cut-off for the general population, while remains still debated, at scientific level, the question about how to define the optimal value for specific diseases, targeted to the aim of prevention and/or treatment.

Conclusion

The measurement of vitamin D levels in the blood is a punctual reference in the bioavailability of the vitamin to the organism. Current technologies allow rapid determination at low cost that might positively influencing the clinical making decision in many important diseases, by supplementation when the vitamin D low levels are well documented. In the clinical setting, should exists a close collaboration between the laboratory and the clinicians to better define the optimal levels according to the patient’s course and on the basis of the disease. Therefore it is desirable that remarks should appear in the lab report. The findings highlight may be the start point for conducting large-scale health screening to identify those patients at risk of DM using vitamin D dosage.

References


