

CLINICAL STUDY

A Study of Vitamin D Insufficiency in Postmenopausal Type 2 Diabetic Women

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Abstract

Background

Type 2 diabetic elderly women are at increased risk for osteoporotic fractures. Low levels of vitamin D increases this risk. We aimed to measure levels of 25 hydroxy vitamin D (25 OH-D) in these patients to help assess the level of risk.

Patients and Methods

In this cross sectional case-control study, the serum concentrations of 25 OH-D were measured by high performance liquid chromatography (HPLC) in 60 ambulatory, postmenopausal, type 2 diabetic female patients under oral anti-diabetic treatment. Thirty control females were comparable for weight, age and years since menopause. Calcium and Vitamin D intake (obtained by 24 hour dietary recall), sun exposure, parathyroid hormone (PTH), serum calcium, phosphorus and alkaline phosphatase were also assessed.

Results

The prevalence of 25 OH-D insufficiency was significantly higher in diabetic patients than in control subjects (38.3% vs. 20%, $p < 0.01$). About 13% of diabetics with vitamin D insufficiency had high PTH levels. While most of the control group with 25 OH-D insufficiency had elevated PTH levels. Out of 66 diabetic women, only 11.7% had adequate vitamin D intake and 30% had adequate calcium intake. Similarly, in non-diabetic women, only 13.3% had adequate vitamin D intake and 26.7% had adequate calcium intake. However, a highly significantly percentage of diabetic women (43.4%) with inadequate vitamin D intake develop vitamin D insufficiency compared with non-diabetic women (23.1%) ($p < 0.001$). Diabetic patients with 25 OH-D insufficiency tend to be older, with higher BMI, and HBA_{1c} ($P < 0.01$, < 0.01 , < 0.001 respectively).

Conclusion: We documented increased risk of vitamin D insufficiency in type 2 diabetic postmenopausal women in the face of inadequate dietary vitamin D and low sun exposure. This finding might partially explain the increased risk for osteoporosis among this group and warrants consideration of dietary vitamin D supplementation.

Key words: Diabetes mellitus, vitamin D

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Introduction

Vitamin D deficiency has long been recognized as a cause of rickets in children and osteomalacia in adults. More recent is the awareness of a preclinical phase of vitamin D deficiency, known as vitamin D Insufficiency ($< 16 \mu\text{g/L}$)¹. More recent researchers suggest that PTH levels and calcium absorption are not optimized until serum 25 hydroxy vitamin D (25 OH-D) levels reach above $40 \mu\text{g/L}$ that is the "desirable" level and they consider levels between 20 and $40 \mu\text{g/L}$ as sufficient, between 10 and $20 \mu\text{g/L}$ as insufficient and less than $10 \mu\text{g/L}$ as deficient².

Despite the lack of symptoms, it is likely that vitamin D insufficiency has deleterious effects on calcium metabolism and ultimately the skeleton. Vitamin D insufficiency is being increasingly recognized as a common yet easily modifiable risk factor for osteoporosis and could explain the deranged mineral homeostasis and skeletal morphology with increased risk of osteoporotic fracture observed in diabetics³.

In addition, accumulating research suggests that low 25(OH)D concentrations may be inversely associated with impaired glucose tolerance⁴, type 2 diabetes⁵, metabolic syndrome^{6,7}, insulin resistance^{8,9}, and cardiovascular disease (CVD)¹⁰. Additionally, low vitamin D concentrations result in elevations of parathyroid hormone, which has been linked to insulin resistance¹¹⁻¹².

The mechanisms by which vitamin D may affect the risk of type 2 diabetes are not clear. The finding of vitamin D receptors in β -cells¹³ and the finding of impaired insulin secretory capacity in mice lacking a functional vitamin D

receptor¹⁴ indicate an important role for vitamin D in regulating β -cell function.

In a recent large prospective study, a potential beneficial role for both vitamin D and calcium intake in reducing the risk of type 2 diabetes was suggested¹⁵. However, other short-term intervention studies with vitamin D supplementation have shown conflicting results¹⁶⁻¹⁹.

Vitamin D insufficiency is common in general medical patients, including those without apparent risk factors for vitamin D deficiency²⁰ and even in young women in sunny countries²¹. Therefore, the association between Type 2 diabetes and vitamin D insufficiency may be just a coincidence and it is still not clear if patients with diabetes actually differ from non diabetics concerning this common state of vitamin D insufficiency.

Based on these findings, we proposed that type 2 diabetes mellitus may be considered as a risk factor for vitamin D insufficiency. As a means of testing this hypothesis, and because circulating 25(OH)D is the hallmark for determining vitamin D status, the aim of our study was to measure the levels of 25(OH)D and dietary vitamin D and calcium in a case-control cross-sectional protocol involving the ambulatory postmenopausal women with type 2 diabetes to assess the prevalence and predictors of vitamin D insufficiency in these patients and in comparable non-diabetic healthy women.

Patients and Methods

Patients study design

In this cross-sectional case-control study, 60 female patients with Type 2 diabetes were recruited from the Out-patient Department of Kaser Al Eni Hospital, Cairo University, during 2 years period from February 2004 till

March 2006. All participants gave a written consent to participate in this study.

Samples were withdrawn during summer period. Patients were divided into 2 groups: group 1 with normal and group 2 with insufficient-D level. Concerning treatment, 43 were under metformin and glibenclamide, 6 were under rosiglitazone and metformin and 11 were under glibenclamide only. Thirty age, weight and years since menopause matched healthy females who were randomly recruited from the same hospital were selected as the control group. All subjects gave informed consent to participate in this study.

Inclusion criteria

Because diabetic elderly women have the greatest risk to develop osteoporotic fractures, postmenopausal ambulatory females under oral antidiabetic-treatment and with normal renal function were selected.

Exclusion criteria

Patients under insulin therapy or with known causes for hypovitaminosis D as diabetic nephropathy, nephrotic syndrome from any other cause, chronic renal failure, chronic liver disease, and malabsorption were excluded. Those who were taking medications known to alter vitamin D metabolism as antiepileptics or under any active treatment affecting bone as calcium supplements, vitamin D, or multivitamins except vitamin B, corticosteroides, bisphosphonate, and patients with bone fractures, metastatic bone lesions, immobilization, or other severe illnesses (e.g. liver, or heart failure) were also excluded from the study.

For every patient, detailed clinical

history and examination were performed with special emphasis to the following points:

Assessment of sun exposure

Participants were asked how often on average they had been exposed to sunshine during the day. Sun exposure was considered negative if the person avoid outdoors during day hours most days and positive if he was outdoor for ≥ 2 days per week on average.

Assessment of dietary intake

Dietary intake was assessed with the 24-hour dietary recall method. Dietary intake of vitamin D and calcium was calculated by multiplying the frequency of consumption of each food item with the nutrient content of each food²².

Assessment of body mass index (BMI)

BMI was calculated as: $\text{Weight} / \text{Height}^2$ (Kg/m^2). BMI values were applied to the related patients for further analysis of the retrieved data and the age and sex matched groups were studied.

Assessment of diabetic complications

The presence of microvascular or macrovascular complications was confirmed by file review, medical history and examination, and vascular laboratory studies.

Chemical assays

All blood samples were fasting morning samples. Fasting blood glucose, serum creatinine, calcium, phosphate and alkaline phosphatase were measured by standard biochemical methods. Haemoglobin A1c (HbA1c) was determined by HPLC to assess diabetes control (normal below 6.1%).

PTH analysis were determined by solid-phase two site chemiluminescent enzyme immunometric assay,

performed on immulite auto analyses using kit supplied by DPC (Diagnostic Product Corporation, 5700 west 96th street, USA). High PTH was considered if its level was >60 pg/ml).

Plasma 25 OH-D was measured according to Chromosystem reagent kit for high performance liquid chromatography (HPLC). Plasma levels from 21 to 40 µg/L were considered sufficient, from 10 to 20 ng/ml insufficient and <10 µg/L as deficient².

Statistical methods

Statistical evaluation of all data was done on IBM-PC microprocessor computer using SPSS software for windows (Statistical Package for Social Sciences version 13, USA) for data management and analysis and the Microsoft power point for charts. Quantitative data were presented as mean ± SD. Data are means ± SD or frequencies. Skewed variables were logarithmically transformed to improve normality before analysis. For the comparison of the three groups' means, one way analysis of variance (ANOVA) was used followed by Students' Newman Keuls test to detect significant difference. All tests were two tailed and considered significant when p<0.05. The coefficients of correlation between different studied parameters were calculated according to Pearson's method.

Results

Figure 1 shows that the prevalence of 25-OH insufficiency (10-20µg/L) was significantly higher in diabetic patients than in control subjects (38.3%²³) Vs (20%⁶), p<0.01.

No cases of vitamin D-deficiency (<10µg/L) were detected. Figure 1 also shows the relation between vitamin D

insufficiency and high PTH (>60 pg/ml). About 13% (3 out of 23) of diabetics with vitamin D insufficiency had PTH levels above normal range(>60 Pg/ml). While most of the control group with 25 OH-D insufficiency (83.3% (5 out of 6)) had elevated PTH.

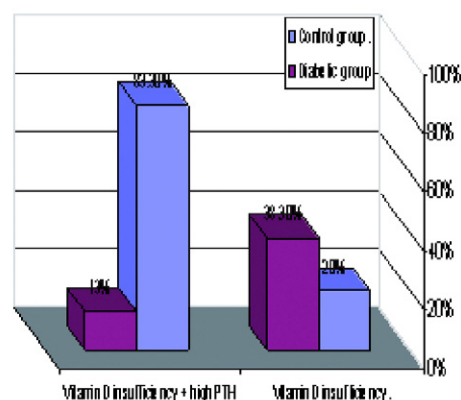


Figure 1: Prevalence % of vitamin D insufficiency in control and patient groups in relation to high PTH

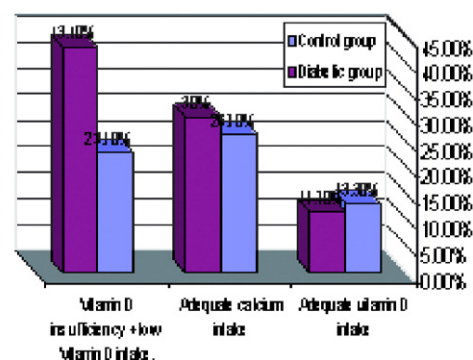


Figure 2: Prevalence of vitamin D insufficiency in control and patient groups in relation to vitamin D intake

In **Figure 2**, based on the latest guidelines set by the Institute of Medicine²³, out of the sixty diabetic women, only 11.7% (7) had adequate vitamin D intake (≥ 400 IU/day), and 30% (18) had adequate calcium intake ($\geq 1,200$ mg/day). Similarly, in the thirty non-diabetic women, only 13.3% (4) had adequate vitamin D intake and 26.7% (8) had adequate calcium intake. However a significant percentage of diabetic women (43.4%; 23 out of 53) with inadequate vitamin D intake develop vitamin D insufficiency compared with non-diabetic women 23.1% (6 out of 26) ($p < 0.001$).

According to serum level of 25 OH-D, diabetics were divided into 2 groups. Table 1 shows comparison between control and diabetic groups. The mean level of 25 OH-D was desirable in the control group (53.4 ± 4.3 $\mu\text{g/L}$), sufficient in diabetic group 1 (33.7 ± 3.8 $\mu\text{g/L}$) with normal PTH level (27.1 ± 1.78 pg/ml) and insufficient in diabetic group 2 (13.1 ± 0.9 $\mu\text{g/L}$) with significantly higher PTH (48.3 ± 8.41 pg/ml) ($p < 0.001$ for all) (**Table 1**). However, the mean PTH in this group was not above normal limit (> 60 pg/ml).

Despite the fact that the control group was selected to be age and weight-matched to the diabetic patients, diabetic group 2 was found to be significantly older (67.2 ± 1.3 years) than other 2 groups (64.6 ± 1.7 years for control group and 59.7 ± 3.1 years for diabetic group 1) ($p < 0.05$ for both) with higher BMI (29.4 ± 3.5 Kg/m² vs 25.8 ± 2.4 & 26.3 ± 1.4 Kg/m² for controls and group 1 respectively) ($p < 0.05$ for all). In Table 1, 11 diabetic patients from group 1 (31.4 %) had microvascular

complications and 4 patients (11.4%) had macrovascular complications. While, the frequency of vascular complication was significantly higher in group 2 with insufficient 25 OH-D {19 patients (67%) with microvascular and 11 patients (44%) with macrovascular} ($p < 0.001$ for both).

Generally, most of the studied women avoid sun exposure during the day and spend most of their time indoors. Frequency of sun exposure was significantly lower in vitamin D insufficiency group 2 (21.7%) than in normal vitamin D group 1 (28.57%) or control group (30%) ($p < 0.05$) (**Table 1**). In Table 1, the diabetic group 2 with vitamin D insufficiency had insignificant lower dietary vitamin D (291.23 ± 56.45 IU/day) and calcium intake (640.3 ± 53.2 mg/day) compared with both diabetic group with normal 25 OH-D (319.17 ± 21.85 IU/day and 654 ± 38.9 mg/day respectively) or control group (342.70 ± 45.90 IU/day & 646.7 ± 40.2 mg/day respectively) ($P > 0.05$ for all).

As shown in **Table 1**, both diabetic groups showed poor diabetic control (significantly high FBG, and HbA_{1c}), more evident in the group 2 with vitamin D insufficiency. This group also showed as expected significantly lower calcium and phosphorous and higher alkaline phosphatase levels (8.8 ± 0.15 , 2.8 ± 0.8 & 160.2 ± 32.1 mg/dl respectively) compared with the other groups (9.71 ± 0.1 , 4.1 ± 0.7 , 134.8 ± 17.8 , 27.1 ± 1.78 mg/dl respectively for diabetic group 2 and 9.86 ± 1.9 , 4.3 ± 0.9 , 131 ± 15.4 respectively for control group).

Table 1: Comparison between control and both diabetic groups

Characteristics	Control group n=30	Group 1 Type 2 diabetic females with sufficient 25 OH-D (21-40 µg/l) n=37	Group 2 Type 2 diabetic females with insufficient 25 OH- D (10-20µg/l) n=23	P-value
Age (years)	64.6±1.7	59.7±3.1	67.2±1.3	p1>0.05^ p2<0.05* p3<0.05*
BMI (Kg/m ²)	25.8±2.4	26.3±1.4	29.4±3.5	p1>0.05^ p2<0.05* p3<0.05*
Duration of DM (years)	-----	15±2.1	16±1.5	p3>0.05^
Frequency of Complications Microvascular: % Macrovascular: %	-----	31.4 (11)% 11.4 (4)%	67% (19) 44% (11)	p3<0.001** p3<0.001**
Frequency of sun exposure (%)	30% (9)	28.57% (10)	21.7% (5)	p1>0.05^ p2,0.05* p3<0.05*
Vitamin-D intake (IU/day)	342.70±45.90	319.17± 21.85	291.23±56.45	#>0.05^
Calcium intake (mg/day)	646.7±40.2	654±38.9	640.3±53.2	#>0.05^
FBG (mg/dl)	74.6±13.0	178.7±51.8	210.5±45.8	p1<0.001** p2<0.001** p3<0.05*
HbA _{1c}	5.3±0.5	8.3±1.2	10.1±1.3	p1<0.001** p2<0.001** p3<0.01*
Calcium (mg/dl)	9.86± 1.9	9.71 ± 0.1	8.8 ± 0.15	p1>0.05^ p2<0.05* p3<0.05*
Phosphorus (mg/dl)	4.3 ± 0.9	4.1±0.7	2.8± 0.8	p1>0.05^ p2<0.001* p3<0.001*
ALP (mg/dl)	131 ± 15.4	134.8±17.8	160.2±32.1	p1>0.05^ p2<0.05* p3<0.05*
PTH (Pg/ml)	28.1± 2.60	27.1±1.78	48.3±8.41	p1>0.05^ p2<0.05* p3<0.05*
25 OH-D (µg/l)	53.4±4.3	33.7±3.8	13.1±0.9	p1<0.05* p2<0.001** p3<0.001**

BMI: body mass index, FBG: fasting blood glucose, HBA1C: glycosylated hemoglobin, ALP: total alkaline phosphatase, PTH: Parathyroid hormone, 25 OH-D: 25 hydroxy vitamin D. P₁: Comparison between control group and diabetic group with normal vitamin D. P₂: Comparison between control group and diabetic group with low vitamin D. P₃: Comparison between diabetic group with normal and group with low vitamin D. #: if p₁, p₂ & p₃ are similar. ^:not significant, *: Significant, **: Highly significant. Normal serum calcium 8.4-10.2 mg/dl. serum phosphorus 2.5-4.6 mg/dl, PTH: 10-60 Pg/ml. Adequate vitamin D intake (≥400 IU/day), calcium intake (≥1,200 mg/day).

Table 2: Odd ratio of Vitamin D insufficiency

Characteristics	25-OH D insufficiency (10-20 µg/L)	Normal 25 OH-D	Odd ratio
Patients with diabetes	23/60 38.3% A	37/60 61.7% B	2.49 p<0.01
Non-diabetic controls	6/30 20% C	24/30 80% D	

Odd ratio= (A/C)/(B/D)

There were negative correlations between 25 OH-D and age ($r=-0.41$, $p<0.01$), BMI ($r=-0.40$, $p<0.01$), and HBA_{1C} ($r=-0.51$, $p<0.001$) (data are not shown). **Table 2** shows that the Odds ratio (OR) of vitamin D insufficiency was 2.49; meaning that a patient who has Vitamin D insufficiency is about 2.49 times more likely to have type 2 diabetes than a patient who has not Vitamin D insufficiency (OR= 2.49).

Discussion

We documented a high prevalence of vitamin D insufficiency in the postmenopausal ambulatory diabetic patients in the face of inadequate dietary vitamin D and low sun exposure and tendency not detected in a comparable non diabetic women (Tables 1&2). Several studies have demonstrated abnormalities in calcium, phosphate, and vitamin D metabolism in diabetic patients. In particular, Isaia and co-workers²⁴ who studied similar group of patients and **both Di-Cesar**²⁵ and Pietschmann and their associates²⁶ concluded that Vitamin D deficiency is more common in Type 2 than in Type 1 diabetes.

While others found that the balance among the major vitamin D metabolites is altered in diabetes and found that low 24,25-dihydroxyvitamin D is the altered metabolite in diabetes²⁷. Others described variations of vitamin D metabolism to include the decreased

synthesis of vitamin D-binding protein by the liver, decreased renal 1 α -hydroxylase activity, and reduced vitamin D-receptor concentrations resulting in a state of peripheral vitamin D resistance²⁸. On the other hand, normal 25-hydroxyvitamin D levels was detected in some other studies in diabetic patients²⁹.

As a rule, serum PTH increases progressively when 25 OH-D falls <30 µg/L²⁹. In this study, although mean PTH was increased in the group with low serum 25OH-D (**Table 1**), PTH was not a sensitive marker of vitamin D insufficiency, as only 13% of diabetics with vitamin D insufficiency had PTH levels above normal range in contrast to most control group (**Figure 1**). This is consistent with others³⁰⁻³³, who found that PTH secretion seems to be lower than expected for the homeostatic needs in diabetes. This state of 'functional hypoparathyroidism' has been confirmed by dynamic challenge studies, such as during citrate induced hypocalcemia,³⁰ or hyperinsulinemic hypoglycemia,³¹ or following an oral glucose tolerance test³². This functional state has been related to magnesium deficiency³³ and has been considered responsible for the low bone turnover that ends in low bone resorption with increased bone mineral density (BMD) seen in type 2 diabetes relative to non-diabetics³⁴. This state has also been

reported to be correlated with the duration of diabetes and the degree of hyperglycemia suggesting that hyperglycemia per se may have an inhibitory action on the synthesis of PTH in diabetes.³⁵ This study confirmed the importance of age as a major determinant of vitamin D status. Results showed that the diabetic group with vitamin D insufficiency was older than other groups (Table 1) and increasing age was negatively correlated with levels of 25 OH-D. Declining levels of 25 OH-D with age have been attributed to impaired vitamin D absorption from the intestine³⁶, as well as a decline in the concentration of the vitamin D precursors that are normally stored in the skin³⁷. Overall, the elderly have reduced capacity to synthesize vitamin D in the skin when exposed to UVB radiation, and are more likely to stay indoors or use sunscreen.³⁷

Results emphasized also the role of sun exposure as vitamin D intake did not differ between the 2 diabetic groups, however the group with lower sun exposure develop hypovitaminosis D (Table 1). In addition our studied women were well covered with excessive wrapping while outdoors. Osteomalacia has been documented in women who cover all of their skin whenever they are outside for religious or cultural reasons.²¹ Vitamin D can be obtained through the diet or it is synthesized in the skin after exposure to the sun. However, because few foods provide a natural source of vitamin D³⁸ and because fortification of foods with vitamin D is often unreliable³⁹, skin synthesis is thought to constitute the major source. In addition, levels of vitamin D and its main circulating metabolite, 25 OH-D, are under the predominant influence of solar ultraviolet B radiation (wavelength 290

to 315 nm).³⁹.

In this work, diabetics differ from non-diabetics to be more prone to develop vitamin D insufficiency in the face of low vitamin D intake if not helped by increased sun exposure (Tables 1,2 & Figure 2). There must be some intrinsic factors unique to type 2 diabetes that could be used as predictors for developing this state of hypovitaminosis. The significant higher age, BMI, glycemic indices and frequency of diabetic complications in the group with vitamin D insufficiency, besides the negative association between 25 OH-D level and BMI and glycemic indices in our diabetics could represent the answer. The inverse relation between serum 25 OH-D and BMI has been observed previously in type 2 diabetes²⁵, postmenopausal women⁴⁰, elderly people⁴¹, and younger obese subjects⁴². Need and associates⁴⁰ suggested that the inverse relation between 25 OH-D and BMI is due a larger body pool of vitamin D and 25 OH-D or slower saturation and mobilization of these compounds from adipose tissue (or both). Once vitamin D is synthesized in the skin or ingested, it is deposited in body fat stores, making it less bioavailable to people with large stores of body fat making obesity a risk factor for vitamin D deficiency⁴². The influence of poor metabolic control on lowering 25 OH-D level was detected by other researchers.

In Need's study⁴⁰ fasting serum glucose increased as 25(OH)D levels fell throughout the range of serum 25(OH)D measured but the greatest increase was observed in those with vitamin D insufficiency. The association between Vitamin D insufficiency and diabetic complications seems to be strong but complex. Some researchers claim that vitamin D insufficiency predispose to

complications mainly cardiovascular events¹⁰. More studies are necessary to determine whether vitamin D insufficiency predicts the occurrence of cardiovascular disease, and to determine whether vitamin D supplementation would be protective against cardiovascular disease in Type 2 Diabetics.

Conclusion

Our findings, confirm some previous evidence demonstrating that vitamin D insufficiency is highly prevalent in postmenopausal women with type 2 diabetes increasing their high risk for fragility fractures. The associated functional hypoparathyroidism leads to low bone turn-over, one of the underlying mechanisms for the so called diabetic osteopenia. The increased age and weight, lower sun exposure, poor metabolic control and presence of vascular complications seem to be the predictors of this state. The significance of these findings seems to be important as this state of hypovitaminosis D could lead to worsening of diabetic control. However, our findings need to be consolidated by larger study as there are some limitations in this study.

Because our study was a cross-sectional one, the causative nature of the associations cannot be established.

Additionally, 24,25 and 1 α ,25(OH)D were not measured in this study. Additional studies are needed to clarify the association between vitamin D insufficiency and type 2 diabetes whether a cause or a consequence and also to study effect of vitamin D repletion on diabetic status. Further evidence based clinical research is required to confirm our findings.

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