IRANIAN DIABETICS MAY NOT BE VITAMIN D DEFICIENT MORE THAN HEALTHY SUBJECTS

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Abstract- There are some reports of decreased serum levels of 25(OH)D in the subjects with impaired glucose tolerance and type 2 diabetes mellitus (T2DM). To assess vitamin D status of the Iranian diabetics, a pilot study was conducted on 90 subjects with either type 1 diabetes mellitus (T1DM) (n= 30), T2DM (n= 30), or apparently healthy subjects (n= 30) during fall and winter of 2005. Serum samples were analyzed for 25-hydroxycholecalciferol using three different methods: high-performance liquid chromatography (HPLC), competitive protein-binding assay (CPBA) and radioimmunoassay (RIA). In this study serum levels of 25(OH)D were categorized as follows: sufficient ≥ 37 nmol/L; 25 nmol/L ≤ mild deficiency < 37 nmol/L; 12.5 nmol/L ≤ moderate deficiency < 25 nmol/L; severe deficiency < 12.5 nmol/L. Results showed that the occurrence of vitamin D insufficiency was almost the same in patients with T1DM and healthy controls. Mean serum level of 25(OH)D in patients with T2DM was significantly higher than in T1DM, as judged by HPLC (58.2 ± 8.5 vs. 35 ± 5 nmol/L, Mann Whitney U-Wilcoxon, *P*= 0.024). Moreover, both CPBA and RIA showed some over-estimation of serum 25(OH)D compared to HPLC. Our findings suggest that, at least in the cold seasons, vitamin D status of the healthy subjects may not be higher than that of T1DM patients.

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Key words: Vitamin D, 25-hydroxycholecalciferol, diabetes mellitus

INTRODUCTION

Vitamin D endocrine system is believed to regulate many aspects of calcium homeostasis, apart from those directly involved in bone metabolism, including cellular differentiation, immune system and adipocyte function among the others (1). The available data on vitamin D and type 1 diabetes mellitus (T1DM) suggest that this hormonal system, as an environmental factor, may have some role in the autoimmune destruction of β-cells and generation of diabetes mellitus (2).

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The effect of vitamin D on glucose metabolism may not be confined to T1DM. There are some reports of inverse correlation between vitamin D status and body fat mass (3, 4). Considering the high prevalence of central obesity in type 2 diabetes mellitus (T2DM), it is expectable to find lowered vitamin D status in this pathology. Indeed, there are some reports of decreased serum levels of 25(OH)D in the subjects with impaired glucose tolerance (IGT) and T2DM (5).

Recently, it has been proposed that vitamin D deficiency may be more common in T2DM than in T1DM (6) and even serum 25(OH)D levels may have predictive value in such long-term diabetic complications as cardiovascular disease (7). However, in most of these studies serum levels of 25(OH)D in the patients, though lower than those of healthy controls, were not actually "deficient". So, the question is raised if in the communities with the

high prevalence of vitamin D insufficiency a significant difference in vitamin D status between normal and diabetic subjects still persists. To answer this question, first we developed a HPLC-based method to assess circulating 25(OH)D in our laboratory (8). Then a pilot study was conducted on vitamin D status of apparently healthy, T1DM and T2DM subjects. We also used two common methods of serum 25(OH)D determination beside HPLC, *i.e.*, radioimmunoassay (RIA) and competitive protein binding assay (CPBA), to compare the results.

MATERIALS AND METHODS

During fall and winter 2005, a total of 90 subjects consisting of 30 patients with T1DM (16 males and 14 females) aged 25.1 ± 9.0 years, 30 age- and sex-matched apparently healthy subjects (16 males and 14 females) aged 29.7 ± 6.8 years and 30 patients with T2DM (9 males and 21 females) aged 52.2 ± 9.0 years were assessed for serum levels of 25(OH)D using three different methods. Known cases of diabetes (duration since diagnosis 1-5 years) were recruited from the Iranian Society of Diabetes. The purpose of the study was described for all subjects and then a written informed consent was taken. The study was approved by Ethics Committee of Shaheed Beheshti University of Medical Sciences. Those who were taking vitamin D supplements were excluded from the study.

Of all subjects, 5 mL of non-fasting blood sample was taken. After an hour at room temperature (RT), all samples were centrifuged at 2500 g at RT and then sera were transferred to fresh tubes in aliquots and kept at -70° C until the day of analysis. All serum samples were analyzed for 25(OH)D using HPLC, RIA and CPBA. The two latter procedures were performed by commercial kits (DRG, Austria) routinely used by many diagnostic laboratories in Iran. As serum 25(OH)D levels < 37 nmol/L are associated with increasing PTH levels and lower bone density (9) and the effects of vitamin D deficiency on serum levels of calcium and phosphate will be obvious when the concentration of the circulating 25(OH)D is less than 25 nmol/L (10), in this study serum 25(OH)D levels were categorized as follows: sufficient, 37 nmol/L or more; mild deficiency, less than 37 nmol/L to 25 nmol/L; moderate deficiency, less than 25 nmol/L to 12.5 nmol/L; and severe deficiency, less than 12.5 nmol/L.

Normality of data distribution was evaluated using Kolmogorov-Smirnov method. Comparison of means was carried out with student *t* test or, when the distribution was not normal, Mann-Whitney U-Wilcoxon. The predetermined upper limit of significance throughout this investigation was $P < 0.05$. All statistical analyses were done with Windows/SPSS 11.5 package.

RESULTS

Results of vitamin D status were different based on method used (Table 1). While based on HPLC

Table 1. Vitamin D status of healthy, type 1 and type 2 diabetic subjects ($n_1=n_2=n_3=30$) assayed by three methods*

		Vitamin D status†			
Method	**Group**	**Sufficeint**	**Mild Deficiency**	**Moderate Deficiency**	**Severe Deficiency**
HPLC	Healthy	7 (23.4)	10 (33.3)	10 (33.3)	3 (10)
	T1DM	9 (30)	5 (16.7)	14 (46.7)	2 (6.6)
	T2DM	20 (66.7)	4 (13.3)	5 (16.7)	1 (3.3)
CPBA	Healthy	10 (33.3)	5 (16.7)	10 (33.3)	5 (16.7)
	T1DM	14 (46.7)	9 (30)	4 (13.3)	3 (10)
	T2DM	17 (56.7)	8 (26.7)	4 (13.3)	1 (3.3)
RIA	Healthy	21 (70)	5 (16.7)	3 (10)	1 (3.3)
	T1DM	16 (53.3)	6 (20)	4 (13.3)	4 (13.3)
	T2DM	19 (63.4)	4 (13.3)	6 (20)	1 (3.3)

Abbreviations: HPLC, high-performance liquid chromatography; CPBA, competitive protein-binding assay; RIA, radioimmunoassay; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.
*Data are given as number (percent).
†Definitions based on serum levels of 25(OH)D: sufficient, 37 nmol/L or more; mild deficiency, less than 37 nmol/L to 25 nmol/L; moderate deficiency, less than 25 nmol/L to 12.5 nmol/L; and severe deficiency, less than 12.5 nmol/L.

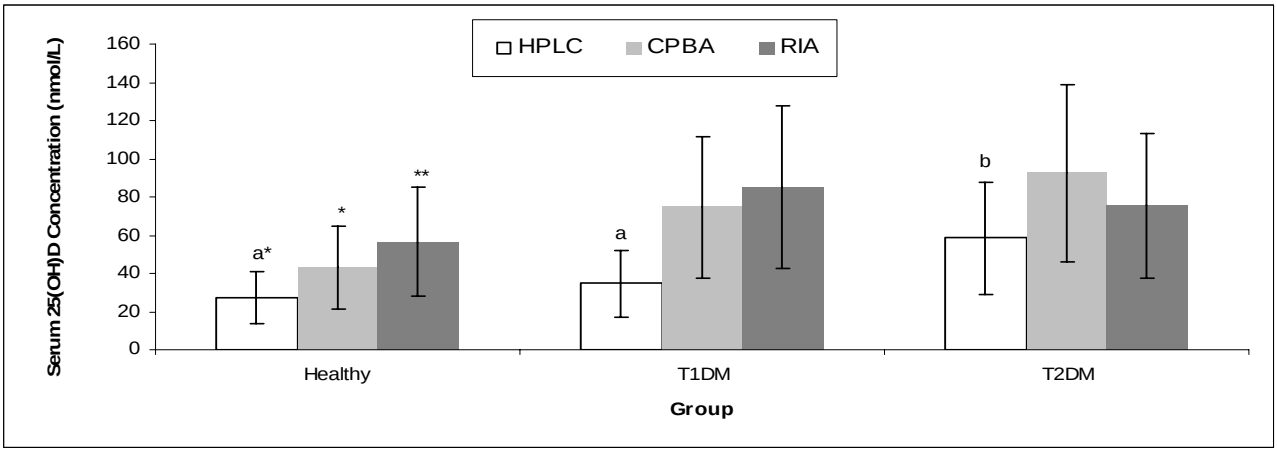

Fig. 1. Comparison of serum 25(OH)D determination in the healthy, type 1 and type 2 diabetic subjects ($n_1=n_2=n_3=30$) using HPLC, CPBA and RIA. Only in the healthy subjects RIA results were significantly higher than those of HPLC ($P< 0.001$) and CPBA ($P= 0.017$) (bars with different number of asterisks are statistically different.). Only HPLC could show significant difference between type 1 and type 2 diabetic subjects (Mann Whitney U-Wilcoxon, $P= 0.024$) (bars with different superscripts are significantly different).

results about 77% and 70% of the healthy subjects and patients with T1DM, respectively, had some degree of vitamin D insufficiency, RIA showed vitamin D insufficiency only in 30% and 47% of healthy and type 1 diabetic subjects, respectively. Results obtained from RIA were commonly higher than those from the other two methods but only in the healthy group was this difference statistically significant (HPLC: 27 ± 2.8 vs. RIA: 56.5 ± 29.5 nmol/L, Mann Whitney U-Wilcoxon, $P< 0.001$) (Fig. 1).

Mean concentration of 25(OH)D in T2DM patients were higher than those in T1DM and healthy subjects but only HPLC results showed significant difference between T1DM and T2DM subjects (35 ± 24.5 vs. 58.2 ± 47.8 nmol/L, Mann Whitney U-Wilcoxon, $P= 0.024$).

DISCUSSION

In two researches conducted separately in Scotland (11) on subjects aged 79.6 ± 7.3 years and in Germany (12) on 996 healthy adults (16-69 years), the occurrence rate of vitamin D insufficiency was found to be 72.6% and 75%, respectively. These numbers are comparable with the prevalence of 79.6% reported from 1210 apparently healthy adults aged between 20 and 69 years from Tehran, Iran (10) and with the occurrence rate of about 77% in the healthy subjects in this study. However, the prevalence of vitamin D insufficiency in 318 students (153 boys and 165 girls) aged 14-18 years from Isfahan, Central Iran, has been reported to be 46.2% (72.1% in females and 18.3% in males) (13).

Though vitamin D status can be influenced by such factors as sun exposure and dietary intake (14), results may also depend on the method used for the assessment. Unreliability of commercial kits has been reported by several investigators (15-18). It has been shown that even the type of the commercial kit in use can influence the results (19). Our findings are in accord with the previous reports that 25(OH)D values by HPLC are around 60% of those values by CPBA (20) and RIA gives even more estimates of the circulating 25(OH)D than CPBA does (21).

There are several reports on lowered vitamin D status in both types of diabetes. In a cross-sectional survey carried out in New Zealand on 5677 subjects aged 40-64 years, serum concentrations of 25(OH)D3 were significantly lower in newly detected cases with diabetes and IGT (n = 238) compared to their healthy age- and sex-matched controls. The authors concluded that low serum 25(OH) levels might somehow predispose to IGT and diabetes (5). This conclusion could not be justified as both diabetics and their healthy counterparts had sufficient circulating 25(OH)D (69 ± 31 vs. 76 ± 34 nmol/L) (5). In another pilot study

the prevalence of vitamin D insufficiency was reported higher in T2DM than in T1DM (6).

The relationship of vitamin D insufficiency with T1DM was further supported by a study recently done in Italy on plasma levels of 25(OH)D3 and $1,25(OH)_2D3$ in 88 patients with newly diagnosed T1DM and 57 healthy age and sex-matched controls. Mean levels of both 25OHD3 and 1,25-(OH)2D3 were found to be significantly lower in patients than in the controls. Here again the investigators concluded that vitamin D3 was an important pathogenic factor in T1DM and suggested vitamin D supplementation not only at birth, but also at diagnosis of type 1 diabetes to favor Th2 immune response and to protect residual β-cells against further destruction (22). The findings drawn out from the nationwide Diabetes Incidence Study in Sweden (DISS) on newly-diagnosed T1DM demonstrated that the plasma 25(OH)D levels were lower at the diagnosis of T1DM than in control subjects, and this may have a role in the development of the autoimmune disorder (23). However, neither the patients nor the healthy subjects were vitamin D deficient (82.5 ± 1.3 vs 96.7 ± 2.0 nmol/L, respectively) (23). It is therefore unlikely that "lowered" serum levels of 25(OH)D, while still far above desirable level, could induce auto-immunity. Decreased circulating 25(OH)D may indicate the increased utilization due to augmented immune reactions as CD4+ T cells have been proposed as targets of vitamin D (24).

High prevalence of vitamin D insufficiency in the Iranian population weakens the possibility of finding any significant difference in the circulating 25(OH)D between healthy and diabetic subjects. Nevertheless, this question can still be raised that whether this extensive vitamin D deficiency in the Iranian population has some role in the increasing occurrence of such auto-immune disorders as T1DM. Our findings warrant further studies on this issue.

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Conflict of interests

The authors declare that they have no competing interests.

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