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Original Article

Vitamin D Deficiency is Associated with the Metabolic Syndrome in Subjects with Type 2 Diabetes

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ABSTRACT

Background and Objectives: There is convincing evidence that subjects concomitantly affected by type 2 diabetes (T2D) and metabolic syndrome (MeS) are at greater risk for cardiovascular disease (CVD). Many metabolic derangements in T2D might be attributed to poor vitamin D status. The purpose of this study was to investigate the associations among vitamin D status, MeS and glycemic status in subjects with T2D.

Materials and Methods: A total of 101 known cases of T2D (39 males, 62 females) were enrolled in a cross-sectional study by convenient sampling. Serum 25(OH)D3, glycemic markers and lipid profile were assessed.

Results: Mean concentration of serum 25(OH)D3 was 42.2±33.8 nmol/L. Prevalence of undesirable vitamin D status (25(OH)D < 50nmol/L) was significantly higher among the subjects with MeS as compared to those without MeS (p=0.020). The subjects with sufficient vitamin D status had 50% lower risk for MeS compared to those who had vitamin D deficiency, and this association remained significant even after additional adjustment for body mass index (BMI), percent of fat mass or waist circumference.

Conclusions: Our data showed that firstly higher vitamin D status is inversely associated with fasting glycemia, and secondly serum 25(OH)D3 predicts MeS risk in the subjects with T2D. Demonstrating the association of hypovitaminosis D with disorders of glucose metabolism and higher risk for development of further complications, notably CVD, may lead to a new target for preventive efforts at the population level.

Keywords: Vitamin D, Type 2 diabetes, Metabolic syndrome, Cardiovascular disease

Introduction

Diabetes is accompanied by many short-term and long-term complications (1). The insidious nature of long-term complications has made them more trouble-making in terms of both health effects and costs allocated (1).

Vitamin D deficiency is a global health problem (2), which has been shown to affect both insulin secretion (3) and action (4). These findings have been the basis for many observational (5-6) and animal (7) studies on the relation of vitamin D and glycemic control. Epidemiological evidence also suggests a potential association between vitamin D insufficiency and metabolic derangements (8-9) including diabetes (6, 10). There is growing evidence suggesting that metabolic syndrome (MeS) causes microvascular complications in the subjects with diabetes (11-12), and part of the increased risk for these conditions may be attributable to suboptimal vitamin D status (13). However, there is no consistency among the results of different studies. In a recent randomized clinical trial, we showed that improvement of the vitamin D status of subjects with type 2 diabetes (T2D) would lead to a significant amelioration of glycemic status (14). Our findings confirmed other reports on the inverse relationship between body fat mass (FM) and vitamin D status (15). Considering the proposed role of vitamin D in the development of both MeS (16) and CVD (17) in non-diabetic subjects, we hypothesized that vitamin D status could be a determinant of such late diabetic complications, mostly by affecting the risk of MeS in the individuals with T2D. In this study, the

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association of vitamin D status with metabolic syndrome (MeS) and glycemic status in a sample of individuals with T2D was investigated.

Materials and Methods

Study design: This cross-sectional study was conducted during the mid-fall to late winter, starting in late October 2009 and ending in late November 2010. During the cold seasons, people usually wear heavy cloths and, especially at Tehran's latitude (36° 21˝N), the sun is at a low angle in the midday. Therefore, dermal synthesis of vitamin D is nearly negligible (18), and people have to rely almost solely on their body stores brought up during the warm seasons, or through the food intake.

All participants were given full information on the objectives of the study. Then they signed an informed written consent. A questionnaire on the history of other diseases, medications and the duration of direct sun exposure was completed. The categorization of the recalled usual number of min/hrs spent in daylight was as: less than 10 min, 10 min to 1 hr, 1-2 hrs, and more than 2 hrs (19). Then 10 mL of the participants' fasting venous blood was taken for the laboratory analysis. The study was approved scientifically and ethically by the Research Council and the Ethics Committee of the National Nutrition and Food Technology Research Institute (NNFTRI), respectively.

Subjects: The total of 101 known cases of T2D (39 males, 62 females) attending the Iranian Diabetes Society (IDS) were enrolled in the study by convenient sampling. Inclusion criteria were: 1) age 30-60 years, and 2) fasting blood glucose ≥ 126 mg/dL on the first visit. Exclusion criteria included: 1) unable or unwilling to participate; 2) receiving medications, which could potentially influence vitamin D metabolism (notably estrogens, and calcitonin) within the last three months; 3) any other concomitant clinical diseases, which could influence vitamin D metabolism (such as renal, hepatic, other endocrinological disorders, and malignancies); and 4) receiving insulin. We also excluded those subjects who were receiving vitamin D, calcium or omega-3 supplements within the last three months as there was a high prevalence of irregular supplement consumption, which made accurate dietary intake evaluations difficult, if not impossible.

Anthropometric and blood pressure measurements: Weight was measured using a digital scale (sensitivity 0.1 kg; 808 SECA, Germany) in light clothing and without shoes. Height was determined by a stadiometer (sensitivity 0.1 cm; SECA, Germany). BMI was calculated using the equation: weight(kg)/height(m)^2. Waist circumference (WC) was measured at the midpoint between the lowest rib and iliac crest using a measuring tape (sensitivity 0.1 cm). Blood pressure (BP) was measured by a digital system (BC 08, Beurer, Germany) while the subject was seated at least for 10 minutes.

Laboratory investigations: Following 12-14 hours of fasting, 10 mL venous blood was collected and kept at room temperature (RT) for 30-60 min in dark. Then clotted samples were centrifuged for 20 min at 2000 *g* at RT. The sera thus recovered were transferred into the fresh microtubes in aliquots, and kept at -80 °C until the day of analysis.

Serum 25(OH)D3 concentration: Serum 25(OH)D3 was assayed by high performance liquid chromatography (HPLC), as described earlier (20). In this study, vitamin D status was defined on the basis of serum concentrations of 25(OH)D3 as sufficient (≥50 nmol/L), insufficient (from ≥27.5 to <50 nmol/L), and deficient (<27.5 nmol/L) (21). Since our previous studies showed no detectable circulating 25(OH)D2 in the Iranian participants (20, 22), serum 25(OH)D3 was considered as the total circulating 25(OH)D.

Glycemic status, liver enzymes, lipid profile and apoproteins: Serum glucose and lipid profile, including triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL) and high-density lipoprotein cholesterol (HDL), as well as alanine aminotransferase (ALT) and aspartate aminotrransferase (AST) were determined using enzymatic methods. Serum concentrations of Apo A, Apo B and Lpa (all from Pars Azmoon Co., Iran) were measured by immunoturbidometric method. All biochemical analyses were done using an autoanalyzer system (Selecta E, Vitalab, the Netherlands) on the day sample collection.

Serum total insulin was determined by immuno-radiometric assay (IRMA) (Biosource, Belgium) and a gamma-counter system (Gamma 1, Genesys, USA).

Glycated hemoglobin (HbA1c) was measured by a colorometric method after an initial separation by ion exchange chromatography (Biosystem, Spain), Insulin resistance was determined by the homeostasis model assessment of insulin resistance (HOMA-IR), which was calculated using the formula:

HOMA–IR = [fasting insulin (mU/l) χ fasting serum glucose (mmol/l)]/22.5 (23).

Advanced glycation end-products (AGEs): Serum AGEs were measured using enzyme linked immunosorbent assay (ELISA) (USDN, China).

Dtermination of body fat mass (FM): Body composition was determined using bioelectrical impedance analysis (BIA) system (Quadscan 4000, BodyStat, UK).

Statistical analysis: Normality of data distribution was assessed by Shapiro- Wilk test. All data were expressed as Mean±Standard Deviation (M±SD). Differences in categorical variables were compared using Chi-square test. To compare continuous variables among the subgroups, *t*-test (normal distribution) and Mann Whitney test (non-normal) were employed. The strength of association between the pairs of variables was assessed by Pearson (r, normal distribution) or Spearman (r_s, non-normal distribution) correlation coefficient. We used backward step-wise logistic regression to identify the statistically significant predictors of diabetic outcomes. All statistical analyses were done using Statistical Package for Social Sciences (SPSS) (version 16). $P<0.05$ was considered significant.

Results

The distribution of 25(OH)D, ALT, AST, AGEs, Lp(a) and SOD was not normal, and even by usual transformations including logarithm, it could not be normalized. Other variables showed normal distribution. As demonstrated in Table 1, serum ALT was higher in men whereas mean BMI, FM, FSG, TC, LDL and HDL were significantly higher in women. The occurrence of vitamin D deficiency and insufficiency was 38.5% and 41.0%, respectively in men and 42.6% and 24.6% in women. However, mean serum 25(OH)D3 (p=0.639) and the distribution of vitamin D status (p=0.178) showed no significant difference between the two genders.

Table 1. Characteristics of the subjects

Variable	Men (n=43)	Women (n=62)	p value[1]	Total
Age(year)	50.1±6.7	50.4±5.5	0.603	50.5±6.0
Diabetes duration(year)	8.8±5.8	8.7±6.1	0.941	8.7±6.0
Weight(Kg)	81.8±13.3	73.0±14.7	*0.002*	76.4±14.7
BMI	28.2±3.8	30.2±5.5	*0.047*	29.5±5.0
WC (cm)	96.0±11.6	97.2±11.7	0.631	96.7±11.6
FM (%)	28.5±9.3	40.2±7.2	*<0.001*	35.6±9.8
SBP (mmHg)	129.0±21.9	129.2±17.5	0.945	129.1±19.2
DBP (mmHg)	75.0±17.9	78.4±11.5	0.249	77.1±14.3
FSG(mg/dL)	163.7±42.5	203.3±63.3	*<0.001*	188.0±59.2
insulin(mU/L)	7.2±3.2	7.9±4.6	0.422	7.6±4.1
HOMA-IR	2.9±1.4	3.5±1.7	0.111	3.2±1.6
HbA1c	7.5±1.4	7.8±1.9	0.310	7.7±1.7
AGE(pg/mL)	555.4±684.3	517.3±522.1	0.764	532.4±588.2
TC (mg/dL)	169.3±40.4	193.7±42.2	*0.005*	184.5±43.0
TG (mg/dL)	168.2±93.8	160.0±80.1	0.673	163.6±85.2
LDL(mg/dL)	79.6±22.1	94.8±27.0	*0.004*	89.0±26.2
HDL(mg/dL)	43.8±8.2	50.9±8.3	*<0.001*	48.2±8.9
Apolipoprotein A(mg/dL)	109.5±30.1	116.9±30.9	0.265	113.7±30.6
Apolipoprotein B(mg/dL)	95.7±27.6	103.4±24.1	0.164	100.0±25.9
Lipoprotein a(mg/dL)	31.4±35.8	27.8±23.4	0.603	29.3±29.1
25(OH)D3 (nmol/L)	39.8±25.0	43.8±38.5	0.639	42.2±33.8
ALT (U/L)	22.6±12.5	19.1±8.1	0.097	20.4±10.1
AST (U/L)	38.7±25.5	25.3±13.4	*0.004*	30.4±19.9

[1]Differences were assessed using *t* test

Abbreviations: ALT: alanine aminotransferase; AGEs: advanced glycated end products; AST: aspartate aminotransferase; BMI: body mass index; DBP: diastolic blood pressure; FM: fat mass; FSG: fasting serum glucose; HDL: high-density lipoprotein cholesterol; HOMA-IR: homeostasis model assessment of insulin resistance; LDL: low-density lipoprotein cholesterol; SBP: systolic blood pressure; TC: total cholesterol; TG: Triglyceride; WC: waist circumference.

Following stratification for MeS according to the NHANS-ATP III criteria (24), 67.3% of the participants were found to have MeS. Sex distribution showed no significant difference between the T2D+MeS and T2D-MeS subgroups (χ^2=0.105, p=0.746). Weight, BMI, FM, insulin, HOMA-IR, AGEs, AST and ALT were all significantly higher but, interestingly, circulating 25(OH)D3 was lower in the subjects with MeS than in those without MeS (Table 2).

Table 2. Characteristics of T2D subjects with and without MeS

Variable	T2D-MeS (n=35)	T2D+MeS (n=70)	p value
Age(year)	51.9±5.9	50.0±6.0	0.138
Duration of disease (yrs)	11.5±6.4	7.4±5.3	0.001
Weight (kg)	66.4±12.5	81.3±13.2	<0.001
BMI	25.9±3.4	31.2±4.8	<0.001
WC (cm)	88.6±9.4	100.6±10.6	<0.001
FM (%)	32.0±8.2	37.4±10.1	0.006
SBP (mmHg)	122.0±17.7	132.6±19.0	0.009
DBP (mmHg)	70.8±15.6	80.2±12.6	0.002
FSG (mmol/L)	10.1±3.31	10.5±3.2	0.487
Insulin(mU/L)	6.0±2.5	8.4±4.5	0.002
HOMA-IR	2.6±1.1	3.5±1.7	0.006
HbA1c	8.0±2.1	7.5±1.5	0.202
AGEs (ng/L)	376.9±316.2	608.8±672.8	0.028
TC (mg/dL)	191.4±48.3	181.3±38.6	0.265
TG (mg/dL)	123.1±62.0	183.3±79.7	0.001
LDL (mg/dL)	92.8±27.0	87.0±23.2	0.237
HDL (mg/dL)	52.2±7.7	46.0±7.7	0.001
Apolipoprotein A (g/L)	112.0±33	114.0±29	0.728
Apolipoprotein B (g/L)	100.0±28	99.0±24	0.894
Lipoprotein a (g/L)	30.0±31.0	28.0±27	0.807
25(OH)D (nmol/L)	53.2±37.8	36.8±30.5	0.026
AST (U/L)	17.0±5.0	22.1±11.5	0.003
ALT (U/L)	22.7±10.4	34.1±22.3	0.001

Abbreviations: ALT: alanine aminotransferase; AGEs: advanced glycated end products; AST: aspartate aminotransferase; BMI: body mass index; DBP: diastolic blood pressure; FM: fat mass; FSG: fasting serum glucose; HDL: high-density lipoprotein cholesterol; HOMA-IR: homeostasis model assessment of insulin resistance; LDL: low-density lipoprotein cholesterol; SBP: systolic blood pressure; TC: total cholesterol; TG: Triglyceride; WC: waist circumference.

Accordingly, the occurrence of undesirable vitamin D status was significantly higher among the subjects with MeS compared to those without MeS (p=0.020) (Table 3). Circulating 25(OH)D3 was found to be inversely correlated with FSG (r_s=-0.257, p=0.010) and FM (r_s=-0.212, p=0.034). No association of 25(OH)D3 with age or other variables was observed.

Table 3. Comparison of vitamin D status based on serum concentration of 25(OH)D3 between patients with and without MeS

	Deficient n (%)	Insufficient n (%)	Sufficient n (%)	p value
Without MeS	9(27.3)	9(27.3)	15(45.5)	
With MeS	32(47.8)	22(32.8)	13(19.4)	0.020*
Total	41 (41)	31 (31)	28 (28)	

* χ^2=7.84

In multivariate logistic regression analysis with certain anthropometric measures, liver enzymes and 25(OH)D3 as independent determinants of MeS risk in T2D in the subjects with sufficient vitamin D status had 50% lower risk for MeS as compared to those who had vitamin D deficiency, and this association remained significant even after additional adjustment for BMI or FM or WC (Table 4).

In multivariate regression analysis, the associations between 25(OH)D3 and glucose level (p=0.016) remained significant after adjustment for FM and weight, indicating that this association was independent of the changes of these anthropometric measurers.

Table 4. Logistic regression analysis for variables associated with risk of MeS

Variables	Odds Ratio	95% CI	p value
Weight* (increase of 1kg)	1.098	1.051-1.14	<0.001
BMI* (increase of 1kg/m^2)	1.36	1.18-1.57	<0.001
WC* (increase of 1cm)	1.12	1.06-1.18	<0.001
FM* (increase of 1%)	1.06	1.01-1.11	0.012
TG* (increase of 1mg/dL)	1.01	1.006-1.02	0.001
HDL* (increase of 1mg/dL)	0.914	0.86-0.96	0.001
HOMA-IR* (increase of 1)	1.46	1.06-2.02	0.020
Vitamin D# (sufficiency vs. deficiency)	0.49	0.29-0.839	0.009

Abbreviations: BMI: body mass index; FM: fat mass; HDL: high-density lipoprotein cholesterol; HOMA-IR: homeostasis model assessment of insulin resistance; LDL: low-density lipoprotein cholesterol;TC: total cholesterol; TG: Triglyceride; WC: waist circumference.
*adjusted for age, sex and duration of disease
#adjusted for age, sex, duration of disease and fat mass

Discussion

High occurrence of undesirable vitamin D status (72%) with a large proportion of sever deficiency (41%) in this study is quite noticeable and comparable with the previous reports from Iran (22) and elsewhere (25-26). The biosocial factors leading to high prevalence of vitamin D deficiency in the general population of Tehran (27-28) do include individuals with diabetes. Among these factors are high latitude, air pollution, living in a metropolitan city with very limited leisure time and outdoor activities, and hence, very short direct sun exposure and the fact that there is no ongoing vitamin D fortification program in Iran.

The inverse association between serum 25(OH)D3 and FSG in the present study is in accordance with some other reports (29,30); however, some studies did not confirm such a relationship (31,32). Though no significant association between circulating 25(OH)D3 and HOMA-IR was found in the present study, in a randomized clinical trial, we recently showed that daily consumption of vitamin D-fortified Persian yogurt drink (doogh) for 12 weeks lowered both FSG and HOMA-IR significantly (14).

Our results did not show any association between 25(OH)D3 and fasting insulin. Actually, reports on the relationship between insulin secretion and 25(OH)D have been inconsistent (33-34). While vitamin D appears to affect the glucose-stimulated insulin secretion, it may not influence basal insulinemia (10). Data from the National Health and Nutrition Examination Survey 1989–1994 (NHANES III) revealed that serum 25(OH)D was inversely associated with diabetes risk, though there was no significant association between 25(OH)D concentrations and HOMA-IR (6).

Our finding on significant negative correlation between 25(OH)D and fat mass (but not BMI) reconfirms other similar reports on the adverse effect of adiposity on vitamin D status (15, 35). Theoretically, deposition in the body fat of vitamin D formed in the skin may reduce its bioavailability (36).

In the present study circulating 25(OH)D was not associated with BMI. The accuracy of BMI in reflecting adiposity as a covariate has been questioned, and when studies have used both dual energy X-ray absorptiometry-derived total body fat and BMI in models to predict 25(OH)D, only total body fat emerged as an independent predictor (15,38).

Of particular interest was that the correlation between serum 25(OH)D and FBS remained significant even after adjustment for weight or fat mass. This finding indicated the direct glycemic optimizing effect of vitamin D in our subjects.

Metabolic syndrome (MS) does have strong links with further comorbidities, notably stroke (38) and CVD (39), which have been considered as the major causes of death in T2D patients (40-41).

In this cross-sectional study of diabetic patients, we observed a strong inverse association of 25(OH)D level with MeS, that did not differ between men and women. A prospective study demonstrated that the baseline vitamin D status in non-diabetic participants was inversely associated with glucose status and MeS risk at the 10-year follow-up (5).

It is important that neither vitamin D status nor the occurrence of MeS showed a gender difference in our subjects. MeS even in non-diabetic subjects may increase the risk of death from all causes, especially from CVD (42). It has been proposed that gender difference in MeS occurrence might have a determining role in gender difference, which is usually observed in CVD (43). Several studies have shown a higher prevalence of CVD in men than in women (44). On the contrary, a study on over 15,000 subjects in Tehran revealed that though the occurrence of at least one CVD risk factor was almost similar in both genders (78% in men and 80% in women), the percentage of women with two or more risk factors was significantly higher than that of men (45). One explanation for the absence of gender difference in the occurrence of poor vitamin D status and MeS in our subjects, despite their higher prevalence in females in the general population, could be that those with poor vitamin D status actually developed MeS and diabetes; hence, gender difference disappeared. This speculation needs to be substantialized by further cohort studies.

The higher prevalence of MeS in Iranian women than Iranian men (35.1% vs 10.7%, $p<0.05$) and in urban residents than in rural residents (24.2% vs 19.5% , $p<0.05$) (46) corresponds to the higher occurrence of vitamin D deficiency in younger and middle-aged females than in age-matched males (27-28) and in urban residents than in rural ones (47). This may, therefore, indicate a determining role for vitamin D status in the development of MeS.

Though MeS may predict CVD risk in general population (48), it has been suggested that MeS might have relatively different outcomes in two genders, i.e. while MeS predisposes women to diabetes and thus to CVD, it increases CVD risk in men mostly independent of diabetes (44). Notwithstanding, in a cohort study, it was shown that though the subjects with both diabetes and

MeS had a greater mortality risk, MeS *per se* (without diabetes) was not an independent predictor of CVD caused death (49). Our data indicated that improvement of vitamin D status, through decreasing the MeS risk, may considerably be effective in prevention of further morbidities in the context of diabetes in both sexes.

The remarkable OR for MeS (0.49) in the subjects with sufficient status of vitamin D as compared to those with vitamin D deficiency remained significant after adjustment for FM. Although we found an inverse association between the serum 25(OH)D and MeS risk, the association was not observed for individual components, except for FSG, suggesting that the association with MeS risk is driven largely by serum glucose. Our results provide further support for an inverse association of 25(OH)D with MeS, independent of gender.

Studies in animals (50-51) and some (3,14) but not all (52) data from humans suggest that vitamin D supplementation could improve glucose intolerance and insulin resistance. However, the possible preventive properties of vitamin D against MeS and its further morbidities, notably T2D and CVD, need to be confirmed by future studies.

We should acknowledge some limitations of the present study. Cross-sectional studies, though pivotal in generating knowledge for further research, cannot establish cause-and-effect relations. Moreover, a relatively limited sample size may have influenced the power to detect some other differences and associations. As MeS, in our sample population, was relatively common, and a logistic regression model was employed to determine OR from the cross-sectional data, overestimation of the magnitude of the associations could be possible.

Conclusion: In conclusion, this study revealed that higher vitamin D status is inversely associated with fasting glycemia among the individuals with diabetes. The findings further suggest that serum 25(OH)D predicts MeS risk in subjects with T2D. Despite mounting evidence linking inadequate vitamin D status with abnormalities of glucose and insulin metabolism, the role of vitamin D is not fully understood, and deserves further investigation in the setting of randomized trials. Demonstration of a causal role of hypovitaminosis D in glucose metabolism and higher risk for the development of further complications, notably MeS and CVD, may lead to a new target for preventive efforts at the population level.

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