Relation of Serum 25 Hydroxy Vitamin D3 Levels with Insulin Resistance in Type 2 Diabetic Patients and Normal Subjects

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Abstract

Hypovitaminosis D is supposed to be associated with diabetes mellitus and metabolic syndrome. Defects in pancreatic β cell function and/or insulin sensitivity are often present, to develop glucose intolerance or type 2 diabetes mellitus. There are accumulating evidence that vitamin D may influence these mechanisms. Aim of our study was to test the hypothesis of an association between hypovitaminosis D and insulin resistance in diabetic patients and normal subjects. We studied 101 type 2 diabetic patients and 60 controls. After detailed physical examination the venous blood was withdrawn, anthropometric measurements were taken, then an indirect measure of insulin resistance was calculated. Study population composed of diabetic and control groups. An indirect insulin resistance index was calculated and compared with other parameters including their 25 hydroxy vitamin D3 levels. In diabetic patients without insulin resistance; fasting blood glucose, fasting insulin and indirect insulin resistance index levels were significantly lower compared to diabetic patients with insulin resistance (p <0.05, <0.01 and <0.01 respectively). We also found that fasting insulin and indirect insulin resistance index levels of the control group without insulin resistance were significantly lower compared to controls with insulin resistance (p <0.001 both). We did not observe any difference in other parameters including vitamin D levels in all groups. These findings suggests that at least in Turkish population vitamin D levels are not associated with insulin resistance both in diabetics and normals.

Keywords*: Diabetes Mellitus, type 2, insulin resistance, vitamin D*

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Introduction

Most tissues have not only Vitamin D receptors, but also the hydroxylase enzyme that is required to convert 25 hdroxy vitamin D (25(OH)D) to the active form, 1,25 dihdroxy vitamin D (1,25(OH)D) [1]. Therefore, vitamin D can affect tissues that are not only involved in calcium homeostasis and bone metabolism. It has been suggested that altered vitamin D status may play a role in the development of diabetes mellitus [2-9], metabolic syndrome [10-12] and cardiovascular disease [13,14].

A high prevalence of hipovitaminosis D was noted in diabetics [15-21]. It was suggested that vitamin D could play a role in the pathogenesis of type 2 diabetes mellitus (T2DM), by affecting either insulin sensitivity or β cell function, or both.

The purpose of the present study was to evaluate the association between vitamin D and insulin resistance both in T2DM patients and normal subjects.

Patients and methods

Patients:

Our study included a total of 101 T2DM patients, aged from 30-80 years, and 60 healthy age-matched people recruited from the outpatient Clinic of Ankara Education and Research Hospital from January 2011 to February 2011.

Subjects with chronic diseases of renal and liver, skin disorders, malabsorption, inflammatory bowel or Celiac disease (in history or nowadays), osteoporosis, osteomalacia and ones taking medications that may interfere serum levels of 25(OH)D were excluded from study.

In all subjects, after detailed physical examination, body weight and height were measured. Waist was measured when fasting, in standing position halfway between costal edge and iliac crest, whereas hip was measured at the greatest circumference around the buttocks, by a non elastic measure. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Petients with HOMA-IR $\geq$2.7 were accepted as inulin resistant [22].

Blood was withdrawn after 12 hour of overnight fasting at 08.30 a.m. for fasting plasma glucose (FPG), hemoglobin A1c(HbA1c), creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), calcium(Ca), phosphorous (P), parathyroid hormone (PTH), thyroid stimulating hormone (TSH), fasting insulin(FI), serum total and HDL cholesterol (HDL-C), triglyceride (TG), and 25 hydroxy vitamin D (25(OH)D) levels.

An indirect measure of insulin resistance was calculated from the fasting plasma insulin (µunite /ml) x fasting plasma glucose (mmol/l)/22.5 formula as homeostasis model assessment-insulin resistance (HOMA-IR).

Systolic and diastolic blood pressure (SBP and DBP) were measured after a 5 min rest in the semi-sitting position with a sphygmomanometer. Blood pressure was determined at least three times at the right upper arm, and the mean was used in the analysis.

Laboratory methods

Plasma glucose, creatinine, AST, ALT, Ca, P, albumin, total cholesterol, TG and HDL-C concentrations were determined by enzymocalorimetric spectrophotometric method in a Roche/Hitachi molecular PP autoanalyser. Low density lipoprotein cholesterol (LDL-C) was calculated by the Friedewald Formula (LDL: Total cholesterol-HDL-TG/5). Insulin was measured by means of DRG Diagnostics (DRG Instruments GmbH, Germany) ELISA kits and FI was measured by TOSOH G7 HPLC system. PTH and TSH was determined with Advia Sentor XP device by chemoluminescence method. For the measurements of 25(OH)D, Waters LC-MS/MS device liquid chromatography mass spectrometry was used.

This study was performed according to the Helsinki decleration 2008. The local ethics comitee approved this study and all the subjects gave written informed consent.

Statistical analysis

Calculations were performed using SPSS version 10.1. Student's test was used to compare the groups in a parametric way (for data showing homogenous dispersion) and Mann Whitney U test was used in a non-parametric way (for data showing non-homogenous dispersion). Data are presented as mean ± SD. A p value of < 0.05 was considered as statistically significant.

Results

Those of 101 type 2 diabetic patients, 61 were female (61%) and 40 were male (29%). In the control group there were 36 (60%) female and 24 (40%) male normal subjects.

All the demographic and laboratory findings of diabetic patients and controls classified according to their HOMA-IR and they were compared and given in Table 1. FBG, FI and HOMA-IR levels of T2DM patients with HOMA-IR< 2.7 were significantly lower compared to those with HOMA-IR ≥2.7 ($p < 0.05$, < 0.01 and < 0.01 respectively). We did not observe any difference in other parameters including 25(OH)D levels between the groups. FI and HOMA-IR levels of the control subjects with HOMA-IR< 2.7 was significantly lower compared to those with HOMA-IR ≥2.7 ($p < 0.001$ both) . We did not observe any difference in other parameters including 25(OH)D levels between the groups.

Table 1. Demographic and laboratory findings of diabetic patients and controls classified according to their HOMA-IR

	T2DM			**Control**		
	HOMA-IR< 2.7 n: 21	HOMA-IR≥2.7 n: 80	P	HOMA-IR < 2.7 n: 50	HOMA-IR≥2.7 n: 10	P
BMI (kg/m2)	28.3 ± 3.4	30.2 ± 4.2	NS	27.1 ± 2.6	27.3 ± 2.0	NS
Waist cir. (cm)	97.9 ± 11.6	97.9 ± 10.4	NS	90.6 ± 7.0	90.1 ± 6.3	NS
Hip Cir.(cm)	103.6 ± 9.6	102.6 ± 9.5	NS	99.6 ± 9.6	101.6 ± 9.4	NS
FBG (mg/dl)	**141.7 ± 46.6**	**110.1 ± 31.4**	**<0.05**	91.8 ± 5.0	94.4 ± 4.8	NS
HbA1c (%)	7.7 ± 1.7	8.3 ± 2.2	NS	5.4 ± 0.2	5.5 ± 0.1	NS
Creat. (mg/dL)	0.9 ± 0.1	0.3 ± 0.1	NS	0.7 ± 0.1	0.8 ± 0.1	NS
AST (U/L)	21.5 ± 5.4	22.7 ± 6.6	NS	22.7 ± 7.0	24.0 ± 8.7	NS
ALT (U/L)	20.8 ± 7.0	25.9 ± 10.1	NS	22.4 ± 10.1	24.9 ± 12.9	NS
Ca (mg/dL)	9.4 ± 0.4	9.2 ± 0.3	NS	9.3 ± 0.3	9.4 ± 0.4	NS
P (mg/dL)	3.3 ± 0.3	3.3 ± 0.4	NS	3.3 ± 0.4	3.2 ± 0.4	NS
PTH (pg/mL)	29.2 ± 23.1	53.0 ± 26.3	NS	54.7 ± 18.5	57.5 ± 18.6	NS
TSH (µU/MI)	1.62 ± 0.7	1.68 ± 1.0	NS	1.8 ± 1.0	2.4 ± 1.5	NS
Albumin (g/dL)	4.3 ± 0.2	4.3 ± 0.3	NS	4.3 ± 0.2	4.3 ± 0.3	NS
FI (µU/ ml)	**6.30 ± 2.2**	**14.7 ± 5.9**	**<0.01**	**6.5 ± 2.5**	**14.8 ± 3.2**	**0.001**
HOMA-IR	**2.0 ± 0.5**	**6.3 ± 3.6**	**<0.01**	**1.4 ± 0.5**	**3.4 ± 0.7**	**<0.001**
SBP (mm Hg)	140.1 ± 10.1	139.0 ± 10.4	NS	130.1 ± 10.4	129.0 ± 11.4	NS
DBP (mm Hg)	92.6 ± 11.5	91.8 ± 9.1	NS	82.6 ± 11.5	89.2 ± 9.2	NS
LDL-C (mg/dl)	120.7 ± 36.6	129.6 ± 38.9	NS	125.8 ± 30.8	119.0 ± 33.7	NS
HDL-C (mg/dl)	42.1 ± 9.9	45.5 ± 10.0	NS	48.0 ± 9.4	43.5 ± 12.5	NS
TG (mg/dl)	226.0 ± 90.2	194.6 ± 95.5	NS	137.0 ± 29.3	163.0 ± 61.5	NS
25(OH)D (ng/ml)	8.1 ± 5.1	10.4 ± 8.1	NS	14.6 ± 7.8	13.2 ± 7.5	NS

BMI: Body mass index, Waist cir: Waist circumference, Hip cir: Hip circumference. FBG: Fasting blood glucose, HbA1c: Hemoglobin A1c, Creat.: Creatinine, AST: aspartate aminotransferase, ALT: alanine aminotransferase, PTH: Parathyroid hormone, TSH: Thyroid stimulating hormone, FI: Fasting insulin, HOMA-IR: Homeostasis model assesment -insulin resistance, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, LDL-C: Low density lipoprotein cholesterol, HDL-C: High density lipoprotein cholesterol, TG: Triglyceride, CRP: C-reactive protein, Hcy: Homocysteine, 25(OH)D: 25 hydroxy vitamin D. Data are presented as mean ± SD. NS: nonsignificant.

Conclusion

Vitamin D insufficiency has long been suspected as a risk factor for type 1 diabetes mellitus (T1DM) based on animal and human studies [2-5]. More recently, there is accumulating evidence to suggest that altered vitamin D homeostasis may also play a role in the development of T2DM [6-9].

In order to develop glucose intolerance and T2DM, defects in pancreatic β cell function and insulin sensitivity are often needed. There is evidence that vitamin D influence these mechanisms. However, vitamin D may also have beneficial effect on glucose metabolism improving systemic inflammation [23-25]. The direct effect of vitamin D on pancreatic β cells may be mediated by binding of its circulating active form 1,25(OH)D to the pancreatic β cell vitamin D receptor [26]. Alternatively activation of vitamin D may ocur within the β cell by the 1α hydroxylase enzyme expressed in those cells.[27] The indirect effects of vitamin D may be mediated via its role in regulating extracellular Ca and Ca flux through the β cell. Insulin secretion is a Ca dependent process [28-30].

Vitamin D may show its beneficial effects on insulin action directly by stimulating the expression of insulin receptor and increasing responsiveness for glucose transport [31]. It was also demonstrated that vitamin D directly activates peroxisome proliferator activator receptor-δ, a transcription factor implicated in the regulation of fatty acid metabolism in skeletal muscle and adipose tissue [32]. Like insulin secretion, Ca is again essential for insulin

mediated intracellular processes in insulin responsive tissues such as skeletal muscle and adipose tissue [33]. Vitamin D may also act on insulin action indirectly by its role in regulating extracellular Ca and ensuring normal Ca influx through cell membranes and adequate intracellular cytosolic a pool. Changes of Ca insulin targeted tissues may contribute to peripheral insulin resistance by impairing insulin receptor phosphorilation leading to signal transduction and decreased glucose transporter-4 activity [34].

Keeping in mind the role of vitamin D on insulin sensitivity we planned to investigate the relation of vitamin D with insulin resistance both in diabetic and normal subjects. Some studies have shown an inverse association between vitamin D and Ca status and insulin resistance [35-42]. There are studies in which no association have been determined [43-47]. Results from randomized trials on the effect of vitamin D supplementation on insulin resistance show either no effect [48-50] or improvement of insulin action with supplementation [51-54].

In the present study, 25(OH)D levels did not differ when the degree of insulin sensitivity concerned in type 2 diabetics. The same result was demonstrated in control subjects. We may explain the absence of the correlation of vitamin D deficiency with insulin resistance with low vitamin D levels in our study population. Similarly, low vitamin D levels diabetics and controls were previously shown in Turkey [55,56].

This report has some limitations: We performed the study in winter season and seasonal variations could have influeced the results. Likewise, the gold standard for the measurement of insulin sensitivity is the use of the euglycemic clamp; we only demonstrated insulin resistance by an indirect metod; HOMA-IR and number of subjects in study group were limited. It is known that there is a relation between VD deficiency and age, our patient and control groups from people aged 30-80 years, but we did not make any correction for age.

We concluded that severe hipovitaminosis D is prevelant both in diabetics and controls. And vitamin D levels are not associated with insulin resistance both in type 2 diabetics and healthy controls. Considering of low vitamin D levels, vitamin D supplementation must be considered immediately in Turkey.

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