Do Low Serum 25-Hydroxyvitamin D Levels Affect Progression of Prediabetes to Type 2 Diabetes?

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ABSTRACT

Objectives: This study aims to investigate 25-hydroxyvitamin D (25(OH)D) levels in patients with glucose and glycated hemoglobin (HbA1c) defined prediabetes and type 2 diabetes mellitus (T2DM).

Patients and methods: Eighty-nine prediabetic patients (44 males, 45 females; mean age 48.9±6.1 years) with impaired fasting glisemia (IFG) with glucose levels between 100-125 mg/dL (5.55-6.98 mmol/L) and HbA1c levels lower than 6.4% (46 mmol/mol), and 78 type 2 diabetic patients (40 males, 38 females; mean age 50.1±6.9 years) with HbA1c levels higher than 6.5% (48 mmol/mol) and glucose levels higher than 126 mg/dL (6.99 mmol/L) were included in the study. All patients were kept under strict control and were allowed to use their oral antidiabetics. Serum levels of glucose, insulin, HbA1c and 25(OH)D were measured in all patients. Insulin resistance was calculated by homeostasis model assessment formula.

Results: While high density lipoprotein cholesterol (p=0.0001), insulin (p=0.013), insulin resistance (p=0.004), and vitamin D levels (p=0.0001) of T2DM patients were significantly lower compared to the IFG group, their HbA1c levels were higher (p=0.0001). There was a negative correlation between vitamin D and HbA1c (r=-0.327, p=0.006), but a positive correlation between vitamin D and insulin (r=0.215, p=0.006), and vitamin D and HOMA-IR (r=0.236, p=0.002). Glycated hemoglobin levels were significantly higher in the T2DM group than in the IFG group (p=0.0001). In the T2DM group, 25(OH)D levels were lower compared to the IFG group (p=0.0001). 25-hydroxy vitamin D levels were inversely associated with HbA1c levels in the T2DM group (p=0.0001).

Conclusion: Vitamin D levels were lower in T2DM group than in IFG group. Our findings indicate that vitamin D supplementation to T2DM patients may improve glycemic control.

Keywords: Adaptive immunity; diabetes mellitus; innate immunity; type 2; vitamin D.

ÖZET

Amaç: Bu çalışmada glikoz ve glikozillenmiş hemoglobine (HbA1c) göre prediabetli ve tip 2 diabetes mellitusu (T2DM) hastaların 25 hidroksi vitamin D (25(OH)D) düzeyleri araştırıldı.

Hastalar ve yöntemler: Glikoz düzeyleri 100-125 mg/dL arasında (5.55-6.98 mmol/L) ve HbA1c düzeyleri %6.4%’ten düşük (46 mmol/mol), bozulmuş açık glikesimi (BAG) olan 89 prediabetik hasta (44 erkek, 45 kadın; ort. yaş 48.9±6.1 yıl) ve HbA1c düzeyleri %6.5’ten yüksek (48 mmol/mol) ve glikoz düzeyleri 126 mg/dL’den yüksek (6.99 mmol/L) 78 tip 2 diabetik hasta (40 erkek, 38 kadın; ort. yaş 50.1±6.9 yıl) çalışmaya alındı. Tüm hastalar siki kontrol altında tutuldu ve hastaların oral antidiyetiklerini kullanmalarına izin verildi. Tüm hastaların glikoz, insülin, HbA1c ve 25(OH)D serum düzeyleri ölçüldü. Insülin direnci, hemostaz model dегerlendirmesi formülü ile hesaplanydı.

Bulgular: Tip 2 diabetes mellitusu hastaların BAG grubuna göre yüksek yoğunluklu lipoprotein kolesterol (p=0.0001), insülin (p=0.013), insülin direnci (p=0.014) ve D vitamini (p=0.0001) düzeyleri anlamlı derecede düşük iken, HbA1c düzeyleri yüksek idi (p=0.0001). D vitamini ile HbA1c arasında negatif (r = -0.327, p=0.006), D vitamini ile insülin (r=0.215, p=0.006) ve D vitamini ile HOMA-IR arasında ise pozitif bir ilişki (r=0.236, p=0.002) vardı. Glikozillenmiş hemoglobinin düzeyleri T2DM grubunda BAG grubuna göre anlamlı derecede yüksek idi (p=0.0001). T2DM grubunda 25(OH)D düzeyleri BAG grubuna göre daha düşük idi (p=0.0001). 25 hidroksi vitamin D düzeyleri T2DM grubunda HbA1c düzeyleri ile ters ilişkilidir (p=0.0001, r=0.215).

Sonuç: D vitamini seviyeleri T2DM grubunda BAG grubundan daha düşük idi. Bulgularımız T2DM hastalarına D vitamini takviyesi yapılmasını glikesim kontrolü iyileştirileceğine işaret etmektedir.

Anahtar sözcükler: Edinsel immünite; diabetes mellitus; doğal immünite; tip 2; D vitamini.
Vitamin D is a hormone that is related to skeletal integrity, and recently, its extraskeletal effects have raised considerable interest. Vitamin D is involved in innate and adaptive immunity, and it enhances the chemotaxis and phagocytic capabilities of innate immune cells. Furthermore, it modifies the phenotype of antigen-presenting dendritic cells (DC) to a more tolerogenic phenotype that favors the differentiation of inducible regulatory T (iTreg) cells instead of the inflammatory T helper (Th) 1 and Th 17 cells.

Previous studies have determined that vitamin D plays a role in the cardiovascular, central nervous, endocrine, and immune systems as well as in cell differentiation and cell growth. Furthermore, its receptor has been found in more than 35 tissues, including endothelial, pancreatic islet, hematopoietic, cardiac, and skeletal muscle cells along with monocytes, neurons, and T lymphocytes that are not involved in bone metabolism.

Vitamin D deficiency appears to be related to the development of type 2 diabetes mellitus (T2DM), and mild to moderate vitamin D insufficiency has been proposed as a risk factor for this disease. Moreover, the higher plasma levels in vitamin D are related to a lower risk for the development of T2DM in high-risk patients. In addition, prediabetes has become a prime topic of discussion in recent years. In cross-sectional and epidemiological studies, the relationship between vitamin D deficiency and DM, metabolic syndrome, obesity-associated as well as this vitamin’s role in insulin resistance, secretion, and the inflammatory process have been reported. Various studies have also shown that deficient serum 25-hydroxy vitamin D (25(OH)D) levels reduce insulin secretion by decreasing calcium absorption, thereby causing secondary hyperparathyroidism and greater peripheral insulin resistance. Hence, vitamin D may be a factor that directly or indirectly affects insulin resistance. Therefore, in this study, we hypothesized that vitamin D deficiency may be prevalent in a population of T2DM patients and that their vitamin D levels may be related to glucose control.

PATIENTS AND METHODS

A total of 89 impaired fasting glycemia (IFG) patients (45 females and 44 males; mean age 48.9±6.1 years) and 78 T2DM patients (38 females, 40 males; mean age 50.1±6.9 years) who came to the outpatient clinics of the Bakırköy Dr. Sadi Konuk Research and Training Hospital were included in our prospective, interventional study. The study participants were classified as prediabetic using the American Diabetes Association (ADA) criteria in which IFG was defined as having fasting plasma glucose levels of between 100 and 125 mg/dL (5.55–6.98 mmol/L). All 89 of the patients in the IFG group had prediabetes and glycated hemoglobin (HbA1c) levels of <6.4% (46 mmol/mol). Furthermore, 78 had HbA1c levels of >6.5% (48 mmol/mol) and fasting plasma glucose levels of >126 mg/dL (6.99 mmol/L). The 78 patients in the T2DM group were known to have diabetes. Insulin resistance was defined as having homeostasis model assessment for insulin resistance (HOMA-IR) levels of >2.5.

Patients who were taking vitamin D and/or calcium supplements and those with bone metabolism disorders or liver or kidney failure were excluded from the study. Informed consent was obtained from all of the study participants, and approval for the study was given by the hospital ethics committee.

Fasting blood samples were drawn via antecubital venipuncture, and the samples were collected directly into serum separator tubes. After coagulation, centrifugation was performed at 1,500 g for 10 minutes. The sera was then separated, stored in aliquots, and kept frozen at −80 °C until the analysis. All of the parameters were measured in the sera except for HbA1c, which was only measured in the whole blood.

Triglyceride, total cholesterol, glucose, blood urea nitrogen (BUN), and creatinine were analyzed using the AU5800 clinical chemistry system (Beckman Coulter, Inc., Brea, CA, USA), and the high-density lipoprotein (HDL) cholesterol levels were determined via the direct enzymatic method without precipitation. The precision values of this method were <5%, <3%, <3%, <5%, <5%, and <5%, respectively. Then the low-density lipoprotein (LDL) cholesterol levels were calculated using the Friedewald formula. Additionally, the insulin and vitamin D levels were measured via the chemiluminescent method utilizing the UniCel® Dxi 800 immunoassay system (Beckman Coulter, Inc., Brea, CA, USA). The intra- and interassay precision for the vitamin D values were <4.6% and <65%, respectively, whereas they were <4.2% and <5.8% for the insulin values. The HOMA-IR was calculated using the formula: HOMA-IR = fasting plasma glucose (mmol/L) × fasting insulin (mU/L)/22.5. The HbA1c levels were also measured using high performance liquid chromatography (HPLC) (Premiere Hb9210™, Trinity Biotech Plc, Wicklow, Ireland).

Statistical analysis

Number Cruncher Statistical Software (NCSS) (Kaysville, UT, USA) was used for all statistical analysis, and the data was written as mean ± standard deviation (SD). An independent samples t-test was used to compare two different groups, and chi-square and Fisher’s exact tests were used to compare qualitative parameters. In addition, Pearson’s correlation test was used to estimate the relationship between the parameters. A p value of
<0.05 was considered to be statistically significant with a 95% confidence interval (CI).

RESULTS

The clinical characteristics and biochemical parameters of the study participants are presented in Table 1. There were no statistical differences between the IFG and T2DM groups with regard to glucose, BUN, creatinine, total cholesterol, triglycerides, or LDL cholesterol (p>0.05). However, the T2DM group had significantly lower HDL cholesterol (p=0.0001), insulin (p=0.013), and HOMA-IR (p=0.014) levels. The patients who had diabetes remained under strict control and were followed up regularly, which may explain these differences. Furthermore, the T2DM group had significantly higher HbA1c whole blood levels (p=0.0001) (Table 1). There were no correlations between vitamin D and the glucose, BUN, creatinine, total cholesterol, LDL cholesterol, HDL-cholesterol, and triglyceride levels (p>0.05), but there was a negative correlation between vitamin D and HbA1c (r=-0.327; p=0.006) and a positive correlation between vitamin D and insulin (r=0.215; p=0.006) and the HOMA-IR levels (r=0.236; p=0.002) (Figure 1).

Moreover, we found that the 25(OH)D levels were lower in the T2DM group (p<0.0001) (Table 1), and linear regression analysis revealed that they were inversely associated with the HbA1c levels in the same group (p=0.0001, r=0.215). We also converted the HbA1c, insulin, HOMA-IR variants via linear regression analysis. Furthermore, univariate analysis showed that the 25(OH)D levels were also related to the HbA1c (p=0.001), insulin (p=0.013), and HOMA-IR (p=0.006) levels (R²= 0.203; corrected R²= 0.197).

DISCUSSION

It is widely known that the pathophysiology of T2DM involves the progressive impairment of insulin secretion, which is connected with the coexisting insulin resistance.[19] Furthermore, several studies have shown a relationship between vitamin D deficiency and a cluster of metabolic abnormalities, known as the metabolic syndrome, which is associated with diabetes and impaired beta cell secretion.[20,21] The concomitant association between vitamin D deficiency and insulin resistance along with the impaired insulin secretion and the related metabolic consequences has generated much speculation regarding the possible role of vitamin D in the pathogenesis of T2DM.[13]

It has been shown that the majority of immune cells express the vitamin D receptor (VDR).[22,23] and Provvedini et al.[24] found evidence of the expression of the vitamin D nuclear receptor together with vitamin D-activating enzymes in both T and B cells. This expression was very low in the resting phase, but after activation and proliferation, the T and B cells were found to significantly upregulate VDR expression.[25-27]

In addition, VDRs have been found in pancreatic beta cells, which in turn express the 1-alpha hydroxylase enzyme.[28,29] Vitamin D facilitates the secretion of insulin in T2DM, and since this vitamin is known to stimulate the expression of the insulin receptor, vitamin D deficiency may be related to insulin resistance.[30,31]

| TABLE 1 |
| Clinical characteristics and biochemical variables of the impaired fasting glycemia type 2 diabetes mellitus groups |
| IFG (n=89) | T2DM (n=78) |
| n Mean±SD | n Mean±SD | p |
| Age | 48.9±6.1 | 50.1±6.9 | 0.243 |
| Gender | | | 0.812 |
| Male | 44 | 40 | |
| Female | 45 | 38 | |
| Glucose(mmol/L) | 6.0±0.4 | 6.1±0.4 | 0.136 |
| Blood urea nitrogen (mmol/L) | 5.7±3.7 | 6.3±4.1 | 0.343 |
| Creatinine (µmol/L) | 79.6±53.9 | 189.2±119.3 | 0.280 |
| Cholesterol (mmol/L) | 4.9±1.1 | 5.0±1.1 | 0.880 |
| Low-density lipoprotein cholesterol (mmol/L) | 3.1±0.8 | 3.0±1.0 | 0.168 |
| High-density lipoprotein cholesterol (mmol/L) | 1.3±0.3 | 1.1±0.2 | 0.0001 |
| Triglycerides (mmol/L) | 1.8±1.5 | 1.9±1.4 | 0.821 |
| HbA1C (mmol/mol) | 3.8±2.0 | 4.9±1.5 | 0.0001 |
| Insulin (pmol/L) | 115.3±19.5 | 77.9±58.1 | 0.013 |
| HOMA-IR | 5.2±5.3 | 3.5±2.6 | 0.014 |
| Vitamin D (nmol/L) | 52.4±25.9 | 34.1±17.7 | 0.0001 |

IFG: Impaired fasting glycemia; T2DM: Type 2 diabetes mellitus; SD: Standard deviation; HOMA-IR: Homeostasis model assessment for insulin resistance.
Our data revealed that the 25(OH)D concentration had a positive effect on the insulin (p=0.013) and HOMA-IR (p=0.006) levels, but it had a negative effect on HbA1c (p=0.001).

Various cross-sectional studies have largely, but not consistently, shown a significantly increased risk of T2DM and impaired glucose metabolism in conditions associated with vitamin D deficiency.[32,33] In the same manner, Oosterwerff et al.[34] found an inverse relationship between vitamin D status and the incidence of T2DM.

The relationship between vitamin D and T2DM and the metabolic syndrome has been debated previously,[35] with vitamin D having been shown to be related to glucose metabolism and the development of T2DM.[36]

Our study had several limitations. It was an observational study; therefore, no conclusions could be made with respect to any course or effect regarding the relationship between vitamin D deficiency and T2DM and IFG. Another issue was that vitamin D “insufficiency” and “deficiency” are defined differently. Vitamin D “insufficiency” is commonly defined as having a 25(OH)D level of between 15 and 20 ng/mL, whereas “deficiency” is defined as having a 25(OH)D level of <15 ng/mL. Thus, patients with vitamin D deficiency for a subset composed of those who are vitamin D insufficient.

Based on these definitions, our IFG group had vitamin D insufficiency while our T2DM group suffered from vitamin D deficiency.[37]

Conclusion

The existing knowledge indicates that maintaining a 25(OH)D level of over 30 ng/mL during the year with no seasonal variations can have multiple real as well as potential health benefits. Furthermore, the structural analogs of vitamin D, which have been tested experimentally, hold great promise for positively influencing the immune system along with insulin and B cell functions, but they appear to suppress the influence of vitamin D on bone and calcium metabolism.[12] Hence, further studies are needed, especially for patients that are at high risk for developing diabetes (i.e., those with IFG and/or glucose intolerance). However, based on the hypothetical mechanism of action of vitamin D, those patients might benefit from the ability of vitamin D to prevent T2DM.

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