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The effects of vitamin D supplementation on endogen amylin hormone, hormonal and biochemical parameters, and insulin resistance in type-2 diabetic patients with vitamin D deficiency in the Kurdistan Region of Iraq.

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Key words: vitamin D; amylin; HbA1c; insulin; C-peptide.

Abstract. The present study was conducted to evaluate the correlations between vitamin D, amylin, c-peptide, insulin, and HbA1c and determine the effects of vitamin D supplementation on the level of these biomarkers in type-2 diabetic patients with vitamin D deficiency. A total of 87 Kurdish type-2 diabetic patients participated in this clinical trial in which biomarkers, including vitamin D (25 hydroxy cholecalciferol), serum amylin, c-peptide, insulin, fasting blood glucose, HbA1c, HDLc, LDLc, total cholesterol, triglycerides, weight, height, waist circumference, and inflammation markers (i.e. IL6, TNF-α, and hs-CRP) were measured. Serum levels of vitamin D were positively correlated with serum Amylin, c-peptide, insulin, and HDL, while it had negative correlations with HbA1c, fasting blood sugar, IL6, TNF-α, hs-CRP, LDL, and triglycerides. After 14 weeks of supplementation with vitamin D (5000 IU/day) there were significant increases in plasma amylin, c-peptide, and insulin concentrations; but the levels of HbA1c, fasting blood glucose, TNF-α, hs-CRP, and IL6 were significantly decreased. It can be concluded that vitamin D supplementation can potentially regulate blood glucose by activating the secretory function of pancreatic B-cells and reducing insulin resistance through a significant reduction in a circulatory level of inflammatory markers.

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Efectos de la suplementacion de Vitamina D sobre la hormona endógena amilina, parámetros hormonales y bioquímicos, e insulina resistencia en pacientes diabéticos tipo 2 con deficiencia de Vitamina D en la región Kurdistan de Irak.

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Palabras clave: vitamina D; amilina; HbA1c; insulina; péptido-C.

Resumen. El presente estudio se realizó para evaluar las correlaciones entre la vitamina D, la amilina, el péptido C, la insulina y la HbA1c y determinar los efectos de la suplementación con vitamina D en el nivel de estos biomarcadores en pacientes diabéticos tipo 2 con deficiencia de vitamina D. Un total de 87 pacientes kurdos, diabéticos tipo 2, participaron en este ensayo clínico en el que se midieron los biomarcadores, incluídos la vitamina D (25 hidroxicolecalciferol), amilina sérica, péptido C, insulina, glucosa sérica en ayunas, HbA1c, HDLc, LDLc, colesterol total, triglicéridos, peso, altura, la circunferencia de la cintura y los marcadores de inflamación (es decir, IL6, TNF-α y hs-CRP). El nivel sérico de vitamina D se correlacionó positivamente con la amilina sérica, el péptido C, la insulina y el HDLc, mientras que tuvo correlaciones negativas con HbA1c, glucosa en sangre en ayunas, IL6, TNF-α, hs-CRP, LDLc y triglicéridos. Después de 14 semanas de suplementación con vitamina D (5000 UI / día) hubo aumentos significativos en las concentraciones plasmáticas de amilina, péptido C e insulina; pero los niveles de HbA1c, glucosa en sangre en ayunas, TNF-α, hs-CRP e IL6 disminuyeron significativamente. Se puede concluir que la suplementación con vitamina D puede potencialmente regular la glucosa en la sangre activando la función secretora de las células B pancreáticas y reduciendo la resistencia a la insulina a través de una reducción significativa en un nivel circulatorio de marcadores inflamatorios.

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INTRODUCTION

A growing research evidence has concluded that type-2 diabetes (T2D) and its complications are significant causes of morbidity and mortality worldwide (1). As a crucial global health condition, 415 million people are afflicted with T2D, and it will increase to 642 million by 2040 (2). Vitamin D deficiency (a prohormone) is also considered as a public health problem throughout the world (3). Vitamin D is an essential fat-soluble secostroid responsible for the maintenance of calcium homeostasis and good physical condition of the bone, and it has also been proved that this vitamin is associated with hypertension, diabetes, metabolic syndrome, cancer, and autoimmune and infectious diseases (4). Observational studies have indicated that vitamin D deficiency is associated with the onset of T2D, its progression, and subsequent macrovascular complications (5-9).

Amylin is a hormone composed of 37-amino acid peptides. It is synthesized and co-localized with insulin in the beta-cells of

pancreatic islets (10). Amylin is co-secreted with insulin from beta-cells of pancreatic islets in response to a meal ingestion. It is also an important hormone for energy balance regulation (11). Vitamin D deficiency has a negative impact on the level of amylin (12). Many clinical trials have been devoted to finding innovative methods for preventing and treating diabetes mellitus, and the focus of these trials has recently been on vitamin D supplementation. Mirhosseini *et al.* (13) found that vitamin D supplementation has a significant positive effect on fasting blood glucose (FBG), HbA1c, and homeostasis model assessment of insulin resistance (HOMA-IR). The results of the studies conducted on both humans and animals have shown that vitamin D status and insulin secretion and resistance are linked because both vitamin D receptor and 1- α-hydroxylase are present in the pancreatic β-cells (14). Based on the literature review, there is a constant association between increased body mass index and decreased serum vitamin D concentrations (15).

Chronic low-grade inflammation, commonly observed in obese patients, is correlated with the development of insulin resistance, which aggregates the risk of T2D (16). Recent data have shown that severe vitamin D deficiency is associated with increased levels of C-reactive protein (CRP), Tumor Necrosis Factor-alfa (TNF-α), and IL-6 (17).

Regarding the high prevalence of T2D among Kurds as well as the premise that T2D may present alongside vitamin D deficiency, the present investigation was aimed to:

1- Conduct an open-label clinical-analytical study on the effects of improved vitamin D status on insulin and amylin secretion, insulin resistance, insulin sensitivity, and pancreatic β-cell functions; 2- evaluate the effect of vitamin D supplementation on the level of Amylin in T2D Kurdish patients (because amylin is a potential hormone in energy expenditure and glucose hemostasis, although it has been ignored in recent studies); and 3- evaluate the effects of vitamin D supplementation on inflammatory markers in patients with both T2D and vitamin D deficiency.

MATERIAL AND METHODS

To take ethical considerations into account, necessary approval was obtained from the Ethical Committee of the Hawler Medical University prior to the beginning of the study. Moreover, all of the participants filled out a written informed consent and they were all free to leave the study at any phase. The study was conducted in the Endocrinology Center located in Sulaymaniyah Governorate, Iraq from December 1, 2017, to June 1, 2018. The Clinical Analysis Department of the College of Pharmacy of Hawler Medical University cooperated to conduct the study. Adult type-2 diabetic patients with vitamin D deficiency were enrolled in the present study. They were of both genders and aged 40 and over. The exclusion criteria consisted of having malnutrition, terminal illnesses, coronary artery, kidney or hepatic disease. Furthermore, pregnant and nursing patients with serum C-reactive protein (CRP) ≥6 mg/L were excluded from the study.

The target patients were enrolled in the Endocrinology Center in Sulaymaniyah Governorate, Iraq. Located in the Northern part of Iraq, Sulaymaniyah is a mountainous governorate characterized by its cold winters, and less sunlight exposure. Each patient was examined by an endocrinologist and the researcher's team. A total of 87 patients (15 males and 72 females) who met the abovementioned inclusion and exclusion criteria were enrolled for 14 weeks of vitamin D supplementation (5000 IU/day), which was added to their treatment regimens. At the beginning of the intervention, the participants were advised to contact the research staff immediately whenever their body reacted unexpectedly to the supplements.

Before and after supplementation with vitamin D_3, the patients underwent an an-

thropometric evaluation including height (m), weight (kg), and waist circumference (cm) measurements.

Moreover, the Quetelet's equation (weight (kg)/height (m) 2) was utilized to calculate the patients' body mass index (BMI). Then, 12-hour overnight fasting venous blood samples were taken from them. The blood samples were divided into two parts; EDTA test tubes were filled with the first part in order to determine the glycosylated haemoglobin (HbA1c) percentage, and the non-coagulant test tubes were filled with the another part in which the sera were centrifuged to be separated (3000 rpm for 15 minutes), and they were kept at -20°C for later measurements within 2 weeks of sampling.

Enzymatic reaction was also employed for spectrophotometric measurement of lipid profile (mg/dL) including total high- and low-density lipoprotein-cholesterol (HDL-c & LDL-c), triglyceride (TG), cholesterol (TC), and fasting serum glucose (FSG). Log of TG/HDL-c was calculated in order to determine the atherogenicity. Moreover, the technology of enzyme-linked immunosorbent assay (ELISA) was utilized to determine fasting serum insulin (mU/L), C-peptide (ng/ml), amylin (pg/ml), IL6, TNF-α, and hs-CRP. Homeostatic model assessment of insulin resistance (HOMA-IR) and Homeostatic model assessment of insulin sensitivity (HOMA-IS) was determined through the following formulas:

$$HOMA-IR = \frac{Fasting\ serum\ glucose\left(\frac{mg}{dl}\right) \times Fasting\ serum\ insulin\left(\frac{mU}{L}\right)}{405}$$

$$HOMA-IS = \frac{1}{HOMA-IR}$$

This study has some limitations including that it did not control for other potential confounders such as anti-diabetic oral agents, other pharmacological therapies and nutrition intervention.

Statistical analysis

The results are expressed as frequencies, percentages, and whenever possible as means ± SD. Data were analyzed using independent and paired samples t-tests, chi-squared and simple (rho) correlation tests. The level of statistical significance was considered $p<0.05$; data analysis was conducted using SPSS version 21.0 software (IBM Corp., Armonk, N.Y., USA) and Microsoft Excel (2007).

RESULTS

The present study was conducted on 87 type-2 diabetic patients with a mean age of 50.5 years. The participants consisted of 72 females and 15 males. Regarding their marital status, all were married except two of them. Only four participants were smokers. Moreover, 44 patients had hypertension, and about 61% of them were living in rural areas (Table I).

After 14 weeks of intervention, vitamin D supplement resulted in a sharp improvement in the level of vitamin D, which in turn led to a significant reduction in fasting blood glucose, and HbA1c, by a non-significant decrease in HOMA-IR. It also led to an improvement in the secretion of fasting serum insulin, and significant improvements were observed in C-peptide and amylin (Table II).

TABLE I
PATIENTS' DEMOGRAPHICS.

Gender		Age±SD /year	Marital status		Residency		Smoking (No.)	Co-morbidity (hypertension) (No.)
Male (No.)	Female (No.)		Single (No.)	Married (No.)	Urban (No.)	Rural (No.)		
15(17.24)	72(82.76)	50.5±7	2(2.3)	85(97.7)	34(39.1)	53(60.9)	4(4.6)	44(50.5)

The results are expressed as number (percentage) and mean±SD.

TABLE II
ASSESSMENT OF GLYCAEMIC STATUS AND RELATED BIOMARKERS.

Biomarkers	Before supplementation	After supplementation	P value
Vitamin D (ng/mL)	13.5 ± 6.34	33.21 ± 10.14	<0.001
Fasting serum glucose (mg/dL)	219.8 ± 64.4	176.6 ± 55.9	
Glycated hemoglobin (HbA1c%)	9.9 ± 1.52	8.44 ± 1.45	
Amylin (pg/mL)	388.0 ± 279.6	426.0 ± 309.1	
Fasting serum insulin (μ unit/mL)	14.2 ± 10.05	16.56 ± 10.08	
HOMA-IR	8.0 ± 7.36	6.96 ± 4	0.125
HOMA-IS	0.125 ± 5.6	0.144 ± 3.5	0.09
C-peptide (ng/mL)	2.3 ± 0.76	2.97 ± 0.9	0.036

The results are expressed as mean±SD. P-value represents the level of significant difference between pre-and post-treatment using two-tailed paired t-test. HOMA-IR: Homeostatic model assessment for insulin resistance. HOMA-IS: Homeostatic model assessment of insulin sensitivity.

Furthermore, after 14 weeks of the 5000 IU/day vitamin D supplementation, the chronic low-grade inflammatory markers (high sensitivity C-reactive protein, interleukin 6, and tumor necrosis factor-α) decreased significantly (Table III).

Waist circumference (WC) and Body Mass Index (BMI) decreased significantly after 14 weeks of vitamin D supplementation. Triglycerides, triglyceride to high-density lipoprotein ratio, and HDL also increased significantly. However, total cholesterol and LDL decreased after supplementation, but these differences were not significant (Table IV).

TABLE III
ASSESSMENT OF INFLAMMATORY MARKERS AND RELATED BIOMARKERS

Biomarkers	Before supplementation	After supplementation	p-value
High sensitivity C-reactive protein (mg/L)	3.04 ± 1.68	2.53 ± 1.42	
Interleukin 6 (ng/mL)	198.3 ± 84.8	176.2 ± 79.6	<0.001
Tumor necrosis factor-α (ng/mL)	187.4 ± 94.7	169.3 ± 95.2	

The results are expressed as mean±SD. P value represents the level of significant difference between pre-and post-treatment using two-tailed paired t-test.

TABLE IV
ANTHROPOMETRIC MEASUREMENTS AND FASTING LIPID PROFILE DATA

Determinates	Before supplementation	After supplementation	P-value
Waist circumference (cm)	100 ± 6.5	98.5 ± 6.6	
Body mass index (kg/m2)	25.7 ± 2.9	25.4 ± 2.9	<0.001
High-density lipoprotein-cholesterol (mg/dL)	38.1 ± 11.8	42.5 ± 10.6	
Triglyceride to high-density lipoprotein ratio	4.6 ± 3.288	3.7 ± 2.371	
Total cholesterol mg/dL)	178.5 ± 40	176.8 ± 37.5	0.534
Triglyceride (mg/dL)	154.8 ± 69.6	143.4 ± 65.2	0.019
Low density lipoprotein-cholesterol (mg/dL)	117.6 ± 31.5	114.6 ± 30.5	0.278

The results are expressed as mean±SD. P value represents the level of significant difference between pre-and post-treatment using two-tailed paired t-test.

DISCUSSION

Considered as a global problem, vitamin D deficiency is observed in both tropical countries and those with temperate climate (18). Furthermore, vitamin D plays an important role in bone homeostasis. Data from observational studies have shown an association between vitamin D deficiency and chronic disorders such as diabetes, autoimmune and cardiovascular diseases (19). Type-2 diabetes is one of the leading non-communicable chronic diseases whose complications have become major causes of morbidity and mortality worldwide (1). It has also been indicated by observational studies, that there is an association between vitamin D deficiency and the onset and progression of T2D, as well as future macrovascular events (5-9). While, a randomized, double blind, placebo-controlled clinical trial among persons at high risk for T2D, found that vitamin D3 supplementation did not result in a significantly lower risk of diabetes than placebo (20).

In the present study, all of the patients had T2D and suffered from vitamin D deficiency. The effects of vitamin D supplement on glycemic status and chronic low-grade inflammatory markers in T2D were evaluated in this study. Vitamin D supplementation for 14 weeks considerably affected glycemic indices, insulin resistance, and systemic inflammation.

Findings of the present study demonstrated that vitamin D (5000 IU/day) supplementation resulted in a significant drop in the percentage of HbA1c and the level of fasting blood glucose, but it increased secretion of amylin and C-peptide remarkably and insulin non-significantly from pancreatic β-cells. Furthermore, the improved vitamin D level after supplementation (5000 IU/day) resulted in decreased HOMA-IR, which indicates the improvement in insulin sensitivity and decreased insulin resistance. Refined vitamin D status negatively affected the markers of inflammation through dropping the circulatory levels of IL6, TNF-α, and hs-CRP. These results are along the lines of other related studies in which vitamin D supplementation improves insulin sensitivity (21) and reduces systemic inflammation(22).

Other study showed that supplementation with vitamin D for 12 weeks, compared with the placebo in patients with DFU, led to a significant reduction in HOMA-IR and HbA1c (23). Moreover, one-year 420 IU/day vitamin D supplementation increased serum 25(OH) D concentration, resulting in beneficial effects on fasting glucose level and insulin resistance (24). However, supplementation with 1000 IU/day vitamin D among healthy overweight women for 12 weeks did not affect the insulin resistance (25).

In terms of inflammation, vitamin D supplementation reduced hs-CRP, IL-6, and TNF-α significantly, and this finding is consistent with other studies (23, 26-29). Matias *et al.* (30) also observed a significant reduction in the serum hs-CRP level at the one-year follow-up of patients with vitamin D deficiency who received high doses of vitamin D3. Although in a study conducted on patients with major depressive disorder, it was found that taking 50,000 IU/week vitamin D supplemention for 8 weeks did not affect hs-CRP level (31).

Witham *et al.* (32) found no reduction in the hs-CRP level after 8 weeks of supplementation with a single dose of 100,000 IU vitamin D3 in women in southern Asia. Pittas *et al.* (33) also found no reduction in the CRP, interleukin 6 (IL-6), and tumor necrosis factor-α (TNF-α) levels. Favorable effects of vitamin D intake on markers of insulin resistance can be explained through its impact on calcium and phosphorus metabolism as well as up-regulation of the insulin receptor gene and increased transcription of insulin receptor genes (34).

One of the strengths of the present study is the assessment of serum biomarkers related to β-cells function such as c-peptide, amylin and 25(OH)D. C-peptide secreted in equimolar concentration to insulin is widely

considered as a better marker of residual β-cells function than insulin due to its longer half-life (35). In addition to C-peptide, amylin, as mentioned before, is a glucoregulatory hormone co-secreted with insulin in response to food intake to complement insulin dependent maintenance of postprandial glucose homeostasis (12). Interestingly, positive associations of insulin, C-peptide, and amylin with 25(OH) D were identified in the results of the present study.

Vitamin D supplementation (5000 IU daily) for 14 weeks led to a significant decrease in FBS and HbA1c by increasing the synthesis of amylin, insulin, and c-peptide. Furthermore, insulin resistance decreased, while insulin sensitivity increased as a result of the significant reduction in IL6 and TNF-α.

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