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ORIGINAL ARTICLE

Pre-Diabetics with Hypovitaminosis D Have Higher Risk for Insulin Resistance

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SUMMARY

Background: **Various studies have been reported on the relationship between vitamin D, whose deficiency has been identified in a pandemic way, and metabolic-endocrine diseases, including insulin resistance. Insulin resistance is an important public health issue since it is a common cause of death as it transforms into metabolic syndrome and type 2 diabetes mellitus (DM). In this study, the aim is to investigate the relationship between the level of serum 25 hydroxy vitamin D (25(OH)D) and insulin resistance.**

Methods: **A retrospective study was carried out including 2,008 patients aged between 18 - 67 chosen from among the patients who had applied to Saglik Bilimleri University Antalya Training and Research Hospital. Patients were divided into three groups as non-diabetic, pre-diabetic, and diabetic according to their blood glucose profile and into three categories according to their 25(OH)D levels. The relationship between serum vitamin D levels and insulin resistance was compared between the groups. Individuals with homeostasis model assessment of insulin resistance (HOMA-IR) > 2.5 were considered to have insulin resistance.**

Results: **The study was composed of 2,008 patients, 1,614 were female (80.4%). Of the participants, 216 (10.6%) were diabetics, 849 (42.3%) were pre-diabetics, and 943 (47.1%) were non-diabetics. It was identified that age, fasting blood glucose, HbA1c, triglyceride (Tg), very-low-density lipoprotein cholesterol (VLDL-C), fasting insulin, and HOMA-IR levels were significantly higher in diabetic patients than in pre-diabetic patients (all $p < 0.001$) and similarly higher in pre-diabetics than in non-diabetics. Tg, VLDL, fasting insulin, and HOMA-IR levels were significantly lower in the group with 25(OH)D ≥ 30 ng/mL. Especially in pre-diabetic individuals, a significant negative correlation was observed between the 25(OH)D level and HbA1c ($p = 0.020$), Tg ($p = 0.001$), VLDL-C ($p = 0.001$), fasting insulin ($p < 0.001$) and HOMA-IR ($p < 0.001$). While high HOMA-IR was positively associated with fasting blood glucose and total cholesterol values (all $p < 0.001$), it was negatively associated with age ($p < 0.001$), LDL-cholesterol ($p < 0.001$), HDL-cholesterol ($p < 0.001$) and 25(OH)D ($p = 0.001$).**

Conclusions: **Diabetic subjects have lower plasma 25(OH)D levels and pre-diabetics with hypovitaminosis D have higher risk for insulin resistance. Thus, HOMA-IR must be well evaluated in pre-diabetic individuals with vitamin D deficiency/insufficiency, if there is associating abdominal obesity.**

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KEY WORDS

Vitamin D, pre-diabetics, insulin resistance

INTRODUCTION

In recent years, vitamin D deficiency has been reported all over the world. Its prevalence varies according to geographic location, season, ethnic origin, and how the

laboratory defines normal levels and the levels of deficiency and insufficiency [1].

The best parameter to show the vitamin D status of the body is serum 25(OH)D level [2]. Although there is no consensus on the optimal level of 25(OH)D, most researchers have found that the levels of vitamin D ≥ 30 ng/mL (75 nmol/L) are sufficient, the levels between 20 - 29 ng/mL (50 - 75 nmol/L) are insufficient and the levels below 20 ng/mL (50 nmol/L) are deficient. In cases where 25(OH)D levels are higher than 150 ng/mL, vitamin D intoxication is mentioned [2-6]. In the studies, it is emphasized that vitamin D is a steroid hormone with neuroprotective and antioxidant effects besides its effects on the skeletal-muscular system, and the relationship between vitamin D and metabolic-endocrine-neuropsychiatric diseases and cancer is examined. Insulin resistance is one of these metabolic-endocrine diseases. It is characterized by a decrease in the response of peripheral tissues to insulin as a result of a decrease in insulin receptor expression [7]. Central obesity and insulin resistance are the two most important causes of metabolic syndrome associated with hypertension, dyslipidemia, impaired glucose tolerance and/or increased fasting blood glucose.

Several theories have been proposed to explain the relationship between vitamin D levels and insulin sensitivity/glucose metabolism. The effects of vitamin D on insulin sensitivity and/or pancreatic beta cell function [8-12] and the polymorphism in vitamin D binding protein (DBP), vitamin D receptor (VDR), and vitamin D 1-alpha hydroxylase genes, resulting in the development of insulin resistance [13] are the most common theories. The relationship between low serum vitamin D levels and increased insulin resistance has been shown in several studies [14-16]. In addition, some studies on animals have shown that insulin release is regulated in those who receive vitamin D supplementation [17-21]. The aim of this study is to investigate whether there is a correlation between insulin resistance and 25(OH)D levels in individuals who visited our outpatient clinics and to evaluate the cases of insulin resistance with regard to homeostasis model assessment of insulin resistance (HOMA-IR).

MATERIALS AND METHODS

Study population

The retrospective study conducted between the dates of January 1, 2016 and March 31, 2018 includes 2,008 patients aged between 18 and 67, who visited the Family Medicine and Internal Medicine outpatient clinics of the Saglik Bilimleri University (SBU) Antalya Training and Research Hospital (TRH) for various reasons. The patients were grouped according to their HOMA-IR and 25(OH)D levels and contacted after obtaining their personal information via our IT Unit. Prior to the study, approval was obtained from Antalya TRH Clinical Research Ethics Committee, and the study was performed in compliance with the Declaration of Helsinki.

Data collection and laboratory measurements

The information about age, fasting blood glucose, liver and kidney function tests, HbA1c, fasting insulin, HOMA-IR values, and lipid parameters that belonged to the study group were recorded in data collection forms. When collecting the blood samples, fasting blood glucose, blood urea nitrogen (BUN), creatinine (cre), alanine aminotransferase (ALT), aspartate transaminase (AST), total cholesterol (total-C), triglyceride (Tg), high-density lipoprotein cholesterol (HDL-C) levels were estimated by the spectrophotometric method and Beckman coulter commercial kits on the Beckman Coulter AU5800 (Beckman Coulter Inc., CA, USA) autoanalyser. HbA1c levels were identified using commercially available high-performance liquid chromatography (Tosoh HLC 723 G8; Tosoh Bioscience, Japan). The levels of 25(OH)D were measured by chemiluminescence method using the Liaison (DiaSorin, MN, USA) device. Very-low-density lipoprotein cholesterol (VLDL-C) was calculated by dividing the triglyceride level by 5, and low-density lipoprotein cholesterol (LDL-C) level was identified according to the formula developed by Friedewald et al. [22], while HOMA-IR was estimated according to the formula fasting plasma glucose (mg/dL) x fasting serum insulin (μU/mL)/405, as indicated by Matthews et al. [23].

Statistical analysis

Statistical analysis was done using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, NY, USA). Pearson's chi-square analysis was performed for categorical variables. The normality assumptions were controlled by the Shapiro-Wilk test. Mann-Whitney *U* test and Student's *t*-test were used for the analysis of non-normally and normally distributed numerical data, respectively. Kruskal Wallis test was used for the comparison of non-parametric variables between the groups, and Bonferroni-Dunn test was used as a post-hoc test for significant cases while One-Way ANOVA with post-hoc Tukey HSD test was used for parametric variables. Multiple logistic regression analyses were performed to identify the association between the study parameters and HOMA-IR. The results were expressed as n (%), mean ± standard deviation (SD) or median (min-max). $p < 0.05$ values were considered statistically significant.

RESULTS

The study included 2,008 patients, 394 of whom were male (19.6%) (mean age = 40.01 ± 12.34) and 1,614 of whom were female (80.4%) (mean age = 39.66 ± 11.72). Of the participants, 216 (10.6%) were diabetics, 849 (42.3%) were pre-diabetics, and 946 (47.1%) were non-diabetics. In addition, 752 participants (37.5%) were diagnosed with hyperlipidemia.

Table 1. Laboratory test results of patients (n = 2,008).

Parameters	Patients' results	
	Mean ± SD	(min - max)
Fasting glucose (mg/dL)	96.22 ± 21.13	(36 - 342)
HbA1c (%)	5.69 ± 0.73	(2.4 - 13.6)
BUN (mg/dL)	11.63 ± 3.35	(2 - 29)
Creatinine (mg/dL)	0.84 ± 0.14	(0.42 - 1.69)
ALT (U/L)	24.76 ± 19.56	(0 - 279)
AST (U/L)	23.27 ± 12.91	(10 - 352)
Total cholesterol (mg/dL)	203.29 ± 46.04	(65 - 578)
Triglyceride (mg/dL)	140.77 ± 105.24	(24 - 1,906)
LDL cholesterol (mg/dL)	125.11 ± 36.66	(24 - 384)
HDL cholesterol (mg/dL)	51.1 ± 12.07	(19 - 111)
VLDL cholesterol (mg/dL)	28.16 ± 21.05	(5 - 381)
Fasting insulin (µU/mL)	10.33 ± 8.14	(1.01 - 104.67)
HOMA-IR	2.52 ± 2.34	(0.22 - 29.46)
Vitamin D (ng/mL)	21.4 ± 14.31	(4 - 150)

Table 2. Comparison of patients who have different glucose profiles.

Parameters Median (min - max)		Non-diabetic (n = 943)	Pre-diabetic (n = 849)	Diabetic (n = 216)	p
Age (years), (mean ± SD)		35.1 ± 10.3 [a]	43 ± 11.8 [b]	47.3 ± 10 [c]	< 0.001
Gender n (%)	male	179 (19.0)	161 (19.0)	54 (25.0)	0.109
	female	764 (81.0)	688 (81.0)	162 (75.0)	
Fasting glucose (mg/dL)		89 (47 - 100) [a]	98 (66 - 125) [b]	114 (36 - 342) [c]	< 0.001
HbA1c (%)		5.3 (2.4 - 5.6) [a]	5.8 (3.3 - 6.4) [b]	6.7 (5 - 13.6) [c]	< 0.001
BUN (mg/dL)		11 (2 - 29) [a]	12 (4 - 25) [b]	12 (5 - 29) [b]	0.001
Creatinine (mg/dL)		0.8 (0.4 - 1.6)	0.8 (0.6 - 1.4)	0.8 (0.5 - 1.7)	0.194
Total cholesterol (mg/dL)		189 (65 - 412) [a]	207 (95 - 578) [b]	214 (107 - 433) [b]	< 0.001
Triglyceride (mg/dL)		102 (28 - 1040) [a]	126 (24 - 1,906) [b]	145.5 (54 - 781) [c]	< 0.001
LDL cholesterol (mg/dL)		115 (24 - 320) [a]	127 (33 - 384) [b]	131 (47 - 325) [b]	< 0.001
HDL cholesterol (mg/dL)		50 (23 - 111)	50 (19 - 102)	48 (24 - 89)	0.071
VLDL cholesterol (mg/dL)		20 (6 - 208) [a]	25 (5 - 381) [b]	29 (11 - 156) [c]	< 0.001
Fasting insulin (µU/mL)		7.5 (1 - 53) [a]	8.8 (1.8 - 104.7) [b]	10.1 (1.5 - 88.6) [c]	< 0.001
HOMA-IR		1.6 (0.2 - 13) [a]	2.1 (0.4 - 29.5) [b]	3 (0.3 - 29.1) [c]	< 0.001
Vitamin D (ng/mL)		19 (4 - 146) [a]	18.7 (4 - 150) [a]	15.6 (4 - 83.3) [b]	0.001

ANOVA with Tukey-HSD test, Kruskal-Wallis with Bonferroni-Dunn post-hoc test. Pearson Chi-square test. Significant differences were presented with underlined line.

Mean serum fasting blood glucose, HbA1c, BUN, creatinine values of the participants were 96.22 ± 21.13 (mg/dL), 5.69 ± 0.73 (%), 11.63 ± 3.35 (mg/dL), 0.84 ± 0.14 (mg/dL), respectively. Their total-C, Tg, LDL-C, HDL-C, VLDL-C values were identified as 203.29 ± 46.04 mg/dL, 140.77 ± 105.24 mg/dL, 125.11 ±

Table 3. Correlation between vitamin D levels and other factors in non-diabetic, pre-diabetic and diabetic patients.

Parameters	Non-diabetic (n = 943)		Pre-diabetic (n = 849)		Diabetic (n = 216)	
	r	p	r	p	r	p
Age (years)	0.107	0.001	0.184	< 0.001	0.119	0.082
Fasting glucose (mg/dL)	-0.059	0.070	0.045	0.187	-0.032	0.644
HbA1c (%)	-0.013	0.701	-0.080	0.020	-0.09	0.189
BUN (mg/dL)	0.021	0.520	0.084	0.014	0.228	0.001
Creatinine (mg/dL)	0.073	0.025	0.176	< 0.001	0.236	< 0.001
ALT (U/L)	-0.050	0.123	0.005	0.882	-0.044	0.517
AST (U/L)	-0.036	0.269	0.023	0.501	-0.105	0.125
Total cholesterol (mg/dL)	-0.019	0.563	-0.031	0.369	0.085	0.215
Triglyceride (mg/dL)	-0.135	< 0.001	-0.111	0.001	-0.068	0.321
LDL cholesterol (mg/dL)	-0.006	0.865	-0.012	0.727	0.079	0.248
HDL cholesterol (mg/dL)	0.086	0.008	0.061	0.076	0.076	0.268
VLDL cholesterol (mg/dL)	-0.135	< 0.001	-0.112	0.001	-0.067	0.330
Fasting insulin (µU/mL)	-0.146	< 0.001	-0.193	< 0.001	-0.099	0.148
HOMA-IR	-0.146	< 0.001	-0.173	<0.001	-0.133	0.051

Spearman correlation test.

Table 4. Comparison of patients characteristics according to serum 25(OH)D levels.

Patients characteristics Median (min - max)		25(OH)D < 20 ng/mL (n = 1,115)	25(OH)D = 20 - 29 ng/mL (n = 548)	25(OH)D ≥ 30 ng/mL (n = 345)	p-value
Age (years) (mean ± SD)		38.9 ± 11.7 [a]	39.9 ± 11.8 [a]	42.3 ± 11.8 [b]	< 0.001
Gender (n (%))	male	231 (20.7)	105 (19.2)	58 (16.8)	0.266
	female	884 (79.3)	443 (80.8)	287 (83.2)	
Fasting glucose (mg/dL)		93 (63 - 342)	93 (36 - 220)	92 (70 - 253)	0.462
HbA1c (%)		5.6 (3.3 - 13.6) [a]	5.6 (2.4 - 9.1) [b]	5.6 (4.5 - 8.8) [b]	0.014
BUN (mg/dL)		11 (5 - 29)	11 (4 - 25)	11 (2 - 29)	0.155
Creatinine (mg/dL)		0.80 (0.4 - 1.7) [a]	0.83 (0.5 - 1.4) [b]	0.84 (0.5 - 1.3) [b]	< 0.001
Total cholesterol (mg/dL)		200 (72 - 578)	198 (102 - 409)	194 (65 - 412)	0.180
Triglyceride (mg/dL)		123 (28 - 1906) [a]	113 (24 - 395) [b]	100 (35 - 420) [c]	< 0.001
LDL cholesterol (mg/dL)		123 (27 - 384)	122.5 (40 - 320)	120 (24 - 304)	0.273
HDL cholesterol (mg/dL)		49 (19 - 102) [a]	50 (28 - 111) [b]	52 (23 - 107) [b]	0.001
VLDL cholesterol (mg/dL)		25 (6 - 381) [a]	23 (5 - 79) [b]	20 (7 - 84) [c]	< 0.001
Fasting insulin (µU/mL)		9 (1 - 88.6) [a]	8.2 (1.3 - 104.7) [b]	6.9 (1.8 - 75.8) [c]	< 0.001
HOMA-IR		2.1 (0.2 - 29.1) [a]	1.8 (0.2 - 29.5) [b]	1.6 (0.4 - 20.4) [c]	< 0.001

ANOVA with Tukey-HSD test, Kruskal-Wallis with Bonferroni-Dunn post-hoc test. Pearson's Chi-square test. Significant differences were presented with underlined line.

36.66 mg/dL, 51.1 ± 12.07 and 28.16 ± 21.05 mg/dL, respectively. Mean fasting insulin, HOMA-IR and 25(OH)D values were 10.33 ± 8.14 (µU/mL), 2.52 ± 2.34, and 21.4 ± 14.31 (ng/mL), respectively (Table 1). Our study identified that age, fasting blood glucose, HbA1c, Tg, VLDL-C, fasting insulin and HOMA-IR

Table 5. Comparison of patients characteristics according to serum cutoff value of vitamin D levels.

Patients characteristics median (min - max)		25(OH)D ≤ 17 ng/mL (n = 853)	25(OH)D = 18 - 29 ng/mL (n = 810)	25(OH)D ≥ 30 ng/mL (n = 345)	p-value
Age (years), (mean ± SD)		38.7 ± 11.8 [a]	39.7 ± 11.8 [a]	42.3 ± 11.8 [b]	≤ 0.001
Gender (n (%))	male	159 (18.6)	177 (21.9)	58 (16.8)	0.091
	female	694 (81.4)	633 (78.1)	287 (83.2)	
Fasting glucose (mg/dL)		93 (66 - 342)	93 (36 - 287)	92 (70 - 253)	0.060
HbA1c (%)		5.6 (3.3 - 13.6) [a]	5.6 (2.4 - 10.1) [b]	5.6 (4.5 - 8.8) [b]	0.001
BUN (mg/dL)		11 (5 - 29)	11 (4 - 25)	11 (2 - 29)	0.188
Creatinine (mg/dL)		0.80 (0.4 - 1.7) [a]	0.83 (0.5 - 1.4) [b]	0.84 (0.5 - 1.3) [b]	≤ 0.001
Total cholesterol (mg/dL)		201 (72 - 578)	198.5 (97 - 463)	194 (65 - 412)	0.179
Triglyceride (mg/dL)		122 (28 - 1906) [a]	116 (24 - 1040) [b]	100 (35 - 420) [c]	≤ 0.001
LDL cholesterol (mg/dL)		123 (27 - 384)	122.5 (33 - 363)	120 (24 - 304)	0.275
HDL cholesterol (mg/dL)		49 (19 - 97) [a]	49 (28 - 111) [a]	52 (23 - 107) [b]	0.007
VLDL cholesterol (mg/dL)		24 (6 - 381) [a]	23 (5 - 208) [b]	20 (7 - 84) [c]	≤ 0.001
Fasting insulin (µU/mL)		9.2 (1 - 79.9) [a]	8 (1.3 - 104.7) [b]	6.9 (1.8 - 75.8) [c]	≤ 0.001
HOMA-IR		2.2 (0.2 - 26.6) [a]	1.8 (0.2 - 29.5) [b]	1.6 (0.4 - 20.4) [c]	≤ 0.001

ANOVA with Tukey-HSD test, Kruskal-Wallis with Bonferroni-Dunn post-hoc test. Pearson Chi-square test. Significant differences were presented with underlined line.

Table 6. Multiple logistic regression analysis showing independent predictors of high HOMA-IR groups.

Variables	Odds ratio (95% CI)	p
Age	0.964 (0.953 - 0.975)	≤ 0.001
Gender (Ref = female)	1.179 (0.897 - 1.55)	0.238
Fasting glucose (mg/dL)	1.069 (1.058 - 1.081)	≤ 0.001
HbA1c (%)	1.214 (0.963 - 1.53)	0.101
Total cholesterol (mg/dL)	1.045 (1.029 - 1.062)	≤ 0.001
Triglyceride (mg/dL)	0.985 (0.914 - 1.062)	0.697
LDL cholesterol (mg/dL)	0.956 (0.940 - 0.971)	≤ 0.001
HDL cholesterol (mg/dL)	0.928 (0.911 - 0.945)	≤ 0.001
VLDL cholesterol (mg/dL)	0.991 (0.979 - 1.003)	0.142
Vitamin D (ng/mL)	0.984 (0.975 - 0.993)	0.001

levels were significantly higher (all $p < 0.001$) in diabetic patients than in pre-diabetic patients, and similarly, higher in pre-diabetics than in non-diabetics. While BUN ($p = 0.001$), Total-C ($p < 0.001$), and LDL-C ($p = 0.001$) values were significantly higher in pre-diabetics and diabetics than in non-diabetics, and 25(OH)D level was identified to be significantly lower in diabetics than in pre-diabetics and non-diabetics (Table 2). It was observed that 25(OH)D was weakly correlated with the age ($r = 0.107$, $p = 0.001$), creatinine ($r = 0.073$, $p = 0.025$), HDL-C ($r = 0.086$, $p = 0.008$), Tg ($r = -0.135$, $p < 0.001$), VLDL-C ($r = -0.135$, $p < 0.001$), fasting insulin ($r = -0.146$, $p < 0.001$), and HOMA-IR ($r = -0.146$, $p < 0.001$) in non-diabetic patients. It was positively correlated with BUN ($r = 0.228$, $p = 0.001$) and creatinine ($r = 0.236$, $p < 0.001$) in DM patients. In prediabetic patients, there was a significant positive correlation between 25(OH)D and age ($r = 0.184$, $p < 0.001$), BUN ($r = 0.084$, $p = 0.014$), and creatinine ($r = 0.176$, $p < 0.001$), whereas it was negatively correlated with HbA1c ($r = -0.080$, $p = 0.020$), Tg ($r = -0.111$, $p = 0.001$), VLDL-C ($r = -0.112$, $p = 0.001$), fasting insulin

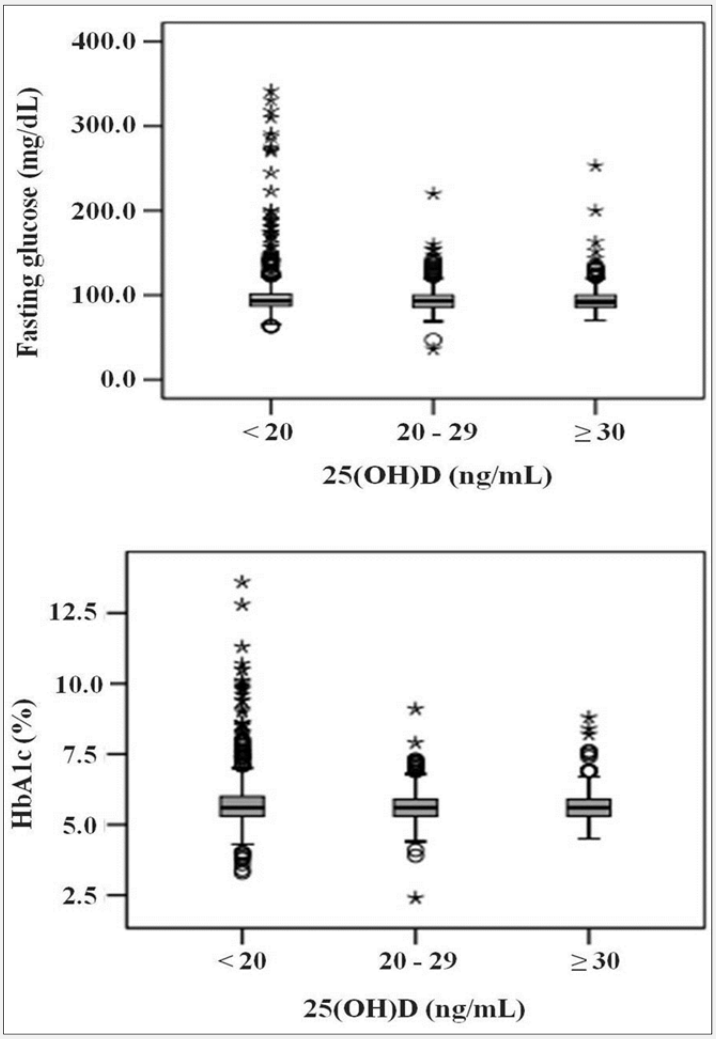

Figure 1. The correlations between 25(OH)D and fasting glucose and HbA1c.

(r = -0.193, p < 0.001), and HOMA-IR (r = -0.173, p < 0.001) (Table 3).

In our study, 25(OH)D < 20 ng/mL was considered as deficient, 20 - 29 ng/mL was considered as insufficient, and ≥ 30 ng/mL was considered as sufficient.

Participants were divided into three groups according to their 25(OH)D levels. The mean age of the group with sufficient 25(OH)D levels was observed to be significantly higher. In addition, Tg, VLDL-C, fasting insulin, and HOMA-IR levels were significantly lower in the group with 25(OH)D ≥ 30 ng/mL when compared to the group with 25(OH)D = 20 - 29 ng/mL, and lower in the same manner in the group with 25(OH)D = 20 - 29 ng/mL when compared to the group with 25(OH)D ≤ 20 (all p < 0.001). While HbA1c levels were significantly lower in the groups with 25(OH)D ≥ 30 ng/mL and 25(OH)D = 20 - 29 ng/mL than in the groups with 25(OH)D ≤ 20 ng/mL (p = 0.014), HDL-C and creatinine levels were higher (p < 0.001). There was no relationship between 25(OH)D level and fasting blood glucose level (Figure 1, Table 4).

The ROC curve analysis showed that by using the optimal Vit D cutoff value of 17 ng/mL calculated by Youden Index, Vit D could predict high HOMA-IR patients with an area under the curve of 0.595 (95% CI = 0.573 - 0.617), sensitivity of 52.89%, and specificity of 62.79% (Figure 2).

In this study, the cutoff value was calculated by the ROC analysis and set as 17 ng/mL for 25(OH)D level. The mean age of the group and HDL-C level were higher in the group with sufficient 25(OH)D levels. Likewise, Tg, VLDL-C, fasting insulin, and HOMA-IR lev-

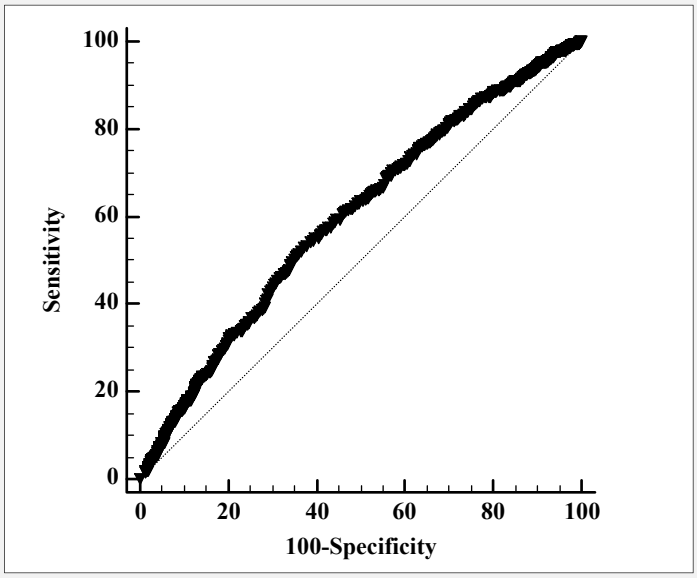

Figure 2. The ROC curve analysing optimal vitamin D cutoff value.

els were significantly higher in the group with 25(OH)D ≥ 30 ng/mL when compared to the group with 25(OH)D = 18 - 29 ng/mL and in the group with 25(OH)D = 18 - 29 ng/mL when compared to the group with 25(OH)D ≤ 17 ng/mL. While HbA1c levels were significantly lower in the group with 25(OH) ≥ 30 ng/mL and 25(OH)D = 18 - 29 ng/mL compared to the group with 25(OH)D ≤ 17 ng/mL (p = 0.001), creatinine level was identified to be higher (p < 0.001) (Table 5).

The results of logistic regression analyses showed that fasting glucose and Total-C were high-risk factors for the development of high HOMA-IR in patients. It was found that high HOMA-IR level was positively associated with fasting glucose (OR: 1.069; 95% CI: 1.058 - 1.081; p < 0.001) and Total-C (OR: 1.045; 95% CI: 1.029 - 1.062; p < 0.001) while it was negatively associated with age (OR: 0.964; 95% CI: 0.953 - 0.975; p < 0.001), LDL-C (OR: 0.956; 95% CI: 0.940 - 0.971; p < 0.001), HDL-C (OR: 0.928; 95% CI: 0.911 - 0.945; p < 0.001) and 25(OH)D (OR: 0.984; 95% CI: 0.975 - 0.993; p = 0.001) (Table 6).

DISCUSSION

In this study we found that lower 25(OH)D concentrations were significantly associated with higher HOMA-IR, Tg, VLDL-C and lower HDL-C levels. These data support the previous studies that have searched the association between vitamin D and metabolic-endocrine diseases.

The associations between vitamin D and insulin resistance in non-diabetics, prediabetics or diabetics have been previously reported. However, there is no data that compares these three groups in the literature. This is one of the strengths of our study, and the other one is that our study was planned to include both summer and winter months with the working group living in Antalya, which is sunny almost 10 months of the year.

In the literature, there are various studies investigating the association between vitamin D levels and insulin sensitivity. Ford et al. divided 8,421 participants into five groups according to their 25(OH)D levels and showed a significant inverse correlation between the groups with low 25(OH)D levels and the incidences of abdominal obesity, hypertriglyceridemia, and hyperglycemia [24]. The study by Lima-Martinez et al. revealed that patients with insufficient/deficient 25(OH)D levels had higher DM risk scores than those with normal 25(OH)D levels, and the DM risk score had a negative correlation with the 25(OH)D levels and a positive correlation with the HOMA-IR index [25]. Similarly, in our study, there was a statistically significant negative correlation between 25(OH)D levels and Tg levels. Fasting blood glucose levels were higher in patients with insufficient/deficient 25(OH)D levels, although not statistically significant. On the other hand, in our study, 25(OH)D levels were determined to be statistically significantly lower in diabetics than in pre-diabetic and non-diabetic patients.

In a prospective study of 524 non-diabetic participants between the age of 40 and 69, Forouhi et al. found a

negative correlation between 25(OH)D levels and insulin resistance [14]. In a randomized and double-blind placebo-controlled study by Cefalo et al., conducted with 18 non-diabetic, vitamin D-deficient volunteers with a BMI > 25, the participants were divided into two groups. The first group was given a hypocaloric diet and a placebo as well as 25,000 IU cholecalciferol for three months, and the second group was only given a hypocaloric diet and a placebo. While significant weight loss was observed in both groups, there was also a significant increase in insulin sensitivity in the group that received vitamin D. Yet, no change in insulin sensitivity was observed in the placebo group [26]. In a study by Need et al., conducted with 753 postmenopausal women who did not receive any drug treatment to affect glucose metabolism, the author found a positive correlation between fasting blood glucose and age, weight, and BMI and a negative correlation between fasting blood glucose and serum 25(OH)D [27]. In our study, although not statistically significant, fasting blood glucose was found to be lower in the group with sufficient 25(OH)D levels compared to the group with insufficient/deficient levels.

Tamadon et al., on the other hand, performed a study similar to Cefalo's with diabetic patients and divided 60 diabetic hemodialysis (HD) patients into two groups. The first group was given 50,000 IU of vitamin D3 in every two weeks for a period of 12 weeks while the second group was given a placebo. After 12 weeks, the group that received vitamin D supplementation had a significantly increased level of serum insulin, decreased HOMA-IR, and increased quantitative insulin sensitivity index (QUICKI). In this study, Tamadon et al. suggested that a 12-week vitamin D supplementation had beneficial effects on serum insulin, HOMA-IR levels, and QUICKI in diabetic HD patients [28]. In a cross-sectional study including 63 patients with Type 2 DM, Minambres et al. showed that the prevalence of obesity or weight gain and the VLDL-C levels were higher in patients with vitamin D deficiency compared to those with normal vitamin D level individuals. They also suggested that vitamin D deficiency was related to metabolic syndrome parameters in type 2 DM patients [29]. Similar to the study of Minambresin, in our study, VLDL-C levels were found to be significantly higher and HDL-C levels were lower in the group with vitamin D deficiency/insufficiency.

In the literature, there are studies showing that vitamin D supplementation has no corrective effect on HOMA-IR [30,31]. Hoseini et al. suggested that the reason why vitamin D supplementation presented no statistically significant response to HOMA-IR was that the study involved a small number of participants and the follow-up period was short. They suggested this as the limitation of their study [30].

In our study, there are some limitations that may influence the results. First, because the study was a retrospective study, the necessary information about patients' BMI, smoking and alcohol habits, the extent of their daily activities could not be obtained. Secondly, the patients were monitored at a single time and in a cross-sectional manner. Third, the HOMA-IR test was used instead of hyperinsulinemic euglycemic clamp which is the most effective method for the evaluation of insulin resistance.

As a result, vitamin D deficiency/insufficiency may be accompanied by metebolic-endocrine disorders. HOMA-IR should be evaluated in these individuals, especially if abdominal obesity is present. Lifestyle changes to those with insulin resistance and metformin or pioglitazone treatment as well as the provision of vitamin D supplementation can be a reliable, cost effecttive method of preventing the development of DM and/or metabolic syndrome.

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Authors have nothing to declare.

References:

1. James WP. 22nd Marabou Symposium: the changing faces of vitamin D. Nutr Rev 2008;66(5):286-90 (PMID: 18454815).
2. Holick MF. Vitamin D deficiency. N Engl J Med 2007;357:266-81 (PMID: 17634462).
3. Wacker M, Holick MF. Vitamin D effects on skeletal and extraskeletal health and the need for supplementation. Nutrients 2013; 5(1):111-48 (PMID: 23306192).
4. Hossein-nezhad A, Holick MF. Vitamin D for health: a global perspective. Mayo Clin Proc 2013;88(7):720-55 (PMID: 23790 560).
5. Pazirandeh S, Burns DL. Overview of vitamin D. In: UpToDate, Waltham, MA. Motil KJ, Drezner MK (Eds). https://www.uptodate.com/contents/overview-of-vitamin-d. Accessed on: Aug 5, 2018.
6. Dawson-Hughes B. Vitamin D deficiency in adults: definition, clinical manifestations, and treatment. In: UpToDate, Waltham, MA. Drezner MK, Rosen CJ (Eds). https://www.uptodate.com/contents/vitamin-d-deficiency-in-adults-definition-clinical-manifestations-and-treatment. Accessed on: Aug 5, 2018.
7. Catena C1, Giacchetti G, Novello M, Colussi G, Cavarape A, Sechi LA. Cellular mechanisms of insulin resistance in rats with fructose-induced hypertension. Am J Hypertens 2003;16(11 Pt 1): 973-8 (PMID: 14573337).
8. Teegarden D, Donkin SS. Vitamin D: emerging new roles in insulin sensitivity. Nutr Res Rev 2009;22(1):82-92 (PMID: 19555 519).
9. Alvarez JA, Ashraf A. Role of vitamin D in insulin secretion and insulin sensitivity for glucose homeostasis. Int J Endocrinol 2010:351385 (PMID: 20011094).

10. Kayaniyil S, Vieth R, Retnakaran R, et al. Association of vitamin D with insulin resistance and beta-cell dysfunction in subjects at risk for type 2 diabetes. Diabetes Care 2011;33(6):1379-81 (PMID: 20215450).

11. Mitri J, Dawson-Hughes B, Hu FB, Pittas AG. Effects of vitamin D and calcium supplementation on pancreatic β cell function, insulin sensitivity, and glycemia in adults at high risk of diabetes: the Calcium and Vitamin D for Diabetes Mellitus (Ca DDM) randomized controlled trial. Am J Clin Nutr 2011;94(2): 486-94 (PMID: 21715514).

12. Chiu KC, Chu A, Go VL, Saad MF. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. Am J Clin Nutr 2004;79(5):820-5 (PMID: 15113720).

13. Sung CC, Liao MT, Lu KC, Wu CC. Role of Vitamin D in insulin resistance. J Biomed Biotechnol 2012;2012:634195 (PMID: 22988423).

14. Forouhi NG, Luan J, Cooper A, Boucher BJ, Wareham NJ. Baseline serum 25-hydroxy vitamin D is predictive of future glycemic status and insulin resistance: the Medical Research Council Ely Prospective Study 1990-2000. Diabetes 2008;57(10):2619-25 (PMID: 18591391).

15. Jackson JL, Judd SE, Panwar B, et al. Associations of 25-hydroxyvitamin D with markers of inflammation, insülin resistance and obesity in black and white community-dwelling adults. J Clin Transl Endocrinol 2016;5:21-5 (PMID: 27833859).

16. Song BM, Kim HC, Choi DP, Oh SM, Suh I. Association between serum 25-hydroxyvitamin D level and insülin resistance in a rural population. Yonsei Med J 2014;55(4):1036-41 (PMID: 24954334).

17. Bourlon PM, Billaudel B, Faure-Dussert A. Influence of vitamin D3 deficiency and 1,25 dihydroxyvitamin D3 on de novo insulin biosynthesis in the islets of the rat endocrine pancreas. J Endocrinol 1999;160(1):87-95 (PMID: 9854180).

18. Norman AW, Frankel JB, Heldt AM, Grodsky GM. Vitamin D deficiency inhibits pancreatic secretion of insulin. Science 1980; 209:823-5 (PMID: 6250216).

19. Tanaka Y, Seino Y, Ishida M, et al. Effect of vitamin D3 on the pancreatic secretion of insulin and somatostatin. Acta Endocrinol (Copenh) 1984;105:528-33 (PMID: 6144227).

20. Cade C, Norman AW. Vitamin D3 improves impaired glucose tolerance and insulin secretion in the vitamin D-deficient rat *in vivo*. Endocrinology 1986;119:84-90 (PMID: 3013599).

21. Clark SA, Stumpf WE, Sar M. Effect of 1,25 dihydroxyvitamin D3 on insulin secretion. Diabetes 1981;30:382-6 (PMID: 7014306).

22. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18: 499-502 (PMID: 4337382).

23. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Teacher DF, Turner RC. Homeostasis model assessment: insulin resistance and b cell function from fasting plasma glucose and insulin concentration in man. Diabetologia 1985;28:412-9 (PMID: 3899825).

24. Ford ES, Ajani UA, McGuire LC, Liu S. Concentrations of serum vitamin D and the metabolic syndrome among U.S. adults. Diabetes Care 2005;28(5):1228-30 (PMID: 15855599).

25. Lima-Martinez MM, Arrau C, Jerez S, et al. Relationship between the Finnish Diabetes Risk Score (FINDRISC), vitamin D levels, and insulin resistance in obese subjects. Prim Care Diabetes 2017;11(1):94-100 (PMID: 27914905).

26. Cefalo CMA, Conte C, Sorice GP, et al. Effect of vitamin D supplementation on obesity-induced insulin resistance: a double-blind, randomized, placebo-controlled trial. Obesity (Silver Spring), 2018;26(4):651-7 (PMID: 29504254).

27. Need AG, O'Loughlin PD, Horowitz M, Nordin BE. Relationship between fasting serum glucose, age, body mass index and serum 25 hydroxyvitamin D in postmenopausal women. Clin Endocrinol (Oxf) 2005;62(6):738-41 (PMID: 15943837).

28. Tamadon MR, Soleimani A, Keneshlou F, et al. Clinical trial on the effects of vitamin d supplementation on metabolic profiles in diabetic hemodialysis. Horm Metab Res 2018;50(1):50-5 (PMID: 28958110).

29. Minambres I, Sanchez-Quesada JL, Vinagre I, et al. Hypovitaminosis D in type 2 diabetes: relation with features of the metabolic syndrome and glycemic control. Endocr Res 2015;40(3): 160-5 (PMID: 25536005).

30. Hoseini SA, Aminorroaya A, Iraj B, Amini M. The effects of oral vitamin D on insulin resistance in pre-diabetic patients. J Res Med Sci 2013;18(1):47-51 (PMID: 23900423).

31. Tai K, Need AG, Horowitz M, Chapman IM. Glucose tolerence and vitamin D: effects of treating vitamin D deficiency. Nutrition 2008;24(10):950-6 (PMID: 18653316).