Evaluation of the relationship between vitamin D level and adropin, IL-1β, IL-6, and oxidative status in women

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Background/aim: Vitamin D, adropin, proinflammatory cytokines, and oxidative stress closely related with metabolic homeostasis and endothelial dysfunction. The aim of the present study is to investigate how vitamin D levels affect serum adropin, IL-1ß, IL-6, and oxidative stress.

Materials and methods: A total of 77 female subjects were divided into 3 groups according to vitamin D levels. Biochemical parameters, adropin, IL-1ß, IL-6, oxidative stress markers were studied in these groups, and the results were compared statistically.

Results: Serum adropin, IL-1ß, IL-6, total oxidant status (TOS) and total antioxidant status (TAS) and oxidative stress index (OSI) levels differed significantly between the vitamin D groups (p < 0.05). A significant positive correlation was detected between vitamin D, and adropin and TAS (r = 0.807; p < 0.001, r = 0.814; p < 0.001, respectively). A significant negative correlation was detected between vitamin D, and IL-1ß, IL-6, TOS, OSI (r = –0.725; p < 0.001, r = –0.720; p < 0.001, r = –0.238; p = 0.037, r = –0.705; p < 0.001, respectively).

Conclusions: Vitamin D could show its effects through vitamin D receptors on tissues or on the ENHO gene in adropin secreting tissues via direct or indirect mechanisms. Proinflammatory cytokines, oxidative stress, and adropin targeted studies could contribute to the prevention and treatment of diseases associated with vitamin D deficiency in future.

Key words: Vitamin D, adropin, IL-1 beta, IL-6, oxidative stress

1. Introduction

While vitamin D is mainly synthesized in the skin under the influence of sunlight in humans, a small portion is obtained from foods. Vitamin D mainly regulates calcium, phosphorus and bone metabolism, and is also known as an immunomodulator hormone [1–3]. Vitamin D deficiency might be due to factors including poor sunlight exposure, insufficient intake of vitamin-containing foods and malabsorption syndromes (Crohn's disease and Celiac disease). Vitamin D has been shown to exert its physiological effects via vitamin D receptors (VDR) present in many tissues and organs [1,4]. Being a worldwide health issue, vitamin D deficiency and insufficiency was associated with many illnesses including osteoporosis, metabolic syndrome, cardiovascular diseases, diabetes, hypertension, endothelial dysfunction, infectious diseases, autoimmune disorders, cancers, and neurological disorders [1,2,5].

Adropin is a peptide hormone coded by the energy homeostasis-associated gene (ENHO) mainly in the liver and brain. It is closely related to glucose and lipid homeostasis and insulin resistance. It is produced in many tissues and organs such as the liver, brain, heart, kidney, pancreas, and gastrointestinal system [6–9]. Adropin has been determined to increase NO release by stimulating endothelial nitric oxide synthase (eNOS) via the interactions between intracellular pathways, improving endothelial cell functions, and protecting the cardiovascular system by providing neovascularization [7,10]. Many studies have reported that serum adropin levels might be associated with obesity, diabetes mellitus, hypertension, cardiovascular diseases, endothelial dysfunction, metabolic syndrome, and several cancers [10–13].

Oxidative stress (OS) is a result of impaired balance between the reactive oxygen species (ROS) formation and antioxidant defense mechanisms. Free radical

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production occurs continuously in all cells as a part of normal cellular functioning. High amounts of free radicals and ROS formed in tissues due to OS interact with intracellular molecules and cause cellular damage by injuring various biological molecules including proteins, lipids, and nucleic acids [14,15]. Excessive endogenous or exogenous free radical production might have a role in many illnesses. Antioxidants prevent free radical-induced tissue damage by preventing the production of or removing the free radicals. While total oxidant status (TOS) reflects the total effect of all oxidants present in plasma and body fluids, total antioxidant status (TAS) reflects the total effect of all antioxidants. Measurement of TOS or TAS is preferred to individual measurements of serum (or plasma) concentrations of different oxidant or antioxidant molecules [16,17]. OS increase leads to the development of metabolic syndrome, endothelial dysfunction, hypertension, diabetes mellitus, cardiovascular diseases, cancer, and kidney and neurological diseases [14,15,18–21].

Interleukin-1 beta (IL-1β) and interleukin-6 (IL-6) are proinflammatory cytokines especially produced by myelomonocytic cells such as monocytes, macrophages, and dendritic cells. They can be the pathogenic precursor to autoinflammatory, autoimmune, infectious, and degenerative diseases by affecting many cells and organs [22,23]. Moreover, they are associated with endothelial dysfunction, metabolic syndrome, insulin resistance, hypertension, diabetes mellitus, oxidative stress, and cardiovascular diseases [18,24–26].

Many chronic illnesses including endothelial dysfunction, diabetes mellitus, cardiovascular diseases, malignancy, autoimmune disorders which, as shown in the studies, were associated with vitamin D were also similarly associated with adropin, IL-1β, IL-6 and oxidative stress. In the literature search we performed, there were limited number of studies individually evaluating the relationship between vitamin D level, and oxidative status or proinflammatory cytokines (IL-1β,IL-6) in some diseases, however, there was no study evaluating the relationship between vitamin D and adropin or evaluating these parameters together. We aimed to evaluate the relationship between the levels of vitamin D and adropin, IL-1β, IL-6 and oxidative status, which, we think might affect the pathological and metabolic conditions seen in vitamin D deficiency.

2. Materials and methods

2.1. Study group
This cross-sectional, case-controlled trial was initiated after approval of Bezmialem Foundation University, ethics committee. Our study included 77 female subjects aged 18 to 65 years who consulted to Bezmialem Foundation University, Internal Diseases Outpatient Clinic between July 2020 and December 2020, did not have a known history of chronic illness, were not pregnant or lactating, did not have a history of surgical operation within the last 6 months, did not use antioxidant medication, vitamin supplement, lipid-lowering agent, tobacco or alcohol, did not use vitamin D within the last 3 months, did not do heavy exercise recently and have normal C-reactive protein (CRP) levels. All study subjects gave written informed consent. The subjects were divided into three groups by their vitamin D level (G1: Vitamin D < 20 ng/mL, deficiency; G2: Vitamin D = 20–30 ng/mL, insufficiency; G3: Vitamin D > 30 ng/mL, normal) [5].

2.2. Blood assay
Venous blood samples were taken from all subjects into gel tubes between 8:00 a.m. and 9:00 a.m. following 12 h of fasting for biochemical parameters, adropin, TAS, TOS, IL-1β and IL-6 tests, and centrifuged at 3600 rpm for 10 min and the sera were separated. The sera of all subjects were transferred into Eppendorf tubes and kept at –80 °C until the study day.

All volunteers underwent a thorough physical examination with their height and weight being recorded. Weight and height were rounded to the nearest kg and cm, respectively, and body mass index (BMI) was calculated [BMI = weight/(height)^2].

2.2.1. Measurement of total antioxidant status and total oxidant status (TAS and TOS) in serum
Total oxidant status and total antioxidant status were determined by a recently developed method, colorimetric assay [16]. The TAS results are expressed in mmol Trolox Equiv./L., and TOS in μmol H_2O_2 Equiv./L. Coefficients of variation values were less than 10%.

2.2.2. Oxidative stress index (OSI) determination
OSI was determined as TOS/TAS ratio (resulting TAS unit was changed to μmol/L) and calculated as follows: OSI (arbitrary unit) = TOS, μmol H_2O_2 Equiv./L/TAS, and mmol Trolox Equiv./L [27].

2.2.3. Measurement of Adropin, IL-1β, and IL-6
Concentrations of adropin, IL-1β, and IL-6 in the serum were measured by a specific commercial ELISA kit according to the manufacturer's instructions (Adropin: E3231Hu, IL-1β: E0143Hu, IL-6: E0090Hu–Bioassay Technology Laboratory, China). Concentrations were determined with a spectrophotometric microtiter plate reader (Varioskan Flash Multimode Reader, Thermo, Waltham, USA) at 450 nm optical density [27].

2.3. Statistical analysis
IBM SPSS (Statistical Package for Social Sciences) statistics 22.0 software was used for the statistical analyses for the study. While evaluating the study data, descriptive statistical methods (mean, standard deviation, median, frequency) were used. Skewness and kurtosis values were used together with the Shapiro-Wilk test to evaluate the normal distribution of the data. While the one-way ANOVA test

was used to compare more than two normally distributed variables, the Kruskal Wallis test was used to evaluate more than two nonnormally distributed variables. Tukey and Games Howell tests were used for post-hoc pairwise comparison of the parameters that were significant after ANOVA and the Kruskal Wallis test. For the assessment of correlation between the data, Pearson's correlation analysis was used for the normally distributed data and Spearman's correlation analysis for the nonnormally distributed data. Results were evaluated within a 95% confidence interval with significance at a p level of <0.05.

3. Results

The mean age of the 77 women participating in the study was 33.79 ± 10.15 years and the mean BMI (body mass index) was 24.90 ± 5.20 kg/m2. The mean age of the groups that we divided into three groups according to their vitamin D levels (G1: Vitamin D < 20 ng/mL; G2:Vitamin D 20–30 ng/mL; G3:Vitamin D > 30 ng/mL) was G1:32.35 ± 10.67, G2:35.14 ± 10.85, and G3:35.41 ± 7.84 were determined. The mean BMI of the three groups was G1:24.41 ± 5.26, G2:26.70 ± 5.79, and G3:23.79 ± 3.81. There was no statistically significant difference between the groups in terms of age and BMI (p: 0.459, p: 0.163, respectively) (Table 1).

There was no statistically significant difference between three groups in terms of glucose, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TC), triglyceride (TG), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), calcium, magnesium, phosphorus, thyroid stimulating hormone (TSH) and free tetraiodothyronine (fT4) (p > 0.05). As for adropin, IL-1β, IL-6, TOS, TAS, OSI, and parathyroid hormone (PTH) levels, a statistically significant difference was detected between the groups (p < 0.05) (Table 1).

In the correlation assessment between vitamin D, and age, BMI, adropin, IL-1β, IL-6, TOS, TAS, OSI and other biochemical parameters, significant positive correlation was detected between vitamin D, and adropin and TAS (r = 0.807; p < 0.001, r = 0.814; p < 0.001, respectively). Significant negative correlation was detected between vitamin D, and IL-1β, IL-6, TOS, OSI and PTH (r = –0.725; p < 0.001, r = –0.720; p < 0.001, r = –0.238; p = 0.037, r = –0.705; p < 0.001, r = –0.524; p < 0.001, respectively) (Table 2). Evaluation of the correlation between vitamin D and adropin, IL-1β, IL-6, TOS, TAS and OSI is shown in Figures 1–6.
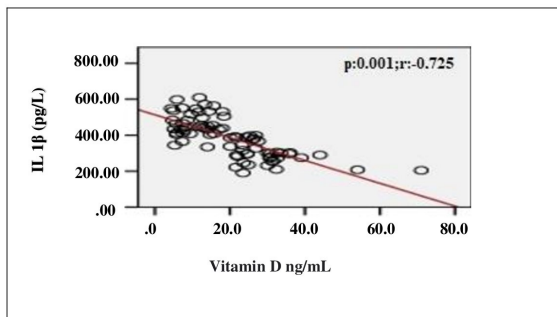

Figure 1. Correlation assessment between vitamin D level, and IL-1β level (n = 77, total subjects). r: Correlation coefficient; IL-1β: Interleukin-1 beta.
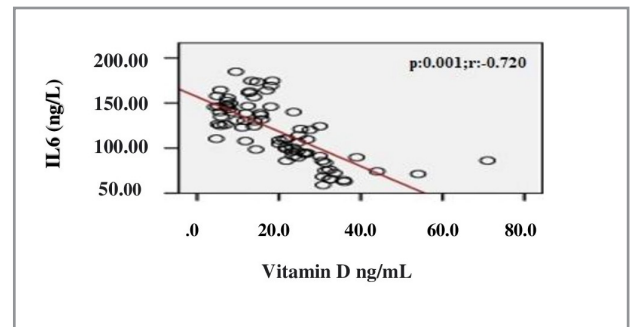

Figure 2. Correlation assessment between vitamin D level and IL-6 level (n = 77, total subjects). r: Correlation coefficient; IL-6: Interleukin-6.
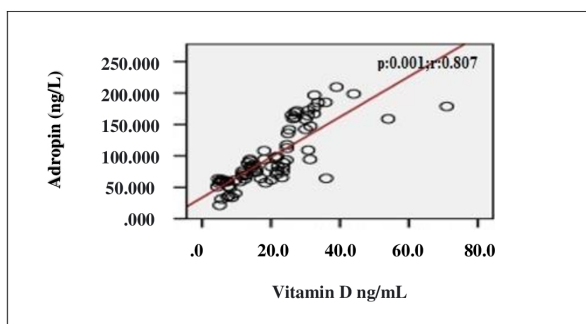

Figure 3. Correlation assessment between vitamin D level and adropin level (n = 77, total subjects). r: Correlation coefficient.
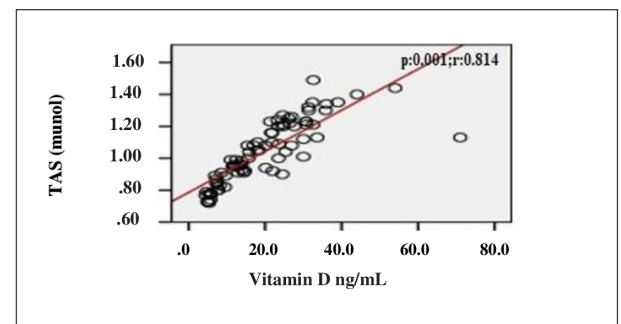

Figure 4. Correlation assessment between vitamin D level and TAS level (n = 77, total subjects). r: Correlation coefficient; TAS: Total antioxidant status.

In the correlation assessment between adropin, and vitamin D, IL-1β, IL-6, TOS, TAS, OSI and PTH, statistically significant positive correlation was detected between adropin and TAS (r = 0.753; p < 0.001), and statistically significant negative correlation was detected between adropin, and IL-1β, IL-6, TOS, OSI and PTH (r = −0.586; p < 0.001, r = −0.659; p < 0.001, r = −0.292; p < 0.010, r = −0.673; p < 0.001, r = −0.511; p < 0.001, respectively) (Table 3). Additionally, comparison of post-hoc analyzes and paired groups is shown in Table 4.

Table 1. Comparison of age, BMI, adropin, IL-1β, IL-6, TOS, TAS, OSI and biochemical parameters of the vitamin D groups.

	Group 1 Vitamin D 20 ng/mL n = 39 Mean ± SD	Group 2 Vitamin D 20–30 ng/mL n = 21 Mean ± SD	Group 3 Vitamin D 30 ng/mL n = 17 Mean ± SD	p
Age (years)	32.35 ± 10.67	35.14 ± 10.85	35.41 ± 7.84	**0.459**
BMI (kg/m^2)	24.41 ± 5.26	26.70 ± 5.79	23.79 ± 3.81	**0.163**
Adropin (ng/L)	64.34 ± 19.08	109.92 ± 37.81	159.35± 38.65	**<0.001***
IL-1β (pg/L)	469.65 ± 67.36	326.67 ± 64.32	268.20 ± 35.25	**<0.001***
IL-6 (ng/L)	143.98 ± 19.46	104.23 ± 12.74	77.41 ± 15.57	**<0.001***
TOS (μmol)	14.14 ± 1.11	13.95 ± 1.63	13.08 ± 1.66	**0.037***
TAS (mmol)	0.89 ± 0.10	1.13 ± 0.11	1.26 ± 0.12	**<0.001***
OSI (AU)	15.95 ± 2.25	12.59 ± 2.69	10.53 ± 2.42	**<0.001***
Glucose (mg/dL)	91.41 ± 6.52	91.52 ± 6.75	88.47 ± 5.37	**0.240**
Creatinine (mg/dL)	0.70 ± 0.68	0.71 ± 0.52	0.72 ± 0.50	**0.770**
AST (U/L)	17.59 ± 6.51	18.57 ± 5.18	17.11 ± 4.15	**0.716**
ALT (U/L)	16.12 ± 9.48	17.95 ± 9.54	15.47 ± 8.47	**0.677**
ALP (U/L)	62.25 ± 18.40	56.95 ± 17.99	61.94 ± 23.94	**0.586**
GGT (U/L)	16.59 ± 11.38	15.38 ± 7.59	14 ± 4.71	**0.626**
TCholesterol (mg/dL)	179.87 ± 43.13	185.38 ± 34.32	195.94 ± 46.03	**0.417**
Triglyceride (mg/dL)	80.69 ± 43.70	74 ± 28.13	70.52 ± 23.55	**0.585**
LDL-C (mg/dL)	105.10 ± 33.83	104.37 ± 29.34	119.75 ± 36.22	**0.269**
HDL-C(mg/dL)	58.33 ± 11.58	64.37 ± 12.95	59.71 ± 17.37	**0.252**
Ca (mg/dL)	9.42 ± 0.39	9.34 ± 0.40	9.42 ± 0.33	**0.743**
P (mg/dL)	3.64 ± 0.57	3.59 ± 0.35	3.59 ± 0.39	**0.886**
Mg (mg/dL)	1.95 ± 0.14	1.92 ± 0.11	1.94 ± 0.13	**0.757**
PTH (ng/L)	68 ± 19.57	55.16 ± 20.19	38.16 ± 7.11	**<0.001***
TSH (mU/L)	1.78 ± 0.94	1.53 ± 0.52	1.43 ± 0.51	**0.216**
FT4 (pg/mL)	12.38 ± 1.19	12.37 ± 1.40	12.37 ± 0.83	**0.999**

Mean ± SD: Mean ± standard deviation; BMI: Body mass index; IL-1β: Interleukin-1 beta; IL-6: Interleukin-6; TOS: Total oxidant status; TAS: Total antioxidant status; OSI: Oxidative stress index; AU: Arbitrary unit; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma glutamyl transferase; TCholesterol: Total Cholesterol; LDL-C: Low-density lipoprotein Cholesterol; HDL-C: High density lipoprotein Cholesterol; Ca: Calcium; P: Phosphorus; Mg: Magnesium; PTH: Parathormone; TSH: Thyroid stimulating hormone; FT4: Free tetraiodothyronine; *Statistical significance (p <0.05).
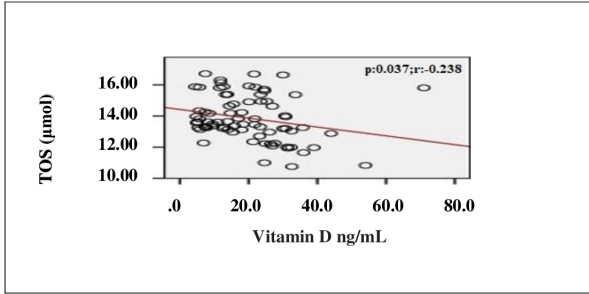
Figure 5. Correlation assessment between vitamin D level and TOS level (n = 77, total subjects). r: Correlation coefficient; TOS: Total oxidant status.
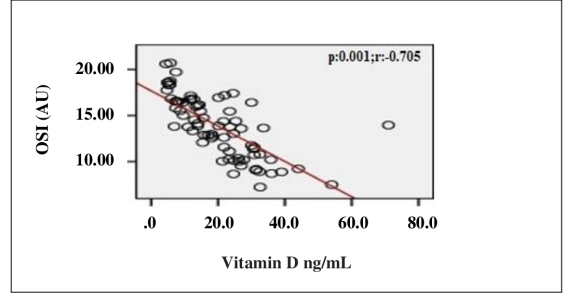

Figure 6. Correlation assessment between vitamin D level and OSI level (n = 77, total subjects). r: Correlation coefficient; OSI: Oxidative stress index.

Table 2. Correlation assessment between vitamin D, and age, BMI, adropin, IL-1β, IL-6, TOS, TAS, OSI and biochemical parameters (n = 77, total subjects).

	r	p
Age (years)	−0.006	0.958
BMI (kg/m²)	−0.064	0.581
Adropin (ng/L)	0.807	<0.001*
IL-1β (pg/L)	−0.725	<0.001*
IL-6 (ng/L)	−0.720	<0.001*
TOS (μmol)	−0.238	0.037*
TAS (mmol)	0.814	<0.001*
OSI (AU)	−0.705	<0.001*
PTH (ng/L)	−0.524	<0.001*
Calcium (mg/dL)	0.015	0.894

r: Correlation coefficient; BMI: Body mass index; IL-1β: Interleukin-1 beta; IL-6: Interleukin-6; TOS: Total oxidant status; TAS: Total antioxidant status; OSI: Oxidative stress index; AU: Arbitrary unit; PTH: Parathormone; *Statistical significance (p <0.05).

Table 3. Correlation assessment between adropin level, and Vitamin D, IL-1β, IL-6, TOS, TAS, OSI and biochemical parameters (n = 77, total subjects).

	R	p
Vitamin D	0.807	<0.001*
IL-1β (pg/L)	−0.586	<0.001*
IL-6 (ng/L)	−0.659	<0.001*
TOS (μmol)	−0.292	0.010*
TAS (mmol)	0.753	<0.001*
OSI (AU)	−0.673	<0.001*
PTH (ng/L)	−0.511	<0.001*

r: Correlation coefficient; IL-1β: Interleukin-1 beta; IL-6: Interleukin-6; TOS: Total oxidant status; TAS: Total antioxidant status; OSI: Oxidative stress index; AU: Arbitrary unit; PTH: Parathormone; *Statistical significance (p < 0.05).

Table 4. Comparison of post-hoc analyzes and pairwise groups.

	G 1	G2	G3	G1 vs. G2	G1 vs. G3	G2 vs. G3
	Vitamin D < 20 ng/mL **n: 39** **Mean ± SD**	Vitamin D 20–30 ng/mL **n: 21** **Mean ± SD**	Vitamin D >30 ng/mL **n: 17** **Mean ± SD**	**P Values**	**P values**	**P values**
Adropin (ng/L)	64.34 ± 19.08	109.92 ± 37.81	159.35 ± 38.65	<0.001	<0.001	<0.001
IL-1 β (pg/L)	469.65 ± 67.36	326.67 ± 64.32	268.20 ± 35.25	<0.001	<0.001	0.012
IL-6 (ng/L)	143.98 ± 19.46	104.23 ± 12.74	77.41 ± 15.57	<0.001	<0.001	<0.001
TOS (µmol)	14.14 ± 1.11	13.95 ± 1.63	13.08 ± 1.66	0.868	0.030	0.148
TAS (mmol)	0.89 ± 0.10	1.13 ± 0.11	1.26 ± 0.12	<0.001	<0.001	0.001
OSI (AU)	15.95 ± 2.25	12.59 ± 2.69	10.53 ± 2.42	<0.001	<0.001	0.029

G1: Group 1, Vitamin D < 20 ng/mL; G2: Group 2, Vitamin D 20–30 ng/mL; G3: Group 3, Vitamin D > 30 ng/mL; IL-1β: Interleukin-1 beta; IL-6: Interleukin-6; TOS: Total oxidant status; TAS: Total antioxidant status; OSI: Oxidative stress index; AU: Arbitrary unit; *Statistical significance (p < 0.05).

4.Discussion

While vitamin D is mainly synthesized in the skin under the influence of sunlight in humans, a small portion is obtained from foods. Vitamin D mainly regulates calcium, phosphorus and bone metabolism, and is also known as an immunomodulator hormone [1–3]. In the literature search, we did not find any study evaluating the relationship between vitamin D level and adropin level.

Our study detected significant difference between the vitamin D groups (G1, G2, G3) in terms of adropin level, and also, a significant positive correlation between vitamin D level and adropin level (p < 0.001). Adropin has been reported to have lipid and glucose homeostasis-regulating, angiogenesis and antiinflammatory effects in addition to preventive effects against insulin resistance and endothelial dysfunction [6,9,10,28,29]. Lovren et al. stated that adropin might have a role in controlling the functions of endothelial cells and protecting the endothelial cells against TNFα-induced apoptosis. They explained this effect of adropin by NOS increase due to increased expression of eNOS via vascular endothelial growth factor receptor (VEGFR2)-phosphatidylinositol 3-kinase-Akt (PI3K-Akt) or VEGFR2-extracellular signal-regulated kinase 1/2 (ERK 1/2) intracellular signal transmission pathways [7,10]. In their study comparing serum adropin levels of a total of 116 type 2 diabetes mellitus (T2DM) patients and 60 control subjects with normal glucose tolerance, Zang H. et al. detected that serum adropin level is lower in T2DM patients, and especially in overweight/obese individuals (compared to the group with normal weight). In the study, it was stated that adropin which is related with glucolipid homeostasis and insulin sensitivity

might have a role in the pathogenesis of T2DM [30]. Topuz M et al. compared the serum adropin levels of the groups of subjects with or without endothelial dysfunction using brachial flow-mediated dilation in patients with T2DM. Serum adropin level was detected to be significantly lower in subjects with endothelial dysfunction compared to controls, and it was stated that low adropin level might be a marker of endothelial dysfunction [29]. Plasma adropin levels of individuals with primary hypertension and normotensive individuals were studied in another study. Plasma adropin levels were detected to be significantly lower in the hypertensive group compared to controls, and it was stated that low adropin level might be associated with hypertension [31]. In their study of two groups consisting of individuals with and without CAD, Zhang C et al. found that serum adropin levels are significantly lower in the CAD group compared to controls [32]. Similar to the studies in the literature, in our study, we believe that low serum adropin levels observed in subjects with vitamin D deficiency might trigger the development and progression of endothelial dysfunction, insulin resistance, hypertension and cardiovascular diseases. We believe that the low adropin level in vitamin D deficiency might be caused by the stimulation level of vitamin D receptors in tissues synthesizing adropin and vitamin D affecting the expression of ENHO gene via direct or indirect mechanisms. More studies are needed to illuminate these possible mechanisms.

In our study, significant difference was detected between vitamin D groups in terms of IL-1β and IL-6 levels, and also a significant negative correlation between vitamin D level, and IL-1β and IL-6 levels (p < 0.001).

Proinflammatory cytokines (IL-1β, IL-6) have multiple effects on many events including the initiation and maintenance of inflammation, endothelial dysfunction, metabolic syndrome, insulin resistance, diabetes mellitus, oxidative stress and cardiovascular events [18,24–26]. It has been stated that vitamin D might play an important role in the modulation of immune/inflammation system by regulating the production of inflammatory cytokines and inhibiting the proliferation of the proinflammatory cells [33]. In their study comparing coronary heart disease (CHD) patients with their control subjects, Liu Y et al. found that vitamin D level is significantly low in CHD, and detected a significant negative correlation between vitamin D, and IL-1β and IL-6. It has been stated that vitamin D deficiency might induce and aggravate CHD by increasing inflammation via NF-κB [34]. In their study on 60 healthy controls and 106 (59 males, 47 females) T2DM patients, Wang W et al. divided T2DM patients into three groups by vitamin D levels (Vitamin D ≤ 20 ng/mL, 20–30 ng/mL and ≥30 ng/mL). They detected that vitamin D level is considerably lower in T2DM patients compared to healthy controls, and has a negative correlation with IL-1β and IL-6 [35]. On the other hand, in their study in healthy women, Azizieh F. et al. divided the participants into 2 groups by vitamin D levels [as >25 nmol/L (10 ng/mL) and <25 nmol/L]. They did not detect a direct significant relationship between serum vitamin D level, and inflammatory markers, IL-1β and IL-6 [36]. Moreover, Peterson C. A. et al. did not detect a significant relationship and correlation between vitamin D concentrations and IL-6 in women exposed to ultraviolet light (UVB) and divided into two groups as high and low vitamin D level [37]. In their study in type 2 diabetic patients, El Hajj C. et al. did not detect a significant decrease in IL-6 levels following vitamin D replacement treatment compared to prereplacement levels [38]. The results of studies by Liu Y. and Wang W et al. were similar to our results. On the other hand, the results of studies by Azizieh F, Peterson C.A. and El Hajj C. et al. were different from our results. We think that this might be because of the fact that the patient groups in the studies were different in terms of vitamin D level. Accordingly, it suggests that as vitamin D deficiency deepens, the secretion of antiinflammatory cytokines will decrease or the secretion of proinflammatory cytokines will increase leading to worsening of inflammation. Furthermore, it shows that vitamin D can exert its antiinflammatory effects through immune cells by lowering IL-1β and IL-6 levels.

There was a significant negative correlation between vitamin D, and adropin level and oxidative stress marker, TOS, and a significant positive correlation with TAS (p < 0.05). Antioxidants prevent or remove excessive endogenous or exogenous free radical production leading to prevention of free radical-induced cellular damage. The increased oxidative stress as a result of the shift in this balance towards the oxidative stress was detected to play a role in the pathogenesis of various conditions including metabolic syndrome, endothelial dysfunction, diabetes mellitus, hypertension, cardiovascular diseases, malignancy, and kidney and neurological diseases [14,15,18–20]. In their study in patients with vitamin D deficiency and healthy controls, Baser H. et al. detected significant increase in TAS level and significant decrease in TOS level following vitamin D replacement. Moreover, a significant positive correlation was found between vitamin D level TAS, and no significant correlation with TOS [39]. In another study, calcium plus vitamin D supplement for 8 weeks to overweight women with vitamin D deficiency and PCOS has been detected to have beneficial effects on inflammatory factor and biological markers of oxidative stress [40]. In their in vitro study comparing nonenzymatic antioxidants (Vitamin E, melatonin and beta-estradiol) with vitamin D, Lin AM. et al. showed that vitamin D has considerably high antioxidant effect [41]. In a study on the effect of vitamin D on decreasing oxidative stress in diabetes mellitus, vitamin D combined with calcium has been shown to be beneficial in reducing oxidative stress in rats with streptozotocin-induced diabetes [42]. In their study comparing the plasma levels of several enzymatic or nonenzymatic antioxidants in 55 diabetic patients and 40 healthy control subjects, Ramakrishna V. et al. demonstrated that plasma antioxidant levels are considerably lower in patients with DM [43]. Adropin has also been demonstrated to have possible effects on oxidative stress. In their study on the brains of young and older rats, Yang C. et al. suggested that adropin level has negative correlation with endothelial dysfunction and oxidative damage markers, thereby, adropin loss in the brain might play a role in the pathogenesis and development of age-related cerebrovascular dysfunction [44]. Similar to the other studies, our study shows that the low level of vitamin D and adropin changes the oxidative/antioxidative balance in favor of oxidative status (high TOS, low TAS). Increased oxidative stress in vitamin D deficiency might be because of reduced synthesis of NO due to both reduced antioxidant effect of vitamin D and low adropin level. Furthermore, we think that increased oxidative stress might have a trigger role in vitamin D deficiency being a risk factor for many chronic illnesses and metabolic disorders.

This study has several limitations. First, the number of patients included is relatively low and it is a single-center study, and second, it is a female-only study. We think that the strength of our study is the combined evaluation of several parameters related with each other.

5. Conclusions

Significantly decreased adropin levels and significantly increased levels of proinflammatory cytokines (IL-1β, IL-6) were detected in vitamin D deficiency with the oxidative/antioxidative balance being changed in favor of oxidative status. Vitamin D could show its effects through vitamin D receptors on tissues via direct or indirect mechanisms. In addition, it may affect adropin release with positive or negative effects on the ENHO gene in adropin secreting tissues. Proinflammatory cytokines, oxidative stress, and adropin targeted studies could contribute to the prevention and treatment of diseases associated with vitamin D deficiency in future. Larger studies are needed to confirm these results.

Informed consent
This study was approved by the Bezmialem Vakıf University Ethics Committee with the approval no. 713006642-050.01.04-

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