Turkish Journal of Medical Sciences

Volume 52 | Number 4 Article 39

1-1-2022

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

MEHMET ZORLU

ABDÜSSELAM ŞEKERCİ

MUHAMMED TUNÇ

ERAY METİN GÜLER

BEDİA GÜLEN

See next page for additional authors

Follow this and additional works at: https://journals.tubitak.gov.tr/medical

 Part of the Medical Sciences Commons

Recommended Citation

ZORLU, MEHMET; ŞEKERCİ, ABDÜSSELAM; TUNÇ, MUHAMMED; GÜLER, ERAY METİN; GÜLEN, BEDİA; KARATOPRAK, CUMALİ; KISKAÇ, MUHARREM; and ÇAKIRCA, MUSTAFA (2022) "Evaluation of the relationship between vitamin D level and adropin, IL-1ß, IL-6, and oxidative status in women," *Turkish Journal of Medical Sciences*: Vol. 52: No. 4, Article 39. https://doi.org/10.55730/1300-0144.5424
Available at: https://journals.tubitak.gov.tr/medical/vol52/iss4/39

This Article is brought to you for free and open access by TÜBİTAK Academic Journals. It has been accepted for inclusion in Turkish Journal of Medical Sciences by an authorized editor of TÜBİTAK Academic Journals. For more information, please contact academic.publications@tubitak.gov.tr.

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

Authors
MEHMET ZORLU, ABDÜSSELAM ŞEKERCİ, MUHAMMED TUNÇ, ERAY METİN GÜLER, BEDİA GÜLEN, CUMALİ KARATOPRAK, MUHARREM KISKAÇ, and MUSTAFA ÇAKIRCA

This article is available in Turkish Journal of Medical Sciences: https://journals.tubitak.gov.tr/medical/vol52/iss4/39

TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2022) 52: 1197-1206
© TÜBİTAK
doi:10.55730/1300-0144.5424

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

Mehmet ZORLU[1,*], Abdüsselam ŞEKERCİ[1], Muhammed TUNÇ[1], Eray Metin GÜLER[2], Bedia GÜLEN[3], Cumali KARATOPRAK[1], Muharrem KISKAÇ[1], Mustafa ÇAKIRCA[1]

[1]Department of Internal Medicine, Faculty of Medicine, Bezmialem Vakıf University, İstanbul, Turkey
[2]Department of Medical Biochemistry, Hamidiye Medical Faculty, University of Health Sciences Turkey, İstanbul, Turkey
[3]Department of Emergency, Faculty of Medicine, İstanbul Medipol University, İstanbul, Turkey

Received: 03.05.2021 • **Accepted/Published Online:** 07.05.2022 • **Final Version:** 10.08.2022

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

* Correspondence: drmehmetzorlu@gmail.com

This work is licensed under a Creative Commons Attribution 4.0 International License.

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.

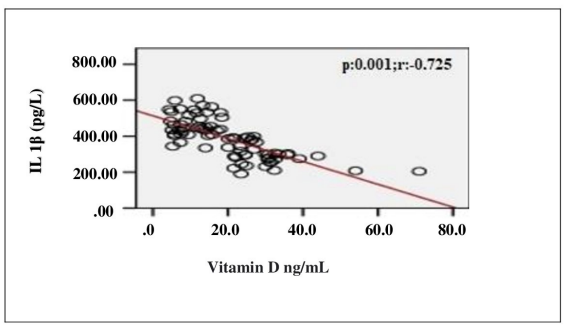


Figure 1. Correlation assessment between vitamin D level, and IL-1β level (n = 77, total subjects). r: Correlation coefficient; IL-1β: Interleukin-1 beta.

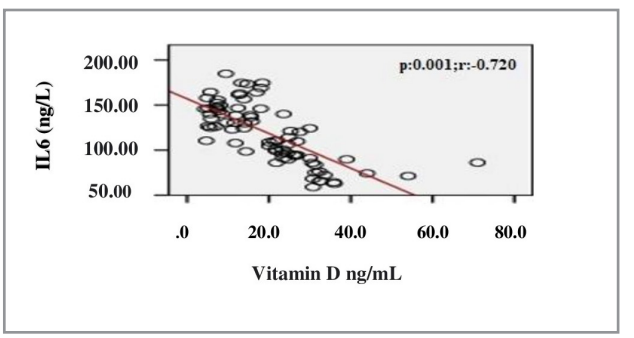


Figure 2. Correlation assessment between vitamin D level and IL-6 level (n = 77, total subjects). r: Correlation coefficient; IL-6: Interleukin-6.

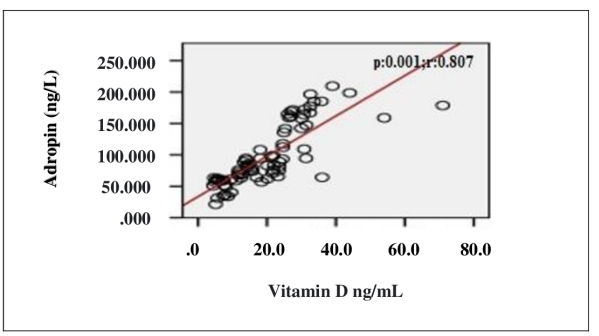


Figure 3. Correlation assessment between vitamin D level and adropin level (n = 77, total subjects). r: Correlation coefficient.

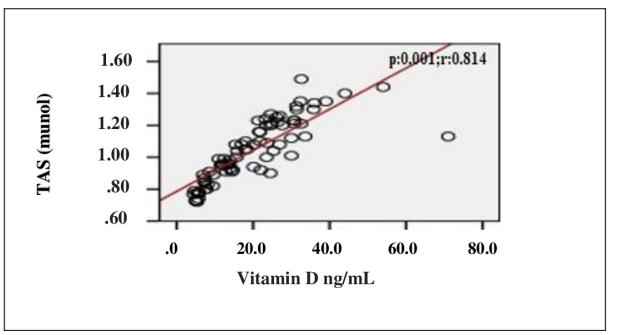


Figure 4. Correlation assessment between vitamin D level and TAS level (n = 77, total subjects). r: Correlation coefficient; TAS: Total antioxidant status.

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).

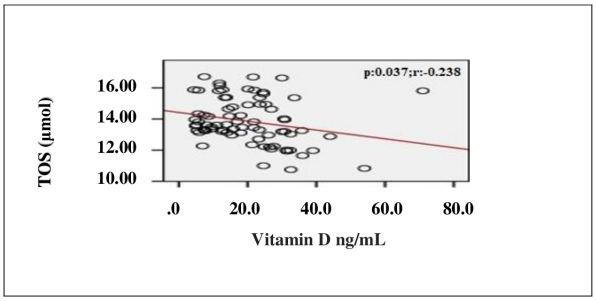


Figure 5. Correlation assessment between vitamin D level and TOS level (n = 77, total subjects). r: Correlation coefficient; TOS: Total oxidant status.

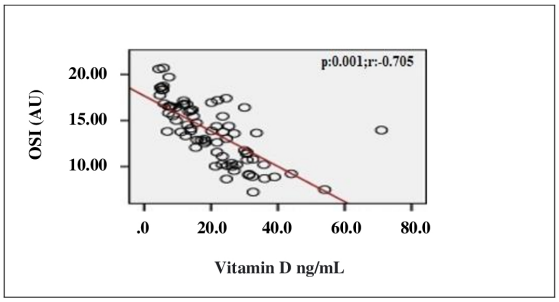


Figure 6. Correlation assessment between vitamin D level and OSI level (n = 77, total subjects). r: Correlation coefficient; OSI: Oxidative stress index.

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/m^2)	–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.

Acknowledgment/disclaimers/conflict of interest

This study received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

The authors declare they have no potential conflict of interest regarding the investigation, authorship, and/or publication of this article.

Informed consent

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

References

1. Kim DH, Meza CA, Clarke H, Kim JS, Hickner RC. Vitamin D and Endothelial Function. Nutrients 2020; 12 (2): 575. https://doi.org/10.3390/nu12020575
2. Charoenngam N, Holick MF. Immunologic Effects of Vitamin D on Human Health and Disease. Nutrients 2020; 12(7): 2097. https://doi.org/10.3390/nu12072097
3. Prietl B, Treiber G, Pieber TR, Amrein K. Vitamin D and immune function. Nutrients 2013; 5 (7): 2502-2521. https://doi.org/10.3390/nu5072502
4. Holick MF. The vitamin D deficiency pandemic: Approaches for diagnosis, treatment and prevention. Reviews in Endocrine and Metabolic Disorders 2017; 18 (2): 153-165. https://doi.org/10.1007/s11154-017-9424-1
5. Holick MF. Vitamin D: a D-Lightful health perspective. *Nutrition Reviews* 2008; 66 (10 Suppl 2): S182-194. https://doi.org/10.1111/j.1753-4887.2008.00104.x
6. Kumar KG, Trevaskis JL, Lam DD, Sutton GM, Koza RA et al. Identification of adropin as a secreted factor linking dietary macronutrient intake with energy homeostasis and lipid metabolism. Cell Metabolism 2008; 8 (6): 468-481. https://doi.org/10.1016/j.cmet.2008.10.011
7. Zhang S, Chen Q, Lin X, Chen M, Liu Q. A Review of Adropin as the Medium of Dialogue between Energy Regulation and Immune Regulation. Oxidative Medicine and Cellular Longevity 2020; 2020: 3947806. https://doi.org/10.1155/2020/3947806
8. Li L, Xie W, Zheng XL, Yin WD, Tang CK. A novel peptide adropin in cardiovascular diseases. Clinica Chimica Acta 2016; 453: 107-113. https://doi.org/10.1016/j.cca.2015.12.010
9. Jasaszwili M, Billert M, Strowski MZ, Nowak KW, Skrzypski M. Adropin as A Fat-Burning Hormone with Multiple Functions-Review of a Decade of Research. Molecules 2020; 25 (3): 549. https://doi.org/10.3390/molecules25204757
10. Lovren F, Pan Y, Quan A, Singh KK, Shukla PC et al. Adropin is a novel regulator of endothelial function. Circulation 2010; 14; 122 (11Suppl): S185-192. https://doi.org/10.1161/circulationaha.109.931782
11. Yosaee S, Soltani S, Sekhavati E, Jazayeri S. Adropin- A Novel Biomarker of Heart Disease: A Systematic Review Article. Iranian Journal of Public Health 2016; 45 (12): 1568-1576.
12. Nergiz S, Altinkaya SO, Kurt Ömürlü İ, Yuksel H, Küçük M et al. Circulating adropin levels in patients with endometrium cancer. Gynecological Endocrinology 2015; 31(9): 730-735. https://doi.org/10.3109/09513590.2015.1065480
13. Oruc CU, Akpinar YE, Dervisoglu E, Amikishiyev S, Salmaslıoglu A et al. Low concentrations of adropin are associated with endothelial dysfunction as assessed by flow-mediated dilatation in patients with metabolic syndrome. Clinical Chemistry Laboratory Medicine. 2017; 55 (1): 139-144. https://doi.org/10.1515/cclm-2016-0329
14. Burton GJ, Jauniaux E. Oxidative stress. Best Practice Research Clinical Obstetrics Gynaecology 2011; 25 (3): 287-299. https://doi.org/10.1016/j.bpobgyn.2010.10.016
15. Young IS, Woodside JV. Antioxidants in health and disease. Journal of Clinical Pathology 2001; 54 (3): 176-186. https:// doi.org/ 10.1136/jcp.54.3.176
16. Erel O. A new automated colorimetric method for measuring total oxidant status. Clinical Biochemistry 2005; 38 (12): 1103-1111. https://doi.org/10.1016/j.clinbiochem.2005.08.008
17. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clinical Biochemistry 2004; 37 (4): 277-285. https://doi.org/10.1016/j.clinbiochem.2003.11.015
18. Srikanthan K, Feyh A, Visweshwar H, Shapiro JI, Sodhi K. Systematic Review of Metabolic Syndrome Biomarkers: A Panel for Early Detection, Management, and Risk Stratification in the West Virginian Population. International Journal of Medical Sciences 2016; 13 (1): 25-38. https://doi.org/10.7150/ijms.13800

19. Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: A review. European Journal of Medicinal Chemistry 2015; 97: 55-74. https://doi.org/10.1016/j.ejmech.2015.04.040

20. Chaudhary P, Pandey A, Azad CS, Tia N, Singh M et al. Association of oxidative stress and endothelial dysfunction in hypertension. Analytical Biochemistry 2020; 590: 113535. https://doi.org/10.1016/j.ab.2019.113535

21. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. The Journal of Clinical Investigation 2004; 114 (12): 1752-1761. https://doi.org/10.1172/JCI21625

22. Murakami M, Kamimura D, Hirano T. Pleiotropy and Specificity: Insights from the Interleukin 6 Family of Cytokines. Immunity 2019; 50 (4): 812-831. https://doi.org/10.1016/j.immuni.2019.03.027

23. Garlanda C, Dinarello CA, Mantovani A. The interleukin-1 family: back to the future. Immunity 2013; 39 (6): 1003-1118. https://doi.org/10.1016/j.immuni.2013.11.010

24. Alexandraki K, Piperi C, Kalofoutis C, Singh J, Alaveras A, et al. Inflammatory process in type 2 diabetes: The role of cytokines. Annals of the New York Academy of Sciences 2006; 1084: 89-117. https://doi.org/10.1196/annals.1372.039

25. Aroor AR, McKarns S, Demarco VG, Jia G, Sowers JR. Maladaptive immune and inflammatory pathways lead to cardiovascular insulin resistance. Metabolism 2013; 62 (11): 1543-1552. https://doi.org/10.1016/j.metabol.2013.07.001

26. Stienstra R, Tack CJ, Kanneganti TD, Joosten LA, Netea MG. The inflammasome puts obesity in the danger zone. Cell Metabolism 2012; 15 (1): 10-18. https://doi.org/10.1016/j.cmet.2011.10.011

27. Guler EM, Gokce M, Bacaksiz A, Kocyigit A. Urotensin-II, oxidative stress, and inflammation increase in hypertensive and resistant hypertensive patients. Clinical and Experimental Hypertension 2021; 43 (3): 211-216. https://doi.org/10.1080/10641963.2020.1847128

28. Aydin S, Kuloglu T, Aydin S, Eren MN, Yilmaz M et al. Expression of adropin in rat brain, cerebellum, kidneys, heart, liver, and pancreas in streptozotocin-induced diabetes. Molecular and Cellular Biochemistry 2013; 380 (1-2): 73-81. https://doi.org/10.1007/s11010-013-1660-4

29. Topuz M, Celik A, Aslantas T, Demir AK, Aydin S et al. Plasma adropin levels predict endothelial dysfunction like flow-mediated dilatation in patients with type 2 diabetes mellitus. Journal of Investigative Medicine 2013; 61 (8): 1161-1164. https://doi.org/10.2310/JIM.0000000000000003

30. Zang H, Jiang F, Cheng X, Xu H, Hu X. Serum adropin levels are decreased in Chinese type 2 diabetic patients and negatively correlated with body mass index. Endocrine Journal 2018; 65 (7): 685-691. https://doi.org/10.1507/endocrj.EJ18-0060

31. Gu X, Li H, Zhu X, Gu H, Chen J et al. Inverse Correlation Between Plasma Adropin and ET-1 Levels in Essential Hypertension: A Cross-Sectional Study. Medicine (Baltimore) 2015; 94 (40): e1712. https://doi.org/10.1097/MD.0000000000001712

32. Zhang C, Zhao L, Xu W, Li J, Wang B et al. Correlation of serum adropin level with coronary artery disease. Zhonghua Yi Xue Za Zhi 2014; 94 (16): 1255-1257.

33. Yin K, Agrawal DK. Vitamin D and inflammatory diseases. Journal of Inflammation Research 2014; 7: 69-87. https://doi.org/10.2147/JIR.S63898

34. Liu Y, Peng W, Li Y, Wang B, Yu J et al. Vitamin D Deficiency Harms Patients with Coronary Heart Disease by Enhancing Inflammation. Medical Science Monitor 2018; 24: 9376-9384. https://doi.org/10.12659/MSM.911615

35. Wang W, Zhang J, Wang H, Wang X, Liu S. Vitamin D deficiency enhances insulin resistance by promoting inflammation in type 2 diabetes. International Journal of Clinical and Experimental Pathology 2019; 12 (5): 1859-1867.

36. Azizieh F, Alyahya KO, Raghupathy R. Association between levels of vitamin D and inflammatory markers in healthy women. Journal of Inflammation Research 2016; 9: 51-57. https://doi.org/10.2147/JIR.S103298

37. Peterson CA, Heffernan ME. Serum tumor necrosis factor-alpha concentrations are negatively correlated with serum 25 (OH) D concentrations in healthy women. Journal of Inflammation (London, England) 2008; 5: 10. https://doi.org/10.1186/1476-9255-5-10

38. El Hajj C, Walrand S, Helou M, Yammine K. Effect of Vitamin D Supplementation on Inflammatory Markers in Non-Obese Lebanese Patients with Type 2 Diabetes: A Randomized Controlled Trial. Nutrients 2020; 12 (7): 2033. https://doi.org/10.3390/nu12072033

39. Baser H, Can U, Baser S, Hidayetoglu BT, Aslan U et al. Serum total oxidant/anti-oxidant status, ischemia-modified albumin and oxidized-low density lipoprotein levels in patients with vitamin D deficiency. Archives of Endocrinology and Metabolism 2015; 59 (4): 318-324. https://doi.org/10.1590/2359-3997000000055

40. Foroozanfard F, Jamilian M, Bahmani F, Talaee R, Talaee N et al. Calcium plus vitamin D supplementation influences biomarkers of inflammation and oxidative stress in overweight and vitamin D-deficient women with polycystic ovary syndrome: a randomized double-blind placebo-controlled clinical trial. Clinical Endocrinology 2015); 83 (6): 888-894. https://doi.org/10.1111/cen.12840

41. Lin AM, Chen KB, Chao PL. Antioxidative effect of vitamin D3 on zinc-induced oxidative stress in CNS. Annals of the New York Academy of Sciences 2005; 1053: 319-329. https://doi.org/10.1196/annals.1344.028

42. Alatawi FS, Faridi UA, Alatawi MS. Effect of treatment with vitamin D plus calcium on oxidative stress in streptozotocin-induced diabetic rats. Saudi Pharmaceutical Journal. 2018; 26 (8): 1208-1213. https://doi.org/10.1016/j.jsps.2018.07.012

43. Ramakrishna V, Jailkhani R. Oxidative stress in non-insulin-dependent diabetes mellitus (NIDDM) patients. Acta Diabetologica 2008; 45 (1): 41-46. https://doi.org/10.1007/s00592-007-0018-3

44. Yang C, DeMars KM, Candelario-Jalil E. Age-Dependent Decrease in Adropin is Associated with Reduced Levels of Endothelial Nitric Oxide Synthase and Increased Oxidative Stress in the Rat Brain. Aging and Disease 2018; 9 (2): 322-330. https://doi.org/ 10.14336/AD.2017.0523