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Original research article

Evaluation of the serum prooxidant-antioxidant balance before and after vitamin D supplementation in adolescent Iranian girls

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ABSTRACT

Purpose: Oxidative stress is caused by an imbalance between the antioxidant defenses and pro-oxidant production in favor of pro-oxidant production. Vitamin D has the potential for both pro- and anti-oxidative effects. The aim of this study was to assess the effect of high dose vitamin D supplementation on the prooxidant-antioxidant balance (PAB) in Iranian girls attending High School.

Materials and methods: A total of 464 girls aged 12–18 years were asked to take vitamin D capsules containing 50000IU vitamin D_3 once a week for a period of 9 weeks. All variables were determined at baseline and after 9 weeks of intervention. Fasting blood samples were taken from all subjects. The serum levels of 25OHD were measured using an electrochemiluminescence method. Serum PAB levels were determined using an ELISA reader at a wavelength of 450 nm.

Results: Vitamin D supplementation was associated with an increase in serum PAB ($P < 0.001$) and a reduction in serum LDL-C ($P < 0.001$), total cholesterol ($P < 0.001$) and HDL-C ($P < 0.01$) serum levels in Iranian adolescent girls. The results obtained from the current study show that there were significant improvements in weight ($P < 0.001$), BMI ($P < 0.001$) and FBG ($P = 0.02$) in adolescent girls who had 50–74.9 nmol/L serum 25OHD levels compared to < 50 nmol/L ones after the vitamin D supplementation. There was no significant association between the serum PAB and all biochemical factors ($P > 0.05$ for all variables).

Conclusions: The results showed that vitamin D supplementation has increased the PAB levels in teenage girls.

1. Introduction

Vitamin D (25-hydroxyvitamin D or 25OHD) is a fat-soluble steroid hormone which is often ingested from dietary sources (fatty fish and dairy products), but is also synthesized in the skin following exposure to UVB light [1]. In addition to its well known role in bone health, 25OHD can also protect against the occurrence of many types of cancers in humans [2–4]. 25OHD is involved in calcium homeostasis and takes part in several cellular processes, for example metabolism, proliferation, and differentiation, by both non-genomic function and its known receptors (VDR)-mediated transcriptional effects [5].

About one third to half of the adult population globally (both in developed and developing countries) are affected by vitamin D deficiency [6,7]; in Canada and the USA more than two-thirds of individuals have a suboptimal serum vitamin D concentrations [8,9]. Reports indicate that about 79–81 % of adolescents in Iran are vitamin D deficient [10,11]. Studies in Mashhad have shown a prevalence of 80% deficiency and 12% insufficiency in vitamin D levels [12].

Oxidative stress occurs when there is an imbalance between the production of antioxidant defenses and pro-oxidant production in favor

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of pro-oxidant production [13,14]. There is a fine balance between the elimination and the production of the reactive oxygen species (ROS) [15]. Some studies have shown that the serum prooxidant-antioxidant balance (PAB) assay values are increased in patients with coronary artery disease and type II diabetes, and that the PAB assay can provide a simple surrogate measure of ROS [15–17]. The cellular oxidative condition is controlled by adjusting the level of antioxidant amount/activity and the level of oxidative source exposure/activity [18]. Therefore, reducing exposure to xenobiotics and radiation, suppressing the inflammatory response, suppressing enzyme activity and uncoupling mitochondrial potential can reduce oxidative stress [18].

The antioxidant properties of 25OHD were first reported in an *in vitro* model of ROS-induced neurotoxicity, and it was found to act by regulating the proteins that can reduce oxidative stress [19]. 25OHD can increase glutathione and protect neurons from oxidative degenerative processes [20]. 25OHD can also inhibit iron-related lipid peroxidation of membranes which may prevent free radical-induced cell membrane damage [21]. However, the mechanism of the vitamin D effects on the oxidative stress are not entirely clear. There is some evidence that the effects of 25OHD in breast cancer cells are caused by reducing antioxidant capacity and increasing ROS, and that it can potentiate the effects of ROS-producing drugs [22–25]. The aim of this study was to investigate the effects of a 3-month intervention with high dose vitamin D supplementation on a measure of the balance between serum pro- and anti-oxidants, measured using the PAB assay.

2. Materials and methods

2.1. Study population and design

The study population comprises adolescent girls who were recruited as a part of a previous study as described by Khayyatzadeh et al. [26], from the cities of Sabzevar and Mashhad, in northeastern Iran from among girls aged 12–18 years. Informed consent was acquired from all participants. The study protocol is approved by ethical committee of Mashhad University of Medical Science (MUMS), Iran (IR.MUMS.fm.REC.1395.12). Inclusion and exclusion criteria were described previously [26]. A total of 464 girls aged 12–18 years were asked to take vitamin D capsules containing 50000IU vitamin D_3 once a week for a period of 9 weeks.

2.2. Measurements

2.2.1. Clinical and biochemical features

Anthropometric indices were determined at baseline and after 9 weeks of intervention using standard procedures. Systolic (SBP) and diastolic (DBP) blood pressures were measured using a standard mercury sphygmomanometer on the left arm in the sitting position following a 15 min rest by a standard procedure. Fasting blood samples were taken after 14 h overnight fast from each subject and collected into vacuum tubes in the morning. The serum of blood samples was separated using centrifugation (Hettich model D-78532, New York, USA) and aliquots of serum were frozen at −80 °C for future analysis. Serum levels of high-density lipoprotein-cholesterol (HDL-C) and fasting blood glucose (FBG) were determined using Pars Azmun kits (Karaj, Iran) and the BT-3000 Auto-analyzer (Biotechnica, Rome, Italy) [26].

Serum levels of 25OHD were measured using an electrochemiluminescence method (ECL, Roche, Basel, Switzerland). Serum levels of 25OHD were measured using an assay with an intra- and inter-assay precision CV of 5.7% and 9.9%, respectively.

2.2.2. Serum prooxidant–antioxidant balance (PAB) assay

The serum PAB assay was used as previously described [15–17]. In brief, standard solutions were prepared by using variable proportions (0 to 100%) of 500 μM hydrogen peroxide (30%) (Merck, Massachusetts, USA), that were added to 3 mM uric acid (in10 mM NaOH). Then, 60 mg of 3,3′,5,5′-Tetramethylbenzidine powder (TMB; Fluka) was dissolved in 10 mL DMSO. The TMB cation was prepared by adding 1 ml of the TMB/DMSO solution to 50 mL of acetate buffer (0.05 M buffer; pH 4.5), In the next step, 175 μL of chloramine T (100 mM; Applichem: A4331, Darmstadt, Germany) solution was added to 50 mL of acetate buffer (0.05 M buffer; pH 4.5). The solution was mixed and incubated for 1 h at room temperature in a dark place and shaken gently several times at the end of incubation. In the next step enzyme solution of peroxidase (16.5 μL; Applichem: 230 U/mg, A3791,0005, Darmstadt, Germany) was added to TMB cation solution (50 mL), then the solution was placed into 1 ml aliquots and stored at −20 °C. The TMB solution was prepared by mixing 200 μL of TMB/DMSO with 10 mL of acetate buffer (0.05 M buffer; pH 5.8), mixed well and placed in 4 °C. Then, 10 μl of each sample, blank (distilled water) or standard samples were added to well of plate on ice. Working solution was ready by adding TMB cation (1 mL) to TMB solution (10 mL), and mixed well for 6 min (on a shaker) at room temperature in a dark place and used immediately. 200 μl of working solution added into the wells and incubated for 12 min in dark place at 37 °C. After the incubation, 50 μl of HCl (2 N) was added to all wells, then the absorption was read by an ELISA reader at a wavelength of 450 nm together with a reference wavelength of 620 or 570 nm. A standard curve was presented by absorption readings of the standard samples. The values of the serum PAB are presented in Hamidi-Koliakos (HK) units, which show percentage of hydrogen peroxide in the standard solution. The values of PAB in the subject samples were then assessed via the values acquired from the above standard curve.

2.2.3. Statistical analysis

SPSS statistical package for Windows was used for all calculations (SPSS 20 software, SPSS Inc., Chicago, IL, USA). Statistical significance for the data compared in the adolescent girls before and after vitamin D administration was evaluated using the Student's *t*-test. Descriptive statistics including mean, frequency and standard deviation (SD) were determined for all variables and were expressed as mean ± SD for normally distributed variables. Data received for independent variables was analyzed using Student's *t*-test. All the analyses were two-sided and statistical significance was set at $P < 0.05$.

3. Results

3.1. Clinical and biochemical features

A comparison of the clinical and biochemical variables in adolescent girls pre- and post-intervention is summarized in Table 1 and was previously published [26]. The results show that serum PAB levels were increased ($P < 0.001$), whereas serum concentrations of LDL-C ($P < 0.001$), total cholesterol ($P < 0.001$) and HDL-C ($P < 0.01$) were reduced after vitamin D supplementation (Table 1).

There were no statistically significant differences in the clinical and biochemical factors among adolescent girls according to 25OHD categories at baseline ($P > 0.05$ for all variables; data have not been shown). Whereas, there were statistically significant improvements in weight ($P < 0.001$), BMI ($P < 0.001$) and FBG ($P = 0.02$) in adolescent girls who had 50–74.9 nmol/L serum 25OHD levels compared to those with a value < 50 nmol/L after vitamin D supplementation (Table 2).

Tables 3 and 4 show the comparison of the clinical and biochemical variables in adolescent girls according to BMI and HDL-C categories before and after vitamin D supplementation. The results demonstrate that vitamin D supplementation cause an increase in the serum PAB levels in both groups of adolescents with BMI < 25 and ≥25 (kg/m2) (Table 3). Moreover, vitamin D supplementation has increased the PAB levels in both groups of adolescents with HDL-C < 50 and ≥50 (mg/dl) (Table 4).

Table 1
Comparison of the clinical and biochemical variables in adolescent girls pre- and post-intervention.

Variables (mean ± SD) (n = 464)	Pre-intervention	Post-intervention	P-value
Weight (kg)	53 ± 11	54 ± 11	0.0001
BMI (kg/m^2)	21 ± 4	21 ± 4	0.31
WC (cm)	71 ± 9	70 ± 9	0.0001
Serum PAB (Hamidi-Koliakos (HK) units)	65 (38–103)	79 (45–122)	0.0001
SBP (mmHg)	99 ± 13	98 ± 13	0.37
DBP (mmHg)	65 ± 11	63 ± 11	0.003
FBG (mg/dl)	88 ± 11	85 ± 10	0.0001
Serum HDL-C (mg/dl)	47 ± 9	46 ± 8	0.003
Serum LDL-C (mg/dl)	101 ± 25	93 ± 21	0.0001
Serum Total cholesterol (mg/dl)	163 ± 28	155 ± 26	0.0001
Serum Triglyceride	84 ± 37	82 ± 30	0.11
Serum 25OHD (nmol/l)	11 ± 10	38 ± 17	0.0001
Serum Hs-CRP (mg/l)	1 (0.5–1.6)	1 (0.5–1.8)	0.46
Serum Calcium (mmol/l)	9 (9.2–9.8)	10 (9.4–10.1)	0.0001
Serum Phosphate (mg/dl)	4 (3.6–4.2)	4 (3.8–4.3)	0.0001

Values expressed as mean ± SD. Between groups comparisons were assessed by nonparametric test for all non-normally distributed data (Wilcoxon test). BMI – body mass index; WC – waist circumference; PAB – prooxidant-antioxidant balance; SBP - systolic blood pressure; DBP – systolic blood pressure; FBG – fasting blood glucose; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; 25OHD – 25-hydroxyvitamin D; Hs-CRP – high sensitive C-reactive protein.

Table 2
Comparison of the clinical and biochemical variables in adolescent girls according to 25OHD categories after the vitamin D supplementation.

Variables (mean ± SD)	Serum 25OHD < 50 nmol/L (n = 341)	Serum 25OH 50–74.9 nmol/L (n = 106)	P-value
Weight (kg)	55 ± 11	51 ± 10	0.004
BMI (kg/m^2)	22 ± 4	20 ± 35	0.004
WC (cm)	70 ± 9	68 ± 8	0.06
Serum PAB (Hamidi-Koliakos (HK) units)	77 (43–121)	82 (46–130)	0.55
SBP (mmHg)	98 ± 13	99 ± 13	0.94
DBP (mmHg)	63 ± 11	63 ± 10	0.53
FBG (mg/dl)	86 ± 11	83 ± 10	0.02
Serum HDL-C (mg/dl)	46 ± 9	46 ± 7	0.61
Serum LDL-C (mg/dl)	95 ± 22	93 ± 22	0.48
Serum Total cholesterol (mg/dl)	157 ± 27	153 ± 26	0.11
Serum Triglyceride	84 ± 32	79 ± 29	0.07
Serum 25OHD (nmol/l)	30 ± 12	60 ± 6	0.0001
Serum Hs-CRP (mg/l)	1 (0.5–1.8)	1 (0.5–1.7)	0.63
Serum Calcium (mmol/l)	10 (9.4–10.0)	10 (9.4–10.1)	0.09
Serum Phosphate (mg/dl)	4 (3.8–4.3)	4 (3.8–4.3)	0.89

Values expressed as mean ± SD. Between groups comparisons were assessed by nonparametric test for all non-normally distributed data (Wilcoxon test). BMI – body mass index; WC – waist circumference; PAB – prooxidant-antioxidant balance; SBP – systolic blood pressure; DBP – systolic blood pressure; FBG – fasting blood glucose; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; 25OHD – 25-hydroxyvitamin D; Hs-CRP – high sensitive C-reactive protein.

3.2. Correlation of PAB with the clinical and biological parameters

The correlations between serum PAB with some other cardiovascular disease (CVD) risk factors among the adolescent girls are summarized in Tables 5 and 6. The results obtained from the current study show that there were no statistically significant associations between the serum PAB and any other biochemical factor parameters ($P > 0.05$ for all variables).

4. Discussion

To the best of our knowledge, this is the first study examining the effects of high dose vitamin D supplementation on PAB levels among adolescent girls. The results showed that vitamin D supplementation for 9 weeks caused a statistically significant decrease in serum levels of LDL-C, total cholesterol and HDL-C, and an increase in serum PAB values. Our findings are at variance with previous reports [27–29]. These showed that there was a statistically significant correlation between serum HDL-C and 25OHD before the intervention ($P = 0.002$), while, changes in serum HDL-C were not affected by supplementation ($P = 0.05$). In a cross-sectional study of 17,411 individuals, there was a positive association between serum HDL-C and serum 25OHD after adjustment for confounding factors in adults (25–84 years old) but in this study there was no intervention with vitamin D supplements [28]. Nikooyeh et al., assessed serum 25OHD and HDL-C in the summer and winter, and found that both increased significantly in summer, and again did not have an intervention arm. Moreover, they evaluated these factors in six different cities of Iran but used a small sample (n = 530 in total) [29].

Previous studies have measured separately the total pro-oxidant and anti-oxidant capacities [8]. Whilst, the present study used the serum PAB assay that measures pro- and antioxidant content simultaneously.

There is little evidence for the antioxidant properties of 25OHD. However, it has been reported that 25OHD can induce genes that can regulate redox balance, such as glutathione peroxidase, glucose-6-phosphate dehydrogenase and thioredoxin reductase [30,31].

It is reported that vitamin D has anticancer effects, perhaps via the effects of 25OHD binding to the vitamin D receptors that then translate to the nucleus where it binds to gene regulator regions [32–34]. However, the studies on the effects of 25OHD on the oxidative stress are limited. One study has shown that the prooxidative effect of 25OHD can reduce cancer development [18]. Whilst another study has reported that 25OHD can exert its anticancer effects through its antioxidative properties. A further study suggests that 25OHD may prevent prostate cancer through its transcriptional activation of glucose-6-phosphate dehydrogenase activity [35].

The results of the current study show that high dose vitamin D supplementation for a period of 9 weeks increased the serum PAB levels in adolescent girls. This result may be due to the high dose, or long-term of vitamin D supplementation in this study. There have been no other similar studies that have done a similar research among adolescents, but previous studies have documented that the regulatory mechanisms of 25OHD can be influenced by the treatment time, dosage, cell type, environment and tissue [18].

There were no statistically significant differences in the clinical and biochemical factors among adolescent girls according to 25OHD categories at baseline. Moreover, there were no statistically significant differences in PAB and HDL-C levels among adolescent girls with serum levels of 25OHD < 50 and 50–74.9 nmol/L after the supplementation. Whereas, the vitamin D supplementation caused improvement in the weight, BMI and FBG in adolescent girls who had 50–74.9 nmol/L serum 25OHD levels compared to < 50 nmol/L ones.

Our results show no statistically significant association between serum PAB or other biochemical factors. To further clarify the effects of high dose supplementation of vitamin D in adolescents, it may be important to measure the components that are measured as part of PAB assay such as albumin or uric acid, to assess the importance of this finding on factors that are more explicitly linked with cardiovascular risk [15]. In addition, more randomized controlled trials using different doses and a placebo group are required to determine the effects of vitamin D supplementation on the oxidative stress.

4.1. Study limitations

In the present study, there was no control group based on advise of

Table 3
Comparison of the clinical and biochemical variables in adolescent girls according to BMI categories before and after vitamin D supplementation.

Variables (mean ± SD)	Pre-intervention		P-value	Post-intervention		P-value
	BMI < 25 (kg/m^2) n = 376	BMI ≥25 (kg/m^2) n = 67		BMI < 25 (kg/m^2) n = 362	BMI ≥25 (kg/m^2) n = 67	
Weight (kg)	51 ± 8	71 ± 9	0.0001	51 ± 8	72 ± 9	0.0001
BMI (kg/m^2)	20 ± 3	28 ± 3	0.0001	20 ± 3	28 ± 2	0.0001
WC (cm)	69 ± 7	84 ± 9	0.0001	67 ± 6	83 ± 9	0.0001
Serum PAB (Hamidi-Koliakos (HK) units)	61 (39–98)***	79 (44–116)*	0.04	76 (44–125)	89 (5–120)	0.24
SBP (mmHg)	98 ± 13	105 ± 13	0.0001	98 ± 13	103 ± 13	0.002
DBP (mmHg)	65 ± 12	69 ± 10	0.006	63 ± 11	65 ± 10	0.10
FBG (mg/dl)	87 ± 11	88 ± 11	0.73	85 ± 11	86 ± 11	0.60
Serum HDL-C (mg/dl)	47 ± 9	46 ± 10	0.42	46 ± 8	46 ± 12	0.52
Serum 25OHD (nmol/l)	11 ± 10	9 ± 8	0.16	38 ± 17	36 ± 15	0.35
Serum Hs-CRP (mg/l)	1 (0.4–1.4)	2 (1.1–3.0)	0.0001	1 (0.4–1.5)	2 (1.0–2.7)	0.0001
Serum Calcium (mmol/l)	9 (9.2–9.8)	10 (9.2–9.8)	0.12	10 (9.4–10.1)	10 (9.4–10.1)	0.39
Serum Phosphate (mg/dl)	4 (3.7–4.2)	4 (3.6–4.1)	0.06	4 (3.8–4.3)	4 (3.8–4.3)	0.64

Values expressed as mean ± SD. Between groups comparisons were assessed by nonparametric test for all non-normally distributed data (Wilcoxon test). BMI - body mass index; WC - waist circumference; PAB - prooxidant-antioxidant balance; SBP - systolic blood pressure; DBP - systolic blood pressure; FBG - fasting blood glucose; HDL-C - high-density lipoprotein cholesterol; 25OHD - 25-hydroxyvitamin D; Hs-CRP - high sensitive C-reactive protein.
***(P = 0.0001) and *(P = 0.013) are related to paired tests comparisons (Wilcoxon test) within BMI groups.

our ethics committee. Also, the high dose and short intervention period may have affected the results in this study compared with previous studies on vitamin D supplementation. Moreover, differences in serum 25OHD concentrations in different seasons were not considered. Although it seems that this issue cannot really affect the interpretation of our data, because the study was done over 9 weeks.

5. Conclusions

The results of the present study show that high dose vitamin D supplementation for a period of 9 weeks increases the serum PAB in adolescent girls. More randomized controlled trials with a control group warranted to assess the effects of vitamin D supplementation on the oxidative stress.

Conflict of interests

The authors declare no conflict of interests.

Financial disclosure

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The author contribution

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Table 4
Comparison of the clinical and biochemical variables in adolescent girls according to HDL-C categories before and after the vitamin D supplementation.

Variables (mean ± SD)	Pre-intervention		P-value	Post-intervention		P-value
	Serum HDL-C < 50 mg/dl (n = 262)	Serum HDL-C ≥50 mg/dl (n = 155)		Serum HDL-C < 50 mg/dl (n = 287)	Serum HDL-C ≥50 mg/dl (n = 111)	
Weight (kg)	54 ± 11	52 ± 11	0.02	54 ± 11	53 ± 10	0.31
BMI (kg/m^2)	22 ± 4	21 ± 3.84	0.01	21 ± 4	21 ± 4	0.64
WC (cm)	72 ± 9	70 ± 9	0.04	70 ± 9	69 ± 9	0.26
Serum PAB (Hamidi-Koliakos (HK) units)	65 (36.5–105)***	62 (38.1–90.5)***	0.63	80 (45.8–125.9)	76 (41.2–118.0)	0.34
SBP (mmHg)	99 ± 13	100 ± 12	0.40	98 ± 13	100 ± 13	0.25
DBP (mmHg)	65 ± 11	67 ± 11	0.35	63 ± 10	64 ± 12	0.70
FBG (mg/dl)	86 ± 12	89 ± 11	0.009	84 ± 10	88 ± 11	0.002
Serum 25OHD (nmol/l)	10 ± 9	12 ± 11	0.02	37 ± 17	39 ± 17	0.27
Serum Hs-CRP (mg/l)	1 (0.6–1.8)	1 (0.4–1.4)	0.08	1 (0.4–1.8)	1 (0.5–1.5)	0.93
Serum Calcium (mmol/l)	9 (9.2–9.8)	9 (9.3–9.8)	0.09	10 (9.3–10.0)	10 (9.4–10.3)	0.002
Serum Phosphate (mg/dl)	4 (3.6–4.2)	4 (3.7–4.2)	0.04	4 (3.8–4.3)	4 (3.9–4.4)	0.27

Values expressed as mean ± SD. Between groups comparisons were assessed by nonparametric test for all non-normally distributed data (Wilcoxon test). BMI - body mass index; WC - waist circumference; PAB - prooxidant-antioxidant balance; SBP - systolic blood pressure; DBP - systolic blood pressure; FBG - fasting blood glucose; 25OHD - 25-hydroxyvitamin D; Hs-CRP - high sensitive C-reactive protein.
*** (P = 0.0001) are related to paired tests comparisons within HDL-C groups.

Table 5
Correlation between PAB with some CVD risk factors among the adolescent girls before taking vitamin D, (n = 464).

		PAB arbitrary unit: HK	Serum TC (mg/dl)	Serum TG (mg/dl)	Serum HDL-C (mg/dl)	Serum LDL-C (mg/dl)	Serum Hs-CRP (mg/l)	Serum Calcium (mmol/l)	Serum Phosphate (mg/dl)
Serum PAB (Hamidi-Koliakos (HK) units)	Pearson Correlation	1	0.03	0.02	−0.05	0.00	0.02	−0.02	0.00
	Sig. (2-tailed)		0.60	0.61	0.31	0.99	0.75	0.68	0.98
	N	463	420	433	425	420	402	426	422
Serum Total cholesterol (mg/dl)	Pearson Correlation	0.03	1	0.29**	0.36**	0.82**	0.00	0.22**	0.08*
	Sig. (2-tailed)	0.60		0.0001	0.0001	0.0001	0.94	0.0001	0.02
	N	420	832	832	802	792	574	800	796
Serum Triglyceride (mg/dl)	Pearson Correlation	0.02	0.29**	1	−0.09**	0.18**	0.15**	0.20**	0.16**
	Sig. (2-tailed)	0.61	0.0001		0.007	0.0001	0.0001	0.0001	0.0001
	N	433	832	892	853	843	610	851	845
Serum HDL-C (mg/dl)	Pearson Correlation	−0.05	0.36**	−0.09**	1	0.12**	−0.04	0.17**	0.08*
	Sig. (2-tailed)	0.31	0.0001	0.007		0.001	0.36	0.0001	0.02
	N	425	802	853	853	841	591	826	828
Serum LDL-C (mg/dl)	Pearson Correlation	0.00	0.82**	0.18**	0.12**	1	−0.03	0.22**	0.1[1]**
	Sig. (2-tailed)	0.99	0.0001	0.0001	0.001		0.50	0.0001	0.002
	N	420	792	843	841	843	585	815	817
Serum Hs-CRP (mg/l)	Pearson Correlation	0.02	0.00	0.15**	−0.04	−0.03	1	0.01	0.00
	Sig. (2-tailed)	0.75	0.94	0.0001	0.36	0.50		0.88	0.93
	N	402	574	610	591	585	628	587	576
Serum Calcium (mmol/l)	Pearson Correlation	−0.02	0.22**	0.20**	0.17**	0.22**	0.01	1	0.24**
	Sig. (2-tailed)	0.68	0.0001	0.0001	0.0001	0.0001	0.88		0.0001
	N	426	800	851	826	815	587	857	844
Serum Phosphate (mg/dl)	Pearson Correlation	0.00	0.08*	0.16**	0.08*	0.11**	0.00	0.24**	1
	Sig. (2-tailed)	0.98	0.02	0.0001	0.02	0.002	0.93	0.0001	
	N	422	796	845	828	817	576	844	851

PAB - prooxidant-antioxidant balance; HDL-C - high-density lipoprotein cholesterol; LDL-C - low-density lipoprotein cholesterol; Hs-CRP - high sensitive C-reactive protein.
** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Table 6
Correlation between the changes of PAB with some CVD risk factors among the adolescent girls at baseline and after 9 weeks of vitamin D supplementation, (n = 464).

		PAB arbitrary unit: HK	Serum TC(mg/dl)	Serum TG(mg/dl)	Serum HDL-C (mg/dl)	Serum LDL-C (mg/dl)	Serum Hs-CRP (mg/l)	Serum Calcium (mmol/l)	Serum Phosphate (mg/dl)
Serum PAB (Hamidi-Koliakos (HK) units)	Pearson Correlation	1	−0.02	−0.02	−0.04	−0.01	−0.04	0.00	0.05
	Sig. (2-tailed)		0.62	0.64	0.38	0.84	0.46	0.94	0.28
	N	463	389	401	385	378	367	394	388
Serum Total cholesterol (mg/dl)	Pearson Correlation	−0.02	1	0.13**	0.43**	0.73**	0.00	0.38**	0.05
	Sig. (2-tailed)	0.62		0.0001	0.0001	0.0001	0.91	0.0001	0.28
	N	389	594	590	558	542	438	562	557
Serum Triglyceride (mg/dl)	Pearson Correlation	−0.02	0.13**	1	0.03	0.12**	−0.03	0.18**	0.03
	Sig. (2-tailed)	0.64	0.001		0.51	0.005	0.55	0.0001	0.40
	N	401	590	649	601	587	469	606	600
Serum HDL-C (mg/dl)	Pearson Correlation	−0.04	0.43**	0.03	1	0.26**	−0.087	0.256**	0.07
	Sig. (2-tailed)	0.377	0.0001	0.51		0.0001	0.07	0.0001	0.11
	N	385	558	601	605	587	445	585	588
Serum LDL-C (mg/dl)	Pearson Correlation	−0.01	0.73**	0.12**	0.26**	1	0.02	0.37**	0.06
	Sig. (2-tailed)	0.84	0.0001	0.005	0.0001		0.73	0.0001	0.16
	N	378	542	587	587	589	436	568	571
Serum Hs-CRP (mg/l)	Pearson Correlation	−0.04	0.00	−0.03	−0.09	0.02	1	−0.06	0.12*
	Sig. (2-tailed)	0.46	0.91	0.55	0.07	0.73		0.22	0.01
	N	367	438	469	445	436	501	461	452
Serum Calcium (mmol/l)	Pearson Correlation	0.00	0.38**	0.18**	0.26**	0.37**	−0.06	1	−0.04
	Sig. (2-tailed)	0.94	0.0001	0.0001	0.0001	0.0001	0.22		0.33
	N	394	562	606	585	568	461	635	624
Serum Phosphate (mg/dl)	Pearson Correlation	0.05	0.05	0.03	0.07	0.06	0.12*	−0.04	1
	Sig. (2-tailed)	0.28	0.28	0.40	0.11	0.16	0.01	0.33	
	N	388	557	600	588	571	452	624	629

PAB - prooxidant-antioxidant balance; HDL-C - high-density lipoprotein cholesterol; LDL-C - low-density lipoprotein cholesterol; Hs-CRP - high sensitive C-reactive protein.
** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

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