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ORIGINAL RESEARCH

Correlation of selected stress associated factors with vitamin D deficiency in Jordanian men and women

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Background: To identify stress associated factors for vitamin D deficiency (VDD) in healthy Jordanian people based on serum 25(OH)D levels.

Design: Prospective cohort study.

Methods: Three hundred and seventy-one Jordanian men and women aged 17–52 years, who were identified as VD deficient 25(OH)D <30 ng/mL, were eligible to participate in the study. Serum vitamin 25(OH) D was measured using chemiluminescent immunoassay. Cortisol, parathyroid hormone, calcium, phosphate, fasting lipid profile, and blood glucose were also analyzed. Questionnaires were used to collect lifestyles parameters. Anthropometric parameters including: body mass index (BMI), waist (W) and hip (H) circumferences, W/H ratio (WHR) were also calculated.

Results: The vast majority (91%) of the participants had vitamin D deficiency (25- (OH) D <30 ng/mL). Positive correlations were observed between vitamin D deficiency and the following anthropometric parameters in all study sample; gender (P=0.010), height (P=0.22), height/hip ratio (P=0.015) and waist/hip ratio (P=0.013). Lifestyle parameters that indicated very weak positive correlations with VDD were number of family members (P=0.011) and insufficient exposure to sunlight (P=0.023). The following clinical parameters showed weak or very weak correlations with VDD; serum cortisol (r=0.318), low density lipoprotein (r=0.246) and total cholesterol (r=0.133). Skin color and water pipe tobacco smoking were added to the multivariable stepwise regression analyses as they have been weakly correlated with VDD. These predictors together explained only 12.2% of the variance in serum cortisol levels in the VDD study sample.

Conclusion: A weak positive association between VDD and elevated serum cortisol was observed in this study. Subcutaneous changes may be involved in that association but further studies are needed to clarify a potential role for adrenocorticotropic hormone (ACTH).

Keywords: Vitamin D deficiency, stress, cortisol, smoking, obesity

Introduction

The prevalence of vitamin D deficiency (VDD) has increased dramatically in Mediterranean countries as seen in Jordan[1] Insufficient exposure to sunlight, as a leading cause of VDD, lacking of dietary sources, and malabsorption of the vitamin remain the most relevant factors.[2] Nevertheless, several studies have shown that the prevalence of VDD has increased dramatically in the Middle Eastern populations despite abundant sunshine.[3] Accordingly, main causes of VDD as well as their contributing factors are still not clear among Mediterranean populations including Jordanians.

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Allostatic load is an inability of the body's systems to accommodate adequately stress challenge due to chronic stressThis accompanies the modern lifestyle and impairs normal levels of stress mediators including cortisol (CORT).[4] Therefore elevated CORT levels reflect decreased body's ability to counteract consequences of chronic stress,[5,6] and to support quality of life.[7] Accordingly, fluctuating blood levels of CORT have been correlated with certain individual's behaviors such as heavy cigarettes smoking,[8] heavy coffee consumption,[9] sleep deprivation,[10] overweight,[11,12] and hyperlipidemaia.[13] Subsequently, some recent studies have focused on the association between VDD and lifestyle stressors. For example, significant inverse correlation has been observed between serum 25 (OH)D and postpartum allostatic stress.[14] Similar observations were also noted between CORT and 1, 25-(OH) 2D3 (VD3) levels in preeclampsia (PE) patients.[15] Furthermore, glucocorticoids (GC) administration lowered VD3 synthesis in PE-induced rats.[15] However, clinical studies that correlated VDD with cortisol or stress associated factors were inconsistent. VDD was positively correlated with caffeine consumption habits in healthy male Korean subjects compared to their peers who did not consume caffeine at all.[16] Conversely, a recent study conducted in Saudi Arabia excluded coffee consumption as a contributing factor for VDD.[17] The relationship between VDD and cigarette smoking is also controversial.[18–20] Finally, although prior studies have shown a positive relationship between VDD and obesity biomarkers.[21–23] some of these biomarkers, including waist, hip, weight and body mass index (BMI), are still controversial in terms of the association degree with VDD particularly in relation to lifestyle stressors. Therefore, the purpose of this study was to assess the association between selected lifestyle stressors and the severity of VDD in healthy Jordanians.

Subjects and methods

Research design and participants

This was a prospective observational cohort study carried out at the Applied Science Private University (ASU), Amman, Jordan during the period from October 2015 to May 2017. To avoid some anticipated variations in the study sample, only male and female Jordanian ASU students and employees who live in Amman participated in the study. Anthropometric data of the participants were measured by group of research assistants. Research assistants filled out a questionnaire including anthropometric and lifestyle habits. That was through a face-to-face interview with each participant in this study on the day of blood sample collection. The total number participants who were approached to participate in the study was 407, among which 36 were excluded because their VD levels were within normal values (≥30 ng/mL).[25] The same criteria for medical diagnosis of VDD are widely adopted in clinical diagnostics and trials. The remaining 371 participants composed the final sample that included in the analyses as shown in the schematic diagram of the study design (Figure 1).

Ethical considerations

This study was performed using a protocol no. DRGS-2014–2015-165, approved by the ASU ethics committee for the protection of human subjects. The study was conducted in accordance with the Helsinki Declaration. All participants were provided with an information sheet, which contained details of the experimental protocol. Participants fully understood the purpose of the study as well as the risks involved. Participants were informed of being free to withdraw from the investigation at any stage. All participants provided written informed consent prior to commencement of the study and were asked to complete a health screening questionnaire prior to their participation in the study. For the participants under the age of 18 (2 students) written informed consent has been obtained from the student's guardian so that theses 2 students were able to participate in this study. This was acceptable and approved as an additional annex by the ethics committee according to protocol no. (DRGS 2014–2015-165).

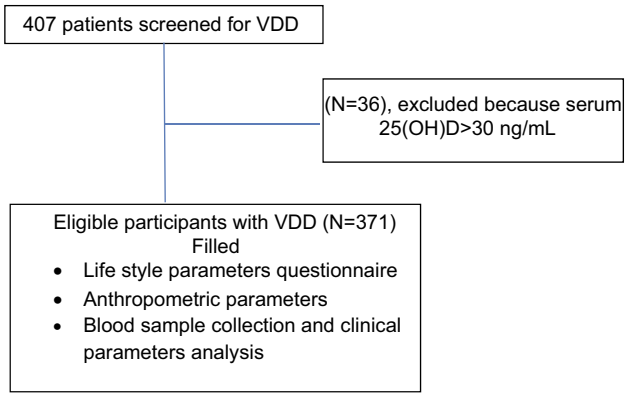

Figure 1 Schematic diagram of the study design.
Abbreviation: VDD, vitamin D deficiency.

Data collection or measurement

Anthropometric data included weight (Wt), height (Ht), BMI, waist (W) circumference, hip circumference (H), W/H ratio (WHR), Ht/W ratio (HtWR) were collected following standard procedures.[21,24] Age, gender, marital status, lifestyle data and clinical status of the participant included information on physical activity, cigarette or water-pipe tobacco smoking (WPTS), morning sun exposure, night sleep period, coffee and tea beverages consumption, family number, living alone or with family, number of family members were collected using a structured questionnaire.

Sample size

We assumed 95% confidence and margin of error 5%. The number of ASU community was approximately 7,000 subjects including employees and students. Accordingly, 370 participants were required for this study. The same method of calculation has been used in a previous study conducted on Jordanian women with VDD in ASU 25 using research advisor's calculator Furthermore, the total number of participants for this study was in accordance with the number of participants recruited in similar recent randomized controlled trials involving females with VDD.[25]

Clinical parameters assays

Serum 25(OH)D assay

Serum 25OHD levels were measured using chemiluminescent immunoassay technology by LIAISON® 25-hydroxyvitamin D Assay (DiaSorin, Stillwater, MN, USA). Specific antibody to VD was used for coating magnetic particles (solid phase) and VD was linked to an isoluminol derivative. Its lower limit of assay was approximately (4 ng/mL), and its intra- and interassay coefficients of variation were 5% and 8.2%, respectively. The assay has a 100% cross-reactivity with both metabolites of 25 (OH)D namely, 25(OH)D2 and 25(OH)D3 and thus measures total serum 25(OH)D content.

Serum assays

Serum CORT levels were measured using an ELISA, EIA-1887 (DRG International, Inc., NJ, USA). The limits of detection of this assay were from 0–800 ng/mL with sensitivity =2.5 ng/mL (6.0 nmo/L) at a 95% confidence limit.

Serum parathyroid hormone (PTH) assay

The serum PTH levels were measured using PTH Intact ELISA KIT (DRG International) using Rayto RT2100C Microplate reader. It is a two-site ELISA for the measurements of PTH. The normal range of PTH ranged from 9–90 pg/mL for serum. Sensitivity of the test was 1.57 pg/mL and it was capable of detecting very low concentration levels.

Serum calcium levels were measured using CALCIUM-ARSENAZO kit (BioSystems, Barcelona, Spain), calcium in the serum reacts with Arsenazo III forming a colored complex that is measured using spectrophotometry (RAL Analyzers ClimaPlus, Barcelona, Spain). Normal serum calcium levels 8.6–10.3 mg/dL, detection limit =0.2 mg/dL. Serum PO4 levels were measured using Phosphorus Phosphomolybdate/UV kit (BioSystems) using spectrophotometry (RAL Analyzers ClimaPlus). The reference values for serum PO4 were 2.5–4.5 mg/dL and the detection limit was 0.13 mg/dL.

Statistical method

The statistical analyses were performed using a statistical software package SPSS, version 21.0 for Windows (IBM Corporation, Armonk, NY, USA). We calculated frequencies as well as means (SD) of predicted VDD overall for each variable. The *P*-values were considered significant at $P<0.05$ and differences between study participants were presented. We calculated Pearson correlations between 25 (OH)D and each one of three categories of parameters; lifestyle, anthropometric and clinical. We used multivariate stepwise regression analysis to identify independent predictors for serum cortisol levels after adjusting for participant characteristics. Normality of distribution for laboratory measurements was tested using the Kolmogorov–Smirnov test and added as a supplementary material (Figure S1). Although the data did not follow normal distribution ($P=0.001$ for serum 25(OH)D), it was approximately close to the normal distribution because of large sample size of the study.

Results

The characteristics of the 371 subjects, aged between 17 and 52, with VDD are presented in Table 1. The mean BMI refers to simple overweight (25.5±5.5) kg/m^2. Means waist and hip circumferences were (84.28+/13.4 and 101.8 ±14.1) cm respectively. Table 1 also shows selected lifestyle parameters.

The mean 25(OH)D serum value was 12.2 ng/mL (SD: 5.7 ng/mL) which classified as vitamin D deficiency Table 2. Calciotropic hormone (PTH), ionized calcium (Ca+2) and phosphate in addition to serum morning cortisol were within physiological limits. Elevated lipid profile parameters (TG,

Table 1 Baseline descriptive statistics of the anthropometric and life style parameters, (N=371)

Parameter	Range	Mean ±SD
Anthropometric parameter		
Age (years)	17.0–52.0	27.4±10.1
Height (m)	1.46 −0.195	1.7±0.01
Weight (kg)	39.0–134.0	69.7±15.3
Body mass index (BMI)	15.8–46.5	25.3±5.6
Waist (cm)	0.37–0.13	84.3±13.4
Hip cm	0.42 −1.53	1.01±0.14
WHR	0.48–2.06	0.83±0.15
HtHR	1.0–0.40	1.66±0.31.5
Lifestyle parameter		
Family number	2–17	6.4±2.4
Coffee consumption (100 mL/day)	0–10	1.2±1.4
Tea consumption/day (100 mL/day)	0–10	1.3±1.3
Mobile using (hours/day)	0–16	2.8±2.6
Water (liter/day)	0.25–5	1.4±0.6

Abbreviations: HT, height; Wt, weight; BMI, body mass index; W, waist; H, hip; WHR, waist/hip ratio; HtHR, height/hip ratio.

Table 2 Baseline descriptive statistics of the clinical parameters, (N=371)

Parameter	Range	Mean ±SD
VD (ng/mL)	3.50–29.5	12.3±5.7
CORT (pg/mL)	4.3–32.0	12.9±4.5
FBG (mg/dL)	54.0–119	88.9±15.7
TG (mg/dl)	59.0–271	143.9±26.7
TC (mg/dL)	117.0–460	270.7±43
LDL (mg/dL)	54.0–302	153.5±22.9
HDL (mg/dL)	18.0–96.0	56.9±8.2
PTH (pg/mL)	9.21–46.2	21.8±10.2
Calcium (mg/dL)	7.8–22.9	11.8±2.4
Phosphorus (mg/dL)	3.2–23	5.9±3.3

Abbreviations: VD, 25(OH)D; CORT, serum cortisol; FBG, fasting blood glucose; TG, triglycerides; TC, total cholesterol; HDL, high density lipoproteins; LDL, low density lipoproteins; PTH, parathyroid hormone.

LDL, HDL and TC) were noted as shown in baseline descriptive of the clinical parameters for all participants in the study (N=371). The majority of participants in this study were females (72%). This is due to the fact that the percentage of females exceeds males in most faculties of the ASU community, particularly the School of Pharmacy. Several positive lifestyles were observed as a predominant percentages of participants in the study including adequate sleep hours during the night (81.7%), morning sun exposure (75.2%), nonsmokers (89.8%) and living with their families (72.8%). Other baseline frequencies and percentages of studied anthropometric and lifestyle parameters are presented in Table 3.

Table 3 Baseline frequencies and percentages of some anthropometric and lifestyle parameters, (N=371)

Parameter	N (%)
Gender	
Male	104 (28.0)
Female	267 (72.0)
Regular exercise (3 times/week)	159 (42.9)
Night sleep (6–8 hours/night)	303 (81.7)
Morning Sun exposure (30 minutes daily)	279 (75.2)
Cigarette smoking (yes)	38 (10.2)
Skin color	
White	179 (48.2)
C-skin	176 (47.4)
Black	16 (4.3)
Living with family (yes)	270 (72.8)

Note: Percentages reflects the positive frequency.

Correlations of lifestyle, anthropometric and clinical parameters with the vitamin D deficiency (VDD)

In addition to gender (P=0.01), anthropometric parameters; Ht, WHR and HtHR showed very weak positive correlations with VDD (P=0.022, 0.013, 0.015), (R=0.119, 0.129, 0.127) respectively (Table 4). Table 5 shows the correlations of associated lifestyle stresses with vitamin D levels. An expected, a significant

Table 4 Correlations of anthropometric parameters with VDD in all subjects

Parameter	R	*T*-test	*P*-value
Gender	–	2.588	0.010*
Age	−0.046	–	0.377
Weight	0.033	–	0.524
Height	0.119	–	0.022*
BMI	−0.030	–	0.570
Waist	0.030	–	0.563
Hips	−0.088	–	0.090
WHR	0.129	–	0.013*
HtHR	0.127	–	0.015*

Notes: Unpaired t-test is used to compare between values of means from study sample of men and women with VDD. *p <0.05.
Abbreviations: VDD, vitamin D deficiency; R, Pearson correlation test; Wt, weight; Ht, height; BMI, body mass index; W, waist; H, hips; WHR, waist/hip ratio; HtHR, height/hip ratio.

Table 5 Correlations of selected lifestyle associated stress parameters with VDD in all subjects

Parameter	R	*t*-test	*P*-value
Family number	−0.131	–	0.011*
Coffee consumption	−0.091	–	0.078
Tea consumption	0.002	–	0.965
Mobile use	0.055	–	0.291
Regular exercise	–	1.782	0.076
Night sleep	–	0.112	0.911
Morning sun exposure	–	−2.286	0.023*
Cigarette smoking	–	−0.188	0.851
Water-pipe tobacco smoking	–	1.231	0.219

Note: *p <0.05.
Abbreviations: VDD, vitamin D deficiency; R, Pearson correlation test; WPTS, water-pipe tobacco smoking.

negative correlation between vitamin D levels and exposure to the sunlight (t= −2.286, P<0.05) was noted. The number of family members of the participant was negatively correlated with vitamin D levels (r= −0.131, P<0.05) indicating that larger family numbers are associated with higher severity of VDD. Regarding clinical parameters correlations (Table 4), obvious positive correlation has observed between mean serum levels of stress hormone (cortisol) and severity of VDD in this study (r=0.318, P<0.001). Otherwise, LDL-C and TC also showed very weak positive correlations with the severity of VDD (r=0.246 and r= –0.133) as listed in Table 6.

Multivariate analysis

Multiple liner regression (stepwise method) was conducted to identify multivariate associations that mediated an increase of serum CORT levels in study participants with VDD. Stepwise regression model of VDD, skin color, and WTS were significantly influenced by serum CORT (R=0.349, R^2=0.122, P<0.0001). The three predictors together clarified approximately 12% of the variance in CORT levels in the study. No further predictive variables were identified (Table 7).

Table 6 Correlations of clinical parameters with VDD in all subjects

Parameter	R	*P*-value
CORT (pg/mL)	0.318	0.000
FBG (mg/dL)	−0.091	0.079
TG (mg/dL)	0.003	0.947
TC (mg/dL)	−0.133	0.011
LDL (mg/dL)	0.246	0.000
HDL (mg/dL)	0.081	0.121

Abbreviations: VDD, vitamin D deficiency; R, Pearson correlation test; CORT, cortisol; FBG, fasting blood glucose; TG, triglycerides; TC, total cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein.

Discussion

This study showed that VDD is associated with morning serum cortisol levels. Although the positive association between VDD and CORT was quite weak, it can be considered as a potential link between VD status and stress level in youth period. To our knowledge this work is the first to describe VDD and CORT as a stress hormone in a representative sample of Jordanian university students and older adults from both genders, despite controversial results on an association of low VD levels with CORT overproduction.[25,26] To some extent our findings were similar to study results that showed a negative correlation between VD and CORT levels in 55 Italian athletes.[26,27] Further, GC may increases the risk of preeclampsia via diminishing VD3[15] The same association was studied in a reverse way where vitamin D3 supplements suppressed HPA-axis activity.[27,28] Similarly, in small-scale study (N=15), daily VD supplementation may reduce cardiovascular disease risk factors including a decrease in 11β-HSD1 activity, as evidenced by the decrease in the CORT/cortisone ratio.[28,29] A linear increase of inflammatory markers including CORT (P=0.025) and decrease of VD levels (P<0.001) was also found across the entire sample of major depressive disorder.[29,30] Although literatures on ethnic variances have demonstrated divergent diurnal CORT rhythms for Caucasians and Latinos, the nature of the variances are inconsistent across studies.[30–32] However, it seems that people with dark skin showed higher levels of CORT as a part of physiological responses to psychosocial stressors and health behaviors such as smoking.[32–34] Consequently, our observations showed thatparticipants with higher skin color had obviously increased VDD. This inverse correlation between skin color and 25(OH)D levels

Table 7 The multivariate association between stresses associated factors and serum cortisol levels by using multiple linear regression (stepwise method)

Parameter	B	R	R^2	*P*-value
VDD	0.253			
Skin color	0.837			
Water-pipe tobacco smoking	1.158	0.349	0.122	0.000

Abbreviations: VDD, vitamin D deficiency; R, Pearson correlation test; R^2, regression; WPTS, water-pipe tobacco smoking.

has previously been noted.[34,35] Therefore we adopt a potential synergistic association between certain related factors of stress and skin color with the severity of VDD. It is well known that adrenocorticotropic hormone (ACTH) has intrinsic melanocyte-stimulating hormone (MSH) activity causing generalized hyperpigmentation as a clinical feature of adrenocortical insufficiency. Because of ACTH secretion is low in secondary adrenal insufficiency, hyperpigmentation does not occur. This can be linked because all of the hypothalamus-pituitary-adrenal (HPA) axis elements are expressed in the skin including ACTH.[35,36]Accordingally, association of stress with darkening of the skin has taken particular attention in recent studies conducted on humans and animals.[37,41] In humans, developed pigmentation was observed in response to a combination of ultraviolat radiation (UV) and low humidity stress to the epidermal barrier.[37] In Mathematical modeling and empirical studies[38] showed that the melanocyte master regulator, microphthalmia-associated transcription factor (MITF) serves to synchronize stress responses and pigmentation. This hypothesis was previously noted, where ACTH activated brown adipose tissue and influenced browning of white adipose tissue whereas corticosterone counteracts ACTH action.[39] Surprisingly, increasing skin pigmentation following severe head trauma was observed in a case of a 67-year-old man. The patient's hormone study showed high levels of ACTH (978 pg/mL) with normal cortisol levels.[40]

Finally, To understand the functional mechanisms of dynamic color in amphibians, injected ACTH used as a maximum stressor model, did not lead to a skin-lightening response.[41] Consequently, the same UV exposure produces less vitamin D in brown-skinned people compared to white-skinned.[42]

In recent decades, water-pipe tobacco smoking (WPTS) has become widely popular among Jordanians particularly in youth. Some previous studies have linked VDD with cigarette smoking.[18,19] Remarkable changes were noted in rat offspring after exposure to tobacco during the lactation period including developed resistance to vitamin D in the adipocyte, HPA-axis dysfunction and visceral lipogenesis.[36,43] An increase in alpha hydroxylase associated with significant reduction of VDR has also been noted in obese induced rats.[44]

Alternatively, some of anthropometric parameters including lipid profile and adiposity measures showed significant correlations with VDD. High prevalence of VDD observed in our sample appears to coexist with hyperlipidemia which is positively associated with stress and cortisol levels. The majority of recent studies suggest a negative relationship between VD status and LDL-c serum levels[38,45] in healthy subjects[39–41,46–48] and in patients.[42,49] In relation to stress, hyperlipidemia in cardiovascular disease patients has contributed to excessive CORT levels.[43,44,50,51]

Al-Dujaili et al[29] reported that, in coronary artery disease (CAD), hyperlipidemia can be ameliorated under effect of VD supplementation via decreasing 11β-HSD1 activity. Metabolically, cholesterol is a precursor molecule for VD as well as CORT synthesis. The branching point in the metabolism is 7-cholesterol (7DHC) from which both VD and cholesterol are formed.[52,53] Accordingly, some relevant research hypothesized a linkage between cholesterol, VD, and steroid hormones in some subclinical cases.[45,54]

VDD has recently been linked to obesity.[46,55] Interestingly, a higher association between waist/hip ratio WHR and VDD observed in our study are consistent with some previous studies.[41,46,47,56] Nevertheless, observations of some small-scale[46,55] studies were partially inconsistent with our findings. In a large-scale study, Al-Daghri et al[56] found that WHR was less significant than other anthropometric measures of obesity regarding the strength of association with VD status. However, the inverse correlation observed between VD levels with waist[49,50,57,58] or hip[51,53,59,60] circumferences in previous studies may result due to subcutaneously visceral adiposity alterations.[39,46,54,61] Similar alterations were also noted in elderly Korean women.[55,62]

Study limitations

The major limitation of this study was that we did not measure serum ACTH levels to evaluate HPA axis and its relationship with VDD.

Conclusion

Overall, our observations confirm the association between the severity of VDD and serum CORT levels as a predictor of HPA axis. This association will be clarified in further studies in which we will assess the role of ACTH on vitamin D metabolism in the subcutaneous layer.

Acknowledgments

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary material

		Vitamin D 30 75 ng/mL
N		371
Normal parameters[a,b]	Mean	12.2399
	SD	5.71713
Most extreme differences	Absolute	0.101
	Positive	0.101
	Negative	-.077-
Kolmogorov–Smirnov Z		1.939
Asymp. Sig. (2-tailed)		0.001

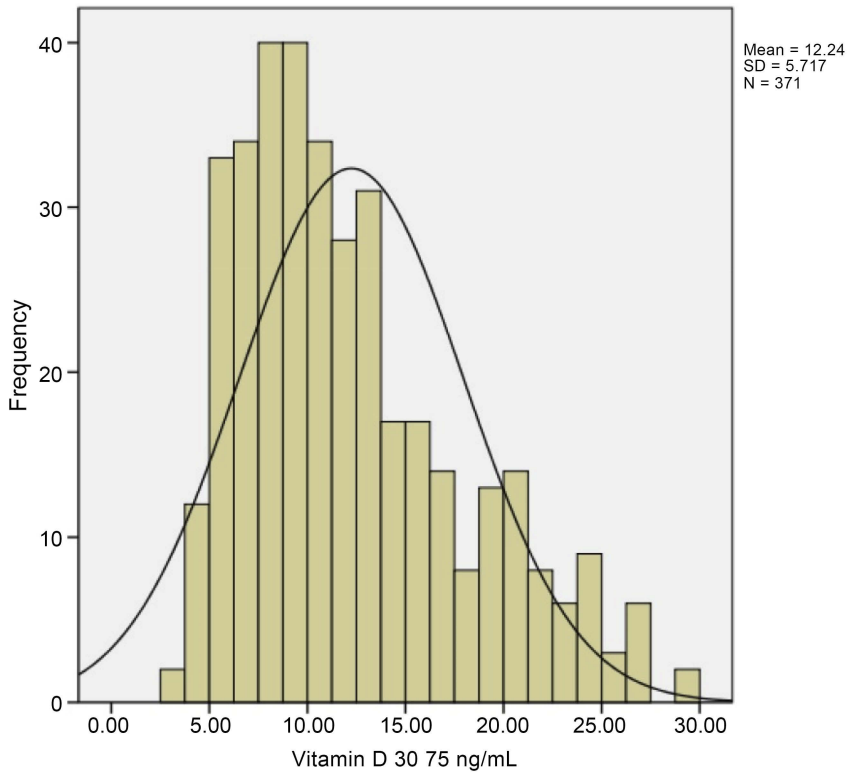

Figure S1 One-sample Kolmogorov–Smirnov test.
Notes: [a]Test distribution is normal. [b]Calculated from data.