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VITAMIN D STATUS AND VARIOUS PREDICTORS IN EGYPTIAN ADOLESCENT FEMALES

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Abstract

The last decade has witnessed a tremendous interest in vitamin D, based on the awareness of the worldwide high prevalence of vitamin D deficiency. This study aimed to investigate vitamin D status among Egyptian adolescent females and their association with various predictors. The study consisted of one hundred two healthy adolescent females enrolled in Helwan University, Egypt. The subjects classified by vitamin D status as, vitamin D sufficient group (G1), Insufficient (G2), and deficient group (G3). Females completed a questionnaire regarding sun exposure duration, use of topical sunscreens and performed anthropometric measures, 25-hydroxycholecalciferol 25(OH)D, vitamin D binding protein (DBP), total calcium, calcium ionized, alkaline phosphatase, inorganic phosphate, PTH, Albumin were all determined. The bioavailable 25(OH)D levels were calculated using 25(OH)D, DBP, and albumin concentrations. 25(OH)D, vitamin D binding protein, levels, calculated free vitamin D, bioavailable vitamin D, serum total calcium, and calcium ionized were significantly decreased in the deficient (G3) and insufficient (G2) in comparison to the sufficient group (G1) ($P<0.01$). Our study demonstrated a high prevalence of vitamin D insufficiency and deficiency about (54.9%) among healthy Egyptian adolescent females.
Keywords **:** vitamin D binding protein (DBP), 25-Hydroxy vitamin D, Egyptian, and adolescent.

Introduction

More than one billion people worldwide have vitamin D deficiency or insufficiency (Talaei *et al*., 2018), particularly high-risk groups, such as infants, children, young adults, pregnant women, lactating mothers, elderly people, and individuals who cover their bodies for cultural reasons (Kunz *et al*., 2018).

Accumulating data from Middle East countries indicate a high prevalence of vitamin D deficiency and insufficiency, although abundant with sunshine, indicating that 70-80% of adolescent girls in Saudi Arabia and Iran had vitamin D levels of <20 ng/ml. Vitamin D deficiency is a common problem among Egyptian adolescent girls (Botros *et al*., 2015). Vitamin D deficiency is extremely higher prevalent in the Middle East than in Northern and Western Europe with an increased risked severe deficiency in adolescents (Lips *et al*., 2019). Vitamin D is a group of steroid hormones produced from both diet and sunlight; it also performs a vital function in innate immunity, cellular functions (Hamedanian *et al*., 2019), calcium homeostasis, bone health, muscles, and the cardiovascular system. Vitamin D3 produced in the skin after ultraviolet radiation, but smaller amounts of vitamin D2 and D3 are obtained via food (Oleröd *et al*., 2017). The presence of vitamin D receptors in most tissues and cells in the human body leads to that vitamin D was identified as a unique hormone (Talaei *et al*., 2018).

Vitamin D is transported in the blood by the vitamin D binding protein (DBP) (Cyprian *et al*., 2019), a 52–58 kDa glycoprotein produced in the liver, belonging to the albuminoidal superfamily (Oleröd *et al*., 2017). The calcium ion is a primary structural component of the skeleton and performs an essential role in muscle contraction, blood coagulation, enzyme activity, neural excitability, secondary messengers, hormone release, and membrane permeability (Caprita, *et al*., 2013).

The objectives of the present study were; to investigate vitamin D status among Egyptian adolescent females and its association with various predictors (vitamin D binding protein (DBP), calculated free vitamin D) and is it directed by demographic attributes (location), seasons, and sun exposure time

Materials and Methods

Study Location

We conducted the study at Helwan University. Geographic coordinates; Latitude & Longitude for Helwan, Egypt in decimal degrees: 29°43'N, 31°12'E. Elevation above sea level: 28 m (Robaa and Hasanean, 2007)

Study Population and Design

One hundred two healthy Caucasians adolescent females aged 18-20 years randomly selected. They were all recruited during the period between September 2017 and May 2018. The study was approved by the ethical committee of the Ministry of Health and Population, Cairo, Egypt; approval No, IORG0005704/IRB0000687. All subjects provided written informed consent before their enrollment in the study, stated that they were in full physical health and answered questionnaires. We excluded studies conducted individuals, those with chronic illnesses (kidney, liver or heart failure), low thyroid function since this may cause depressive symptoms, multiple sclerosis, rheumatoid arthritis, thyroid disease, cancer, Type 1 or Type 2 diabetes mellitus, or polycystic ovaries, pregnant or lactating women, or used any medication known to affect bone metabolism including calcium and vitamin D supplements. One questionnaire including demographics data (i.e. age, living in urban/rural environment, and marital status), a detailed sunlight exposure questionnaire was designed to record the nature of the day (between 6 and 8 AM, between 9 AM and 4 PM, and after sunset), and the use of sunscreens was recorded.

Vitamin D cutoffs have been framed by the Institute of Medicine's (IOM) Report and the Endocrine Society Guidelines (IOM, 2005). According to these guidelines, vitamin D deficiency was defined, a concentration of vitamin D below 10 ng/ml, between 20 and 29.9 ng/ml as insufficiency and a concentration ≥30 ng/mL as sufficient

Specimens collection

Blood samples were taken according to a standard protocol and immediately centrifuged (Hettich D-78532, Tuttlingen, Germany). Venous blood samples were obtained by venipuncture using a minimal tourniquet. Blood samples were stored at room temperature for 10-20 minutes centrifuge at the speed of 2000-3000 rpm for 20-min, and separated samples were stored at -60 ^{o}C for analysis

Anthropometrical measurements

Weight was measured on an electronic digital scale to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm using Leicester height meter. Body mass index (BMI) was computed as weight in kg/height in meter square. BMI classification as following; Underweight (BMI<18.5), Normal weight (BMI 18.5-24.9 kg/m^2), Overweight, (BMI 25-29.9 kg/m2), and Obese (BMI≥30 kg/m2) stated by the World Health Organization (WHO, 2003).

Laboratory investigations

Biochemical measurements were made by routine methods; the serum levels of albumin, total protein, phosphors, Alkaline phosphatase, PTH and total calcium were analyzed with standard laboratory techniques on a (Cobas instrument; Roche Molecular Diagnostics). The quantification of Vitamin D in serum was performed using the solid phase enzyme-linked immunoassay (ELISA), DRG Instruments GmbH, Germany. The Vitamin D binding protein (DBP) concentration was measured using the DBP DuoSetH ELISA kit (R&D Systems, Minneapolis, MN). Calcium ionized by equation Ca^{++}=(6×total Ca–Total Protein/3)/(6+Total Protein). Concentrations of free/bioavailable 25(OH)D were calculated using published mathematical models that include concentrations of vitamin D, DBP, and albumin. Using the following formula (Oleröd *et al*., 2017).

$$\text{Calculated free 25(OH)D (pmol/L)} = \frac{\text{Total 25(OH)D}}{1+(6\times 10^5\times[\text{Albu min}])+(7\times 10^8\times[\text{DBP}])}$$

Where 6×10^5 is the affinity constant between vitamin D and albumin (Kalbumin)

Where 7×10^8 is the affinity constant between vitamin D and vitamin D binding protein

Bioavailable25 (OH) D=albumin bound 25 (OH) D+free 25 (OH) D= (albumin×Kalbumin+1) ×free 25(OH) D

Bioavailable 25 (OH) D (nmol/L) =$6x10^5$ x [Albumin] +1 } x calculated free25 (OH) D (Oleröd *et al*., 2017).

Statistical analysis

The data were analyzed by SPSS statistical software (version 21.0; SPSS Inc., Chicago, IL, USA). Descriptive statistics were expressed as mean and standard deviations. Chi-square (X^2) test and sFisher exact test were conducted for categorical variables. A comparison between groups was done by using the ANOVA test. Correlation analysis was done using Spearman's correlation test to evaluate the association between variables. For all inquiries, a probability (P) less than 0.05 were considered significant. Linear regression analysis was conducted to detect predictors of vitamin D status among the adolescent females

Results

Characteristics of the subjects

The mean age was (18.7±0.6) years; the students (53.9%) were of normal weight based on BMI classification as conferred in the table (1). Our study indicates a high prevalence of vitamin D insufficiency and deficiency of about (54.9%). Seasonal differences in vitamin D concentrations became obvious in each group as shown in fig (1).

Regression analysis of the Subjects:

A multiple backward linear regression analysis was calculated to predict vitamin D levels based on different seasons, and sun exposure time. A significant regression equation was found as follows F (4, 97) =61.76, P<0.01, R^2=0.71. (Test using alpha =0.05). A significant regression equation was found as follows F (3, 98) =83.18, P<0.001, R^2=0.71.

Discussion

Form the study population we found that (54.9%) of them having vitamin D insufficiency and deficiency while (45.1%) having vitamin D sufficiency. Our findings are in agreement with earlier studies (Metwalley *et al*., 2016), from Egypt showed that about 63% of healthy adolescent girls had vitamin D deficiency, despite the availability of sunlight throughout the year, and were explained by traditional and cultural circumstances in addition to a genetic predisposition. Another study (Amr *et al*., 2012) in Egypt of adolescent girls (54.7%) had vitamin D deficiency**.**

In the present study, we found that the mean anthropometric values were in the normal range with no statistical difference, between the investigated groups (G1, G2, and G3). All mean biochemical values were in the normal range, except for (25(OH)D, vitamin D binding protein, calculated free vitamin D, bioavailable vitamin D, serum total calcium, and calcium ionized) which were significantly decreased in the deficient (G3) and insufficient group (G2) in comparison to the sufficient group (G1) (P<0.01). Vitamin D deficiency results in a decrease in intestinal calcium absorption, resulting in a decline in calcium concentrations in the serum.

Active calcium absorption decreases when the 25(OH)D concentration is less than 8 ng/ml. The active 25(OH)D metabolite binds to the vitamin D receptor (VDR) in the intestinal cell and stimulates the active calcium transport from the intestine to the circulation (Lips., 2012).

Besides, a higher insignificant level of Alkaline Phosphatase (ALP) and phosphorus were detected in the deficient (G3) and insufficient (G2) in comparison to the sufficient group (G1). The negative association of 25(OH)D with phosphorus in the study subjects indicating an osteomalacic effect on bone and the higher level of phosphorus in adolescent females indicated that bone turnover was increased. Moreover, PTH results showed none statistically decreased level of the deficient (G3) and insufficient (G2) in comparison to the vitamin D sufficient group (G1). The present study findings showed that a low level of vitamin D does not always increase the serum PTH

level. 25(OH)D did not qualify as an independent risk for the occurrence of overt hypothyroidism in adolescent females. Therefore, the serum concentration of PTH may not be used clinically as an indicator of vitamin D deficiency and regardless of calcium and PTH results; serum vitamin D should be measured if vitamin D deficiency or insufficiency is predicted. Our findings are in disagreement with (Manish *et al*., 2012) who reported that the classical actions of vitamin D influence mineral metabolism suppressing parathyroid hormone (PTH) production.

The most salient outcome of this research analysis was the strength of season as a predictor of the vitamin D level. So, the consequence of vitamin D deficiency or insufficiency on health the core of the debate on what levels define vitamin D adequacy rests on. A significant difference in the season between the studied groups (G1, G2, and G3), P=0.03. 25(OH)D concentrations were significantly lower in subjects taking in autumn. Our finding is in agreement with earlier studies confirming that cultural factors, inadequate sun exposure, and lack of sun exposure that may have contributed to such a high prevalence, which indicated that sun exposure is among the top predictors (Botros *et al*., 2015). Solar ultraviolet (UV) intensity is affected by latitude, altitude, time of day, and season, as well as by environmental factors, including air pollution, cloud cover, and natural ozone levels, that reduces incident UV radiation (Patwardhan *et al*., 2018). The WHO report for the ambient air pollution database in Cairo and the Delta region considered to be among the areas with the highest ambient air pollution (Botros *et al*., 2015). Total sun exposure duration varied widely across subjects (P =0.05). Our finding is in agreement with the previous study in Egypt with adolescent females showed that sun exposure time was significantly higher in girls with adequate vitamin D levels compared to those with inadequate and deficient vitamin D. Exposure of at least 18% of body surface area (BSA) for at least 37 minutes/day is enough to achieve adequate vitamin D levels in a sunny climate as Egypt. Vitamin D levels correlated positively with the sun exposure time in a cohort study of adolescent girls (Amr *et al*., 2012). Vitamin D deficiency has been identified as a problem in healthy Egyptian females and is often related to limited sun exposure (Brouzes *et al*., 2019).

On the other hand, there was a significant difference among participants (P=0.04) of using sunscreens. Sunlight exposure as a viable option for vitamin D deficiency management remains low, possibly because of recommendations for sun avoidance. Our finding is in agreement with (Jafari *et al*., 2016) reported that usage of sunscreens may affect vitamin D synthesis. The major source of vitamin D in humans is cutaneous synthesis.

Conclusion

The results indicate a high prevalence of vitamin D insufficiency and deficiency about (54.9%) which could be a serious concern for an adolescent female in Egypt. The primary causes of vitamin D deficiency vary and may include factors such as ethnicity, lifestyle, living conditions, and physical movement. Therefore, both governmental and nongovernmental health professionals and health policymakers should pay attention to increase the awareness of vitamin D deficiency and its adverse effects on an adolescent female in Egypt.

The study showed a statistically proven relationship between vitamin D levels and sun exposure time. Moreover, vitamin D showed a significant positive correlation with serum total calcium, calcium ionized, vitamin D binding protein (DBP), calculated free vitamin D, and bioavailable vitamin D. The concentration of vitamin D in was significantly negatively correlated with serum phosphorus

Recommendation

Public health initiatives are invested in raising awareness about the importance of physical exercise, supplementing a sufficient amount of vitamin D, to reduce the prevalence of vitamin D insufficiency or deficiency in Egypt. The concentration of vitamin D in the serum should be routinely measured, vitamin D modulates, improves, and sustains the immune and defense system.

A comprehensive program including extensive awareness of the importance of sunlight exposure and improved dietary supplies of calcium and vitamin D as well as the inclusion of food fortification is recommended to prevent vitamin D deficiency.

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Conflicts of interest

The authors did not receive any financial or non-financial benefits from any commercial entity in support of this article.

Table 1: Comparison between the anthropometric, biochemical analysis, vitamin D profile, seasons, demographics, BMI classification, exposure to the sun duration, and using a sunscreen of all the studied subjects

Variables	Sufficient group (G1)	Insufficient group (G2)	Deficient group (G3)	P-value
	n=46	n=37	n=19	
Age (year)	18.8±0.7	18.8±0.5	18.4±0.5	0.09
BMI (Kg/m2)	25.2±4.4	24.2 ± 3.8	23.6 ± 3.6	0.2
25(OH)D (ng/ml)	38.5±10.6	20.4±4.5[a]	7.22±1.0[b,c]	<0.01
DBP(Ug/ml)	144.6±25.7	111.2±26.1[a]	106.9±26.1[b]	<0.01
Calculated free 25(OH)D (Pmol/L)	46.6±14.1	31.3±9.9[a]	11.3±2.4 [b,c]	<0.01
Bioavailable 25(OH)D (nmole/L)	16.7±5.2	11.4±3.6[a]	4.0±0.9[b,c]	<0.01
Total Ca (mg/dl)	9.6±0.6	9.1±0.6[b]	8.7±0.7[c]	<0.01
Ca^{++} (mg/dl)	4.1±0.3	4.0±0.3	3.7±0.4b	<0.01
Alkaline phosphatase (U/L)	132.1±38.4	148.43±53.8	143.1±34.3	0.2
Phosphorus (mg/dl)	4.3±0.5	4.5±0.9	4.7±0.8	0.2

PTH (Pg/ml)		41.5±12.4	40.3±16.1	34.7±14	0.2
Albumin (g/dl)		4±0.2	4.0±0.2	3.9±0.3	0.2
Seasons	Autumn	12(26.1%)	14(37.8%)	9(47.4%)	0.03
	Spring	29(63%)	13(35.1%)	5(26.3%)	
	Winter	5(10.9%)	10(27%)	5(26.3%)	
demographics (Region of residence)	Rural	21(45.7%)	23(62.2%)	7(36.8%)	0.14
	Urban	25(54.3%)	14(37.8%)	12(63.2%)	
BMI classification	Underweight	2(4.3%)	2(5.4%)	1(5.3%)	0.5
	Normal	22(47.8%)	21(56.8%)	12(63.2%)	0.6
	Overweight	16(34.8%)	13(35.1)	4(21.1%)	
	Obese	6(13.0%)	1(2.7%)	2(10.5%)	0.5
Exposure to the sun duration	6-8 AM	0(0%)	0(0%)	2(10.5%)	0.05
	9 AM to -4 PM	45(97.8%)	36(97.3%)	17(89.5%)	
	Before sunset	1(2.2%)	1(2.7%)	0(0%)	
Using sunscreen creams	No	5(10.9%)	0(0%)	0(0%)	0.07
	Yes	41(89.1%)	37(100%)	19(100%)	0.04

Data are shown as the mean ± standard deviation for continuous variables and n (%) for categorical variables. a-c Data in the same row with different superscript letters are significantly different from each other ($P \leq 0.05$ is significant; one-way analysis of variance and Bonferroni test). Multiple comparisons; a: significance between G1 and G2, b: significance between G1 and G3, c: significance between G2 and G3. n: the number of observations. BDP: Vitamin D binding protein, BMI: body mass index

Table 2: Correlations between vitamin D (ng/ml), and different predictive variables:

Variables	Vitamin D (ng/ml)	
	Correlation Coefficient (rs)	P-value
BMI (Kg/m^2)	0.16	0.09
Total Ca (mg/dl)	0.3	<0.01
Ca^{++} (mg/dl)	0.3	<0.01
Phosphorus (mg/dl)	–0.2	0.03
DBP (Ug/ml)	0.53	<0.01
Calculated free 25(OH)D (Pmol/L)	0.86	<0.01
Bioavailable 25(OH)D (nmole/L)	0.8	<0.01
Albumin (g/dl)	–0.38	<0.01
Exposure to the sun duration	0.53	<0.01

P-Value of ≤0.05 is significant, r_s: Spearman's rho correlation coefficient, BMI: body mass index, protein, BDP: Vitamin D binding protein, Total 25(OH)D: Total 25-hydroxy vitamin D

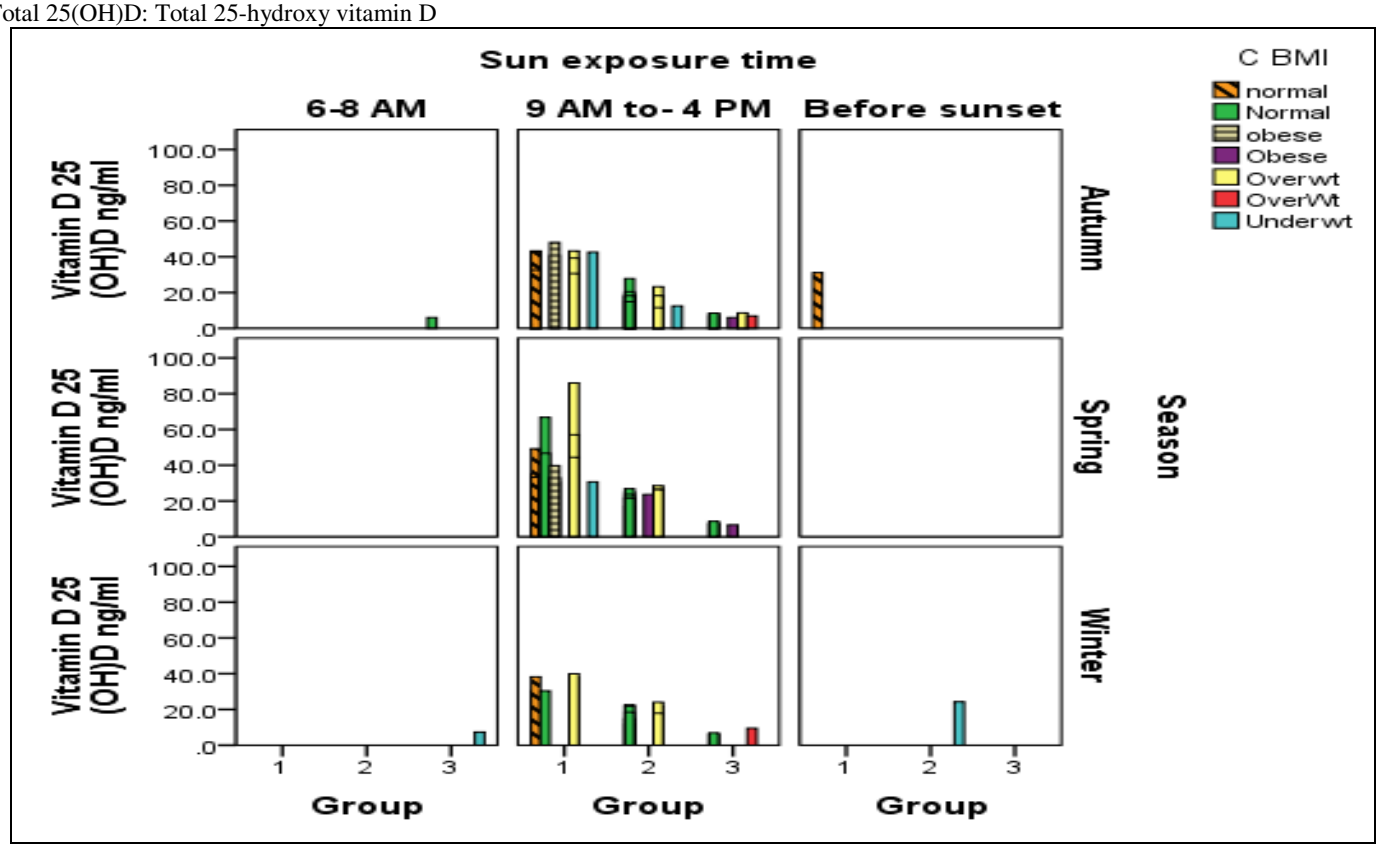

Fig. 1 : Mean serum vitamin D 25 (OH) D levels (ng/ml) in each season with body mass index classification

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