Plasma Levels of 25-Hydroxyvitamin D and Dress Style in a Sample of Egyptian Female University Students

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Abstract: Background: Sunlight exposure is the most important source of vitamin D. Nevertheless, there are indications of a high prevalence of vitamin D deficiency in a number of sunny countries. Concealed clothing, as it blocks the absorbance of UV light, is hypothesized to be the cause of impairment of vitamin D production.The objectives of the present study were to determine the prevalence of vitamin D deficiency and whether vitamin D status is related to the dress style among apparently healthy female university students in a prototype of Egyptian governorates *which enjoy* a good deal of *sunny* weather. **Methods:** A random sample of 120(90 females; 30 males) apparently healthy undergraduate students, Zagazig University, Sharkia, Egypt, were enrolled in a cross sectional study. Females were divided according to their dress style into three groups, Western, Hegab and Nekab dress style group. Vitamin D status was determined in terms of plasma 25(OH) D levels using electrochemiluminescence immunoassay (ECLIA). **Results:** Mean serum 25(OH)D level was 23.7 ±12.681 ng/mL. Levels did not differ between males and females or between females grouped by dress style. Using a cutoff point of 30 ng/mL, 74.6% of the sample (61.7% and 79.2% of males and females, respectively) had low vitamin D status. **Conclusions:** This study demonstrates a high prevalence of low vitamin D status among university students in Zagazig, Egypt. Results, however, are not in support of the hypothesis that concealing clothing is the cause of the vitamin D insufficiency. Thus, other factors must be sought to explain the low vitamin D levels despite the sufficient solar source of this vitamin.
[Maggie M. Fawzi, Enas Swelam and Nagwa S. Said Plasma. **Levels of 25-Hydroxyvitamin D and Dress Style in a Sample of Egyptian Female University Students**. Life Sci J 2012;9(2):763-767] (ISSN:1097-8135). http://www.lifesciencesite.com. 113

Keywords: 25(OH) D; Dress Style; Egypt; Hegab; Nekab; Vitamin D.

1. Introduction

The steroid hormone vitamin D, also known as the "sunshine vitamin," plays a major role as a precursor in calcium homeostasis of the human body. By far, the most important source of vitamin D is sunlight exposure. Hence, limited skin exposure to sunlight is presumed to be the cause of vitamin D deficiency(**Holick, 2005**).The liver and other tissues metabolize vitamin D, whether from the skin or oral ingestion, to 25-hydroxyvitamin D (25(OH)D) which is the principal circulating form of vitamin D. Further metabolism of 25(OH)D to the active metabolite 1,25-dihydroxyvitamin D occurs mainly in the kidneys (**Henry, 2011**).Although 1,25(OH)D is the principal hormonal form of vitamin D, it is not the ideal measure for vitamin D status. This is mainly because the plasma half-life of circulating 1,25(OH)D is only 4-6 hours and the 1,25(OH)D circulating levels are thousand fold less than those of 25(OH)D (**Anderson *et al.,* 2003**). By contrast, 25(OH)D, the principal circulating form of vitamin D has a half-life of approximately 2-3 weeks. Therefore, measurement of 25(OH)D is widely considered a robust 'gold standard' indicator of vitamin D status (**Hollis, 2005; Hollis and Horst**, **2007; Springbett *et al.,* 2010**).

Low levels of vitamin D metabolites are associated with malabsorption of calcium, which results in bone loss and variety of many other conditions including cancers of prostate, colon and breast, hypertension, lack of proper immune modulation and diabetes (**Khazai *et al.,* 2008**).This is especially true for people more prone to vitamin D deficiency, including people of darker skin color photo proteced by melanin (**Brok *et al.,* 2011**). Because of recent recognition of its broad pathophysiological importance, vitamin D has become a hot topic in medical research during the last decade (**Grant, 2006; Bacchetta *et al.,* 2010;**).

There appears to be an unrecognized epidemic of vitamin D deficiency in many parts of the world (**Holick and Chen, 2008**). However, there are wide regional differences in the 25(OH)D levels with an observed latitude effect, which has already been addressed in several countries by implementing dairy fortifications policies (**Rucker *et al.,* 2002, Kull *et al.,* 2009;**). Most of the studies concerning vitamin D status have focused on Western countries situated at temperate latitudes, though studies in the East have shown similar observations. Thus, a greater prevalence of the disease has been reported in the northern regions of China where the intensity of summer ultraviolet (UV) light is less than in the south (**Strand *et al.,* 2009**).This suggests that vitamin D deficiency is simply the consequence of inadequate exposure of the skin to the sun. Given the significant role of sunlight in vitamin D synthesis, one should expect low prevalence of vitamin D deficiency in tropical countries. Contrary to expectation, however, a number of studies conducted in sunny countries such as Tunisia (**Meddeb *et al.,* 2005**), Morocco (**Allali *et al.,* 2009**) Lebanon (**Gannagé-Yared *et al.,* 2000**), Saudi Arabia (**Al Faraj and Al Mutairi, 2003**) United Arab Emirates (**Al Anouti *et al.,* 2011**) and Iran (**Hovsepian *et al.,* 2011; Kaykhaei *et al.,* 2011**) have reported a high prevalence of vitamin D deficiency. One plausible presumption of the high prevalence of vitamin D deficiency in the Middle Eastern countries, despite the abundant sunlight almost throughout the year, has been related to clothing style. Concealed clothing, such as veiling worn by women, which is the cultural norm in most of the Middle Eastern countries, is hypothesized to be the cause of impairment of vitamin D production as it blocks the absorbance of UV light.

Egypt, like other Middle Eastern countries, *enjoys* a good deal of *sunny* weather, and likewise, the available few studies are hinting that Egyptian females may be, nevertheless, at high risk of vitamin D insufficiency (**El Badawy *et al.,* 2009**). Also, it is not yet ascertained whether vitamin D insufficiency is because of insufficient sunlight exposure as a result of concealing clothes. Egyptian women are dressed in different styles ranging from, as locally called,"Nekab" (totally covering the whole body, including the hands and face) and "Hegab" (covering the whole body but excluding the hands and face) to the western dress styles. Laboratory *in vitro* and *in vivo* studies have shown that clothing inhibits the production of previtamin D and serum vitamin D respectively (**Matsuoka *et al.,* 1992; Salih, 2004**).

The objectives of the present study were to determine the prevalence of vitamin D deficiency by measuring plasma levels of 25(OH)D, and to find out whether vitamin D status is related to the dress among apparently healthy female university students in an Egyptian governorate.

2. Subjects and Methods

Subjects

A random sample of 130 (96 females; 34 males) apparently healthy undergraduate students, Zagazig University, Sharkia, Egypt, were enrolled in a cross sectional study. Female subjects were classified by their dress style into three groups: the "Nekab" dress group where subjects have their whole body totally covered but eyes, the "Hegab" dress group where subjects have their body covered except hands and face, and the Western dressgroup.

Exclusion criteria were chronic illnesses, chronic use of medications (including vitamin D supplementation) or failure to give written informed consent. The study was approved by the ethics committee of Faculty of Medicine, Zagazig University, Zagazig, Egypt.

Methods

All subjects were studied within the same period of one month (April, 2011) to minimize seasonal variations in the levels of vitamin D. A study questionnaire was completed for each participant to collect socio-demographic data. Dress style (for women), skin color, and milk product daily consumption were also recorded. A full clinical examination and clinical history were taken from all subjects.

Anthropometrical measurements

Height and weight were measured with the patient standing in light clothes and without shoes. Body mass index (BMI) was calculated as body weight divided by height squared (kg/m^2).

Determination of plasma 25(OH)D levels

Plasma samples were obtained in EDTA tubes, mixed and centrifuged for 15 minutes at 2500 rpm. Plasma samples were stored at -20°C until time of assay. Plasma levels of 25(OH)D were assayed in duplicate,with quality control samples,using electrochemiluminescence immunoassay (ECLIA) performed on the Cobas e601 immunoassay analyzer (Roche Diagnostics GmbH, D-68298 Mannheim, Germany),(detectable range: 3.00-70.0 ng/ml.). Values below the limit of detection are reported as < 3.00 ng/ml. Values above the measuring range are reported as > 70.0 ng/ml. A value of 30 ng/ml is defined as vitamin D sufficiency. A value of < 20 ng/ml is defined as vitamin D deficiency and values between 20 and30 ng/ml are defined as vitamin D insufficiency.

Statistical Analysis

Continuous data were expressed as mean (±standard deviation) and were compared by use of Student's t-test or one-way analysis of variance (ANOVA). Categorical data were expressed as frequencies and percentages, and were analyzed with the two-tailed chi-square test. Data were analyzed using SPSS version 11.0.1. Software (SPSS for Windows, 2001). Two-tailed p values <0.05 were considered statistically significant.

3. Results

Mean age of participants was 21.1 ±2.17 and 20.8 ±1.96 years for male and female students, respectively. A comparison between the characteristics of these subjects by gender is shown in Table 1. A significant difference was found between males and females as regard BMI and daily consumption of milk products.

Mean plasma 25(OH)D concentration was 23.7 ±12.681 ng/ml. As shown in Table 2, mean plasma 25(OH)D level was not significantly different between males (26.6±12.19) and females (22.8±12.75). Also, no differences were noted when categorical comparisons were made by stratifying plasma 25(OH)D levels into deficiency, insufficiency and sufficiency levels. Using a cut-point of 30 ng/ml, 74.6% of this sample (61.7% and 79.2% of males and females, respectively) had low vitamin D status.

Characteristics of female subjects, classified by their dress style, are shown in Table 3. Students dressed in Western style clothing were the youngest. Otherwise there were no significant differences between the three female groups along parameters studied. Table 4 shows that plasma 25(OH)D levels were not different between these groups. Seventy five percent,81.4% and 82.3% of females with Western, Hegab and Nekab dress style, respectively, had plasma 25(OH)D concentrations indicative of deficiency or insufficiency levels.

Table 1. Characteristics of the study sample.

Parameter	**Male** (N= 34) Mean (±SD)	**Female** (N= 96) Mean (±SD)	**Total** (N= 130) Mean (±SD)	**Analysis**
Age (yr)	21.1 (±2.17)	20.8 (±1.96)	20.9 (±2.01)	t=0.638; *p*=0.525
BMI[(1)]	26.1 (±6.53)	30.3 (±6.15)	29.2 (±6.49)	t=3.313; *p*=0.001*
	N (%)	N (%)	N (%)	
Skin type				
Fair	5 (14.7)	22 (22.9)	27 (20.8)	
Intermediate or dark	29 (85.3)	74 (77.1)	103 (79.2)	χ^2=1.029; *p*=0.310
Milk product daily consumption				
Yes	12 (35.3)	16 (16.7)	28 (21.5)	
No	22 (64.7)	80 (83.3)	102 (78.5)	χ^2=5.155; *p*=0.023*

*Significant (1) BMI= Body mass index

Table 2. Distribution of 25-hydroxyvitamin Dlevels in plasma,by gender.

25-OHD*	Male (N= 34)	Female (N= 96)	Total (N= 130)	**Analysis**
Mean (±SD) ng/ml	26.6(±12.19)	22.8(±12.75)	23.7 (±12.681)	t=1.512; *p*=0.133
	N (%)	N (%)	N (%)	
<20 ng/ml	6 (17.6)	31 (32.3)	37 (28.5)	
<30 ng/ml	15 (44.1)	45 (46.9)	60 (46.1)	
≥ 30 ng/ml	13 (38.3)	20(20.8)	33 (25.4)	χ^2=4.929; *p*=0.085

*OHD= Hydroxyvitamin D.

Table 3. Characteristics of the female subjects, by dress style.

Parameter	Western (N= 36) Mean (±SD)	Hegab (N= 43) Mean (±SD)	Nekab (N= 17) Mean (±SD)	**Analysis**
Age (yr)	19.7 (±1.72)	21.1 (±1.87)	20.9 (±2.04)	F=3.476; *p*=0.035*
BMI[(1)]	29.7 (±6.63)	30.6 (±6.23)	30.8 (±5.03)	F=0.287; *p*=0.751
	N (%)	N (%)	N (%)	
Skin type				
Fair	8 (22.2)	8 (18.6)	6 (35.3)	
Intermediate or dark	28 (77.8)	35 (81.4)	11 (64.7)	χ^2=1.937; *p*=0.380
Milk product daily use				
Yes	5 (13.9)	8 (18.6)	3 (17.6)	
No	31 (86.1)	35 (81.4)	14 (82.4)	χ^2=0.328; *p*=0.849

*Significant (1) BMI= Body mass index

Table 4.Distribution of 25-hydroxyvitamin Dlevels among females,by dress style.

25-OHD*	Western (N= 36)	Hegab (N= 43)	Nekab (N= 17)	**Analysis**
Mean (±SD) ng/ml	24,5(±13.86)	21.4(±12.03)	22.5(±12.76)	F=0.586; p=0.558
	N (%)	N (%)	N (%)	
<20 ng/ml	11 (30.6)	15 (34.9)	5 (29.4)	
<30 ng/ml	16 (44.4)	20 (46.5)	9 (52.9)	
≥ 30 ng/ml	9 (25.0)	8 (18.6)	3 (17.7)	χ^2=0.832; p=0.934

*OHD= Hydroxyvitamin D.

4. Discussion

Below latitude of approximately 35° North, UVB radiation is sufficient for vitamin D3 synthesis all year round (**Pierrot-Deseilligny and Souberbielle, 2010; Tsiaras and Weinstock, 2011**).The latitude of Zagazig, Egypt, where this study was carried out, is 30°N.There should be, therefore, no problem with the solar source of vitamin D. However, this study, in keeping with a number of previous studies in other countries sharing Egypt a sunny climate (**Mishal, 2001; Elsammak *et al.,* 2011**) demonstrates a high prevalence of low vitamin D status (61.7% and 79.2% of males and females, respectively) among university students in Zagazig, Egypt. Yet, in contrast to other studies which claim that covered dressing style causes vitamin D insufficiency (**Guzel *et al.,* 2001; Budak *et al.,* 2004; Allali *et al.,* 2009**) we found no differences in plasma 25(OH)D levels between groups, classified by dress style. Thus, our findings do not support the hypothesis that concealing clothing is the mechanism by which vitamin D production is impaired in this sunny country. By contrast, it could be that excess sun exposure itself is the factor responsible for the decrease in vitamin D status. As a regulatory process of vitamin D synthesis in face of continued skin exposure to UVB radiation, pre-vitamin D_3 is broken-down and vitamin D_3 is converted to inactive photoproducts and hence, an increase in vitamin D status is controlled (**Tsiaras and Weinstock, 2011; Webb and Engelsen, 2008**).

Moreover, it should be noted that in the present study, vitamin D levels did not differ between males and females or between females with Western dress style and those with more concealing dress styles, i.e., those wearing Hegab or Nekab. More than 60% of males and 75% of females with Western dress style had low plasma 25(OH)Dlevels. Thus, several reasons other than sun exposure could be involved in such reduction of vitamin D levels among Egyptian university students. Further studies are required to search for these reasons.

Our study had a number of limitations. First, we examined the plasma vitamin D in students of Zagazig University only. Thus, we cannot extrapolate our results to students in other universities or to other populations. Second, we did not measure parathyroid hormone, Ca and PO4levels to exclude any cases of secondary hyperparathyroidism. Yet, many studies have indicated that such serum biomarkers may not reflect the true status of an individual's vitamin D status. Third, while we measured baseline plasma 25(OH)D, we did not have details for sun exposure data of the participants, including holidays in the sun. Evaluation of the impact of dress style on vitamin D status might have been limited by the possibility that many students were spending most of their time indoors. Finally, a larger sample would have been preferred.

Conclusions

This study, in Zagazig, Egypt, in support of those in other Middle East countries, demonstrates a high prevalence of low vitamin D status among university students. Yet, in contrast to other studies we failed to confirm that concealing clothing is the cause of the vitamin D insufficiency. Thus, despite having sufficient solar source of vitamin D, other factors do play a role in these low levels. Further research is needed in this area of interest. While importance of vitamin D supplementation, perhaps not only for conservatively dressed females, is obvious, the most efficacious strategy to improve vitamin D levels remains a subject for further study.

Conflict of interests: None.

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5. References

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5/2/2012