Original Communication

Vitamin D, Parathormone and Associated Minerals among Students in Zagazig District, Sharkia Governorate, Egypt

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Abstract: *Aim:* To determine the prevalence of vitamin D deficiency and associated factors among students of age 13–18 years. *Methods: Design:* Community-based cross sectional survey. *Setting:* Two schools were selected using multistage sampling techniques. *Sampling:* Cluster sampling of all enrolled students (550 students). *Outcome Measures:* Serum levels of 25-hydroxyvitamin D (25 OHD), parathyroid hormone and calcium. Data was collected about nutritional intake, physical activity and lifestyle variables that are potential risk factors for hypovitaminosis D. *Results:* Hypovitaminosis D prevalence was 23.8 %, of which 5.3 % was deficiency and 18.5 % insufficiency. Serum 25 OHD levels inversely correlated with parathyroid hormone levels (r = -0.206, P = 0.00). Low calcium and ionized calcium levels were 40.6 % and 45.9 %, respectively, and significantly correlated with vitamin D levels. Female students have significantly higher levels of hypovitaminosis D compared to males (29.3 % to 15.0 %, respectively) and the level of vitamin D significantly improved with increased age. Exposure to sun had a significant effect on vitamin D levels, and physical activity, soft drink consumption and smoking did not. Multinomial regression analysis revealed that age, sun exposure and Ca level were the only significant independent predictors of hypovitaminosis D among the studied group. *Conclusion:* Our findings revealed that hypovitaminosis D is a prevalent health problem in adolescents, especially girls, who were at higher risk, and increased age and sun exposure improved vitamin D status among the studied group. There is therefore a need to consider vitamin D supplementation for school children together with increased awareness through a health education program.

Key words: vitamin D deficiency, parathormone, calcium, ionized calcium, adolescents

Introduction

Optimal vitamin D status is very important for calcium absorption and bone growth, especially during childhood and adolescence [1]. Despite this, vitamin D deficiency and insufficiency are still reported as a frequent problem in children and adolescents worldwide [2, 3] with high prevalence among healthy adolescents in some countries [3, 4, 5]. There is growing evidence from studies of adolescents [3, 4, 5, 6] and elderly persons [7] that vitamin D deficiency constitutes an important health problem that needs special consideration.

Vitamin D metabolites are important for the normal development of bone and the metabolism of its constituent ions. Vitamin D deficiency in adolescents might have a deleterious effect on their growth [8]. Recent results from studies of adolescents provide evidence of a possible adverse effect of vitamin D deficiency and insufficiency for bone health [9]. Vitamin D may also play a role in the prevention of some health problems, such as diabetes mellitus, multiple sclerosis, hypertension and cancer [10]. Numerous epidemiological publications support the extra skeletal benefits of vitamin D [11, 12, 13]. Benefits of vitamin D supplementation have been reported for some conditions, including diabetes and the metabolic syndrome, [14] and for neoplasia [15, 16]. Although most of these studies are preliminary, they indicate potential benefits that may be of great significance in the future. Although the etiology of osteoporosis is multifactorial, it is believed that secondary hyperparathyroidism, as a result of a more marginal vitamin D deficiency, is a significant contributing factor [9].

Vitamin D is deficient in the ordinary diet and the major source of vitamin D is vitamin D3, synthesized in the skin when exposed to ultraviolet light [17]. Regular exposure to sunlight is not considered important in Egypt and many other countries. Despite abundant sunlight, high daytime temperatures [18] and cultural beliefs, exposure to sunlight is minimal and not regular.

There are several vitamin D supplements, cholecalciferol (vitamin D3) 1000 IU or 25 microgram is the supplement most commonly used; multivitamin supplements with 32 – 200 IU per tablet are not adequate to treat or prevent vitamin D deficiency and calcitriol (1,25-dihydroxyvitamin D3) is generally not suitable for the treatment of vitamin D deficiency as it has a narrow therapeutic window resulting in an increased risk of hypercalcaemia or hypercalciuria [19].

Some studies have been published, about the prevalence of Vitamin D among some groups of adolescents in Egypt, but not at the community level [20]. We therefore undertook this study to determine the prevalence of vitamin D deficiency among students of age 13 – 18 years attending schools in Sharkia Governorate, Egypt.

Our objective was to determine the status of vitamin D, parathormone and other related minerals among students of age group 13 – 18 years in Zagazig District, Sharkia Governorate, Egypt. We also wanted to identify factors within the adolescent lifestyle that are predictors of hypovitaminosis D.

Subjects and Methods

The study was carried out as a cross sectional survey over a period of four months; from December 2011 to March 2012.

Study Population and Sampling.

The total population of the age group 13 – 18 in Zagazig district is 94,228. The expected frequency of the factor under study, calculated from the pilot study, was 25 % with a 95 % confidence interval and power of the test at 80 %; the calculated sample size was 287. Since we are using multistage cluster sampling, we doubled the sample to reach 574.

According to the Ministry of Education; Zagazig district is divided into 16 localities. One locality was selected randomly from which two schools were included; one preparatory (Talhet burden) and one secondary (Hassan-Eloksh). The selected schools were mixed (boys and girls). All students present were included in the study (550 students). Our inclusion criteria were that that students were aged 13 – 18 years and attending one of the two selected schools. The response rate from the targeted sample was high and almost equal to 100 % as the students were highly motivated to participate in the study for many reasons. Their parents or legal guardians were informed in advance and we obtained informed written consent after explaining the objectives of the study and the benefits to be gained from the study. They were informed about the laboratory test results and encouraged by giving them vitamin samples. Finally, even though some students were absent on the first visit they attended on the subsequent visits as a result of word of mouth communication by those students who

attended the first visit. The final number of students whose blood samples underwent laboratory analysis was 466, as the other blood samples were insufficient to carry out laboratory investigations.

Data Collection

A structured questionnaire was used to collect data about the students' socio-demographic characteristics, their daily intake of foods rich in calcium, and their degree of exposure to sun, physical activity, soft drink consumption and smoking. Their daily intake, in the previous month, of foods rich in calcium was assessed through a structured questionnaire guided by the Youth Adolescent Questionnaire (YAQ), which is an adaptation for adolescents of the Harvard Food Frequency Questionnaire (HFFQ) [21] and was modified to include popular foods in the Egyptian rural community (dairy products including milk, yogurt, karish cheese, eggs and bolty and boori fish). Frequency of consumption was measured as times per day, times per week, less than once per week and never. Portion sizes were presented in household units.

Assessment of physical activity was through a structured questionnaire guided by the International Physical Activity Questionnaire for Adolescents [22], which asked about types of activity, and duration per week.

Each of the variables was coded into three levels. Data about exposure to sun included: whether there was exposure, duration/day and week, and time of exposure. Total calcium daily intake was calculated and those who consumed 900 mg or above were considered to consume sufficient amounts of calcium.

Laboratory Measurements

One blood sample (5 ml) was obtained from each student included in the study. All tests were performed in the central laboratory of the Zagazig faculty of Medicine. Parathormone was collected in EDTA tubes, while calcium and vitamin D were collected in plain tubes. The specimens were transported vertically in lockable rigid containers to the central laboratory of the Zagazig Faculty of Medicine within 45 minutes of collection in order to ensure that centrifugation and separation of the specimen took place within an hour. All serum samples were stored at −20 degrees until use.

Serum 25 OHD level was measured by direct ELISA kit supplied by DRG International, Inc. USA, where the coefficients of variations (CV) were 13 % to 19 % [23] and the parathyroid hormone by PTH ELISA kit supplied by Calbiotech and CV was less than 10 % [23].

The cutoff points for 25 OHD were defined as; (1) Deficient: < 30 nmol/l (12 ng/ml), (2); Insufficient: ≥ 30 nmol/l but below 50 nmol/l (≥ 12 ng/ml but below 20 ng/ml); and (3) Sufficient: ≥ 50 nmol/l (20 ng/ml) [24]. The parathormone cutoff point of normal level was from 8.8 to 76.6 pg/ml, below 8.8 pg/ml is hypoparathyroidism and above 76.6 pg/ml is considered hyperparathyroidism [25].

The ionized calcium level was calculated according to the following formula [26]:

Ionized calcium = 0.25 x [0.9 + (0.55 x total calcium) – (0.3 x albumin)]. This equation calculates ionized calcium (mmol/L) from total calcium (mg/dl) and albumin (g/dl). The amount of total calcium varies with the level of serum albumin, a protein to which calcium is bound. The biologic effect of calcium is determined by the amount of ionized calcium, rather than the total calcium.

The cutoff points of total serum calcium level were defined as; hypocalcemia (< 9 mg/dl), normal level (9–10.5 mg/dl) and hypercalcemia (> 10.5). The cutoff points for ionized calcium were as follows: low (< 1.1 mmol/l), normal (1.1–1.4 mmol/l) and high ionized Ca level (> 1.4 mmol/l) [27].

Statistical Analysis

Statistical analysis was conducted using SPSS software version 11. Vitamin D level was coded: 1 = deficient 2 = insufficient 3 = sufficient. A Chi-square test was used to detect significant differences between groups and correlations were conducted to find the relationship between the vitamin D level and the levels of related minerals and of parathormone. Prevalence was calculated as the percentage of those suffering from deficiency and insufficiency of vitamin D among the studied sample. Multinomial regression analysis was carried out to determine the associated risk factors with hypovitaminosis D.

Pilot Study

Before starting data collection a pilot study was conducted with 40 students who were not included in the study to test the applicability and the clarity of the questionnaire, and changes were made accordingly.

Table I: Socio-demographic characteristics and calcium intake levels of the interviewed students

Items	Number (T = 550)	%
Sex:		
-Male	220	40.0
-Female	330	60.0
Age: (years)		
13-	228	41.5
15-	192	34.9
17+	130	23.6
Range	13–18.5	
Mean ± SD	15.3 ± 1.7	
School:		
Preparatory	319	58.0
Secondary	231	42.0
Calcium Intake Level:		
Adequate (≥ 900 mg/day)	259	47.6
Low (< 900 mg/day)	285	52.4

Ethical considerations and administrative approach

Before carrying out the study administrative permission was obtained from the Director of the Ministry of Education in Sharkia Governorate and also from the directors of schools. Informed written consent was received from students' legal guardians before participating in this study. Permission from the Institutional Review Board (IRB) of the faculty was obtained and the study was documented and given an IRB number: 445/29–5-2012.

Results

The total number of students included in the study was 550. The majority were in the age group 13–17 years (more than 75 %). Females constituted 60 % of the sample and 58 % were in preparatory school (Table I).

The blood samples of 84 students were insufficient for testing. Approximately 23.8 % of the sample suffered from low levels of vitamin D (5.3 % deficiency and 18.5 % insufficiency). 69.1 % of the students had normal levels of parathormone while 5.1 % had hypoparathyroidism and 25.8 % had hyperparathyroidism. The students who had normal levels of total calcium and ionized calcium constituted 24.9 % and 31.1 % of the sample, respectively, and those who had low levels formed 40.6 % and 45.9 % respectively of the total sample. Approximately 34.5 % and 23.0 % of the sample had high levels of calcium and ionized calcium (Table II).

Approximately 29 % of female students had low levels of Vitamin D, which was significantly higher than in males (15.0 %) (Figure 1). The level of Vita-

Table II: Vitamin D level, parathormone level and related minerals.

Items	Number (T = 466)*	%
1. Vitamin D level:		
– Deficient (< 30 nmol/l)	25	5.3
– Insufficient (≥ 30 nmol/l to < 50 nmol/l)	86	18.5
– Sufficient (≥ 50 nmol/l)	355	76.2
Mean ± SD	34.7 ± 19.4	
2. Parathormone level		
– Hypoparathyroidism (< 8.8 pg/ml)	24	5.1
– Normal (8.8–76.6 pg/ml)	322	69.1
– Hyperparathyroidism (>76.6 pg/ml)	120	25.8
Mean ± SD	62.3 ± 51.1	
3. Ca level:		
– Hypocalcemia (< 9 mg/dl)	189	40.6
– Normal (9–10.5 mg/dl)	116	24.9
– Hypercalcemia (> 10.5)	161	34.5
Mean ± SD	9.7 ± 2.7	
4. Ionized Ca level:		
– Low Ionized Ca level (< 1.1 mmol/l)	214	45.9
– Normal Ionized Ca level (1.1–1.4 mmol/l)	145	31.1
– High Ionized Ca level (>1.4 mmol/l)	107	23.0
Mean ± SD	1.4 ± 0.3	

NB: There were 466 blood tests used: the other samples were insufficient for inclusion.

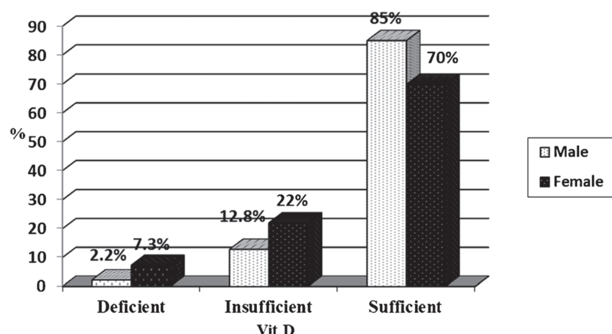

Figure 1: Vitamin D level by gender of the participants. $p < 0.01$

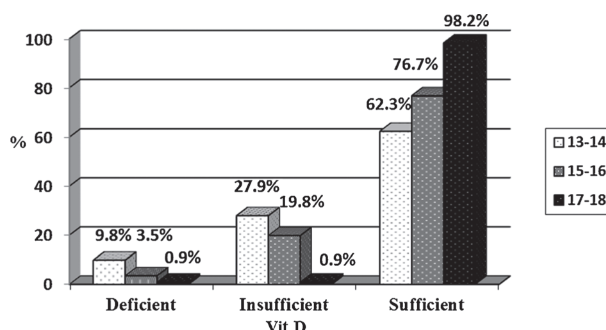

Figure 2: Vitamin D level by age group of the participants. $p < 0.01$

min D was significantly lower among younger ages (13–14), at 9.8 %, compared to the two older groups, at 27.9 % and 62.3 % (Figure 2).

The relationship between vitamin D level and parathormone revealed that those who had vitamin D deficiency and insufficiency had hyperparathyroidism (32.0 % and 44.2 % respectively). Among those who had normal levels of vitamin D approximately 72.7 % had normal levels of parathormone. The difference between groups was statistically significant (Table III). The correlation coefficient revealed an inverse significant correlation between both vitamin D and parathormone values and levels (Table III, Figure 3). There is a significant positive correlation between the level of vitamin D and the level of total calcium, but not with ionized calcium (Table III).

Exposure to sun was significantly associated with the levels of Vitamin D, as those who had vitamin D deficiency and insufficiency were not exposed to sunlight (8.4 % and 36.8 % respectively). Knowledge of the importance of vitamin D, physical activity, soft drink consumption and smoking had no statistically significant effect on the level of vitamin D (Table IV).

Finally, multinomial regression analysis revealed that age, sun exposure and Ca levels were the only significant independent predictors of hypovitaminosis D among the studied group, while other factors as; sex, smoking, soft drinks, sports and ionized calcium weren't considered significant independent variables affecting Vitamin D level (Table V).

Discussion

Vitamin D insufficiency is widespread [28] and is present almost worldwide. From the findings of our study it is evident that vitamin D deficiency constitutes a public health problem among the studied group. This group, students 13–18 years of age, is important as it represents our future workforce. Vitamin D is important for calcium absorption and bone growth and accretion, in

Table III: Relationship between Vitamin D levels, related minerals and parathormone.

Vitamin D level	Deficient 25 (%)	Insufficient 86 (%)	Sufficient 355 (%)	r*	p
Parathormone:					
– Below normal	0 (0.0)	1 (1.2)	23 (6.5)		
– Normal	17 (68.0)	47 (54.6)	258 (72.7)	−0.206	0.00
– High	8 (32.0)	38 (44.2)	74 (20.8)		
Calcium:					
– Below Normal	17 (68.0)	36 (41.8)	136 (38.3)		
– Normal	4 (16.0)	25 (29.1)	87 (24.5)	0.112	0.01
– High	4 (16.0)	25 (29.1)	132 (37.2)		
Ionized Calcium:					
– Below Normal	12 (48.0)	43 (50.0)	159 (44.8)	0.028	0.54
– Normal	6 (24.0)	25 (29.1)	114 (32.1)		
– High	7 (28.0)	18 (20.9)	82 (23.1)		

*Spearman‘s correlation.

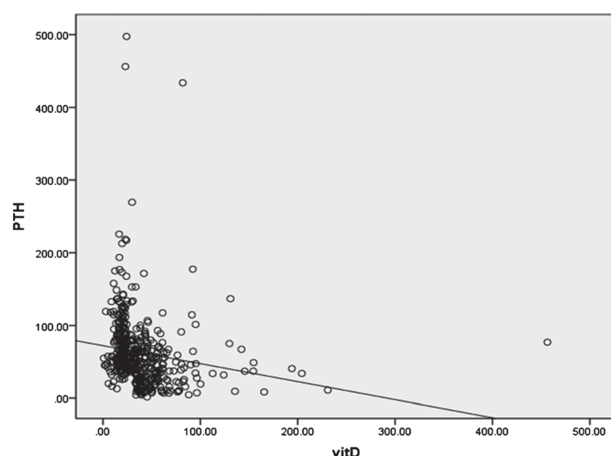

Figure 3: Scatter plot for the relationship between vitamin D and PTH hormone.

addition to skeletal effects, including the maintenance of normal bone turnover, mineralization during adulthood, and prevention of rickets in children [29].

In our study, we used a cutoff point of 25 HO vitamin D to be (1) Deficient if: < 30 nmol/l (12 ng/ml); (2) Insufficient: ≥30 nmol/l but below 50 nmol/l (≥ 12 ng/ml but below 20 ng/ml); and (3) Sufficient: ≥ 50 nmol/l (20 ng/ml) [24]. The prevalence of vitamin D deficiency and insufficiency was approximately 23.8 %. The prevalence in our study is lower than the prevalence in China and Mongolia, especially in children, of whom up to 50 % are reported to have low levels of vitamin D [30]. In a study carried out in Lebanon, 52 % of the students had vitamin D insufficiency [31]. Hypovitaminosis D is also prevalent in children [32] and the elderly [33] living in Latin America. It still constitutes a problem for approximately a quarter of our sampled students, despite ample sunshine throughout the year: this is similar to the results of studies published in the past decade in Sub-Saharan Africa and the Middle East, where between a third and a half of individuals have low levels of serum 25-hydroxyvitamin D [28].

Girls were significantly more affected than boys in our study, 29.3 % to 15.0 %, respectively, a ratio of approximately 2:1. This is much lower than in the study carried out in Saudi Arabia, where prevalence among females reached up to 80 %, [34] and similar to the study conducted in Lebanon, where prevalence among girls was 32 % compared a range of 12 % to 9 % among boys [31, 35, 36]. These findings are expected, as most of the food we eat is deficient in Vitamin D, and only 10–20 % of Vitamin D is acquired through nutritional means, while cutaneous synthesis under the action of sunlight provides us with 80–90 % of our requirements [37]. Despite the fact that the only source of vitamin D in Egypt is exposure to sunlight, as there is no fortification of foods with vitamin D in Egypt [38] and our country is a sunny country, there is lack of exposure to the sun for multiple reasons, such as the hot weather, fear of skin cancer and a lack of awareness of the importance of exposure to direct sunrays. The veiling, cultural and lifestyle factors of eastern communities, which prefer keeping females

Table IV: Association of awareness and lifestyle aspects among the studied group and Vitamin D level.

	Deficient (%)	Insufficient (%)	Sufficient (%)	P
Awareness:				
– Know about Vit. D (N = 257)	12 (4.7)	46 (17.9)	199 (77.4)	0.69
– Don't know about Vit. D (N = 209)	13 (6.2)	40 (19.1)	156 (74.7)	
Sun Exposure:				
– No (N = 166)	14 (8.4)	61 (36.8)	91 (54.8)	0.000
– Sometimes (N = 40)	8 (20.0)	9 (22.5)	23 (57.5)	
– Yes (N = 260)	3 (1.1)	16 (6.2)	241 (92.7)	
Soft drink intake:				
– No (N = 67)	4 (6.0)	8 (12.0)	55 (82.0)	
– Sometimes (N = 122)	8 (5.5)	25 (20.5)	89 (73.0)	0.542
– Yes (N = 277)	13 (4.7)	53 (19.1)	211 (76.2)	
Sport:				
– No (N = 105)	6 (5.7)	20 (19.1)	79 (75.2)	
– Sometimes (N = 108)	4 (3.7)	22 (20.3)	82 (76.0)	0.912
– Yes (N = 253)	15 (6.0)	44 (17.4)	194 (76.6)	
Smokers:				
– No (N = 454)	25 (5.5)	83 (18.3)	346 (76.2)	0.824
– Ex-smoker (N = 6)	0 (0.0)	2 (33.3)	4 (66.7)	
– Smoker (N = 6)	0 (0.0)	1 (16.7)	5 (83.3)	

Table V: Multinomial regression analysis of predictor variables on Vitamin D level among studied group.

Effect	-2 Log Likelihood of Reduced Model	Chi-Square	Degree of Freedom	Sig.
Intercept	468.811	0.000	0	.
Age group	529.309	60.497	4	0.000
Sex	470.711	1.899	2	0.387
Smoker	477.307	8.634	4	0.071
Sun exposure	480.500	11.688	4	0.020
Soft drink	469.414	1.118	4	0.963
Sport	474.193	3.920	4	0.250
Ca level	496.360	27.549	4	0.000
Ionized Ca level	475.959	7.148	4	0.128

indoors, are additional factors in vitamin D deficiency in females.

We noted a significant improvement in the level of vitamin D with increased age in our study. A possible explanation for this might be increased awareness, or more importantly in our opinion, a greater proportion of life outdoors as parents worry less as their children grow older, and thus allow more activities outside the house. They also become engaged in more sports. This in turn allows more exposure to the sun, which has a strong association with the levels of vitamin D The exposure of bare skin to direct sunlight is needed for vitamin D synthesis [30]. This was revealed in our study, where low levels of Vitamin D were associated with absent or interrupted sun exposure, which agrees with other studies in Malaysia and Turkey [39, 40].

The of awareness of the students regarding the importance of vitamin D did not significantly affect their vitamin D levels. Other known risk factors for vitamin D deficiency, identified from other studies, include drinking soft drinks, smoking and a lack of physical activity, but these were not clear in this study. This might due to the difficulty in taking proper histories from the youngsters participating in the study, as they do not find it of importance to record their consumption of soft drinks, smoking or lack of physical activities. The main aim of this study was to determine the prevalence of vitamin D deficiency, as to our knowledge, no community based study has been carried out among Egyptian adolescents.

Measuring parathormone levels in our study demonstrated an inverse correlation with levels of vitamin D, which was similar to most other published studies [30].

The total amount of calcium varies with the level of serum albumin, a protein to which calcium is bound. The biologic effect of calcium is determined by the amount of ionized calcium, rather than the total calcium. Ionized calcium does not vary with the albumin level, and therefore it is useful to measure the ionized calcium level when the serum albumin is not within normal ranges, or when a calcium disorder is suspected despite a normal total calcium level [28]. In the present study, higher percentages of the students were having lower levels of total and ionized calcium levels (40.6 and 45.9 %) respectively. An explanation for this situation may be that our study was conducted in rural areas and in public schools where the socioeconomic state and income level is not high, which is reflected in the types and amounts of food consumed, as 52.4 % had a low calcium intake (< 900 mg/day), and a higher percentage of the students (approximately 60 %) gave a history of consuming soft drinks, which in turn affects calcium levels. As dietary calcium deficiency leads to secondary vitamin D deficiency, the level of total calcium significantly correlates with the level of vitamin D in our study, while the ionized calcium did not show the same correlation. These results are similar to those of a related study conducted in India [41].

Our results from multinomial regression support our suggestion that more exposure to sun rays with increased age, is the most important factor responsible for vitamin D status.

The present study has certain limitations. There were many difficulties with the application of food frequency structured questionnaire during the pilot study that forced us to switch to a structured questionnaire that was more suitable for, and acceptable to the target group. Information on nutrition and activity was obtained by self-report from the students, which has possibilities of recall bias. Our study was, however, the first community-based study conducted in Egypt to measure the prevalence of vitamin D and factors affecting it.

Conclusion

Hypovitaminosis D is a health problem among adolescents in Zagazig district, Egypt as it reaches approximately 23.8 % among the whole group. Girls were at higher risk than boys, mainly due to cultural and lifestyle practices. An increase in age and sun exposure are associated with an improvement of vitamin D status. Parathyroid hormone and total calcium levels significantly correlated with vitamin D, while ionization was not. The inverse relationship with parathyroid hormone suggests that low levels of vitamin D can have a deleterious effect on skeletal growth among adolescents.

There is a need to consider vitamin D supplementation for school children in order to improve biochemical findings related to Vitamin D deficiency, together with encouraging increased awareness through a proper health education program about sun exposure, healthy diet and lifestyle. Further studies are needed in other areas in Egypt to confirm these findings and identify the risk factors responsible for the occurrence of vitamin D deficiency.

Acknowledgements

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The authors declare that there are no conflicts of interest.

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