Original research

Vitamin D deficiency and anemia among pharmacy students

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Abstract: Prevalence of hypovitaminosis D is not restricted to the elderly and hospitalized population. Worldwide, the rate of prevalence of vitamin D deficiency has grown up rapidly in adults over the past decades. Among Libyan population including young students may has a high risk of vitamin D deficiency. The aims of this study were to examine vitamin D status among pharmacy students of Sebha University and to study the hematological profile as well as correlation of vitamin D deficiency with incidence of anemia among the students. This study was carried out on Pharmacy students from 13th January to 12th March, 2020. This is a cross-sectional study designed to determine vitamin D status among healthy young pharmacy students studying at the Sebha University. The blood samples were collected randomly from 62 pharmacy students to analysis complete blood count and 25-hydroxyvitamin D. The concentration of hemoglobin on total student was 12.5 ± 1.9 g/dl which was normal according to the WHO level (12.0 g/dl). Out of total, 36 students (59.1%) were found to have normal hemoglobin concentration (13.7 ± 1.4 g/dl) and 26 students (40.9 %) were found to have low hemoglobin concentration (10.8 ± 1.1 g/dl). Other blood profile as HCT, MCV, MHC and MCHC where statistically significant lower but the counts of RBCs, WBC and platelets were not on anemic group comparing to normal group. The present study reported that majority of pharmacy students in male and female blood donors have low vitamin D levels which represent (87.0%). Out of the total participants (n = 54) who have low vitamin D (n = 49, 79.0%) were classified under vitamin D deficiency category while (n = 05, 08.0%) of students had vitamin D insufficiency. In conclusion, prevalence of hypovitaminosis D (low 25-hydroxyvitamin D) among the pharmacy students at Sebha University was highly occurrence with concomitant a high rate of prevalence of anemia. Thus, vitamin D deficiency at this age represents a public health problem that should be addressed.

Introduction

Worldwide the rate of prevalence of vitamin D deficiency has a grown up rapidly in adults during the over two decades [1, 2]. Vitamin D deficiency, or hypovitaminosis, most commonly occurs in people when they have in-adequate sunlight exposure (in particular sunlight with adequate

ultraviolet B rays, UVB) [3] and do not intake foods that are rich in vitamin D [4]. Vitamin D deficiency has differently been defined from country to country. Epidemiological studies showed that low 25-hydroxyvitamin D (25[OH]D) concentrations are associated with various acute and chronic diseases, thus raising a high interest in vitamin D [5]. Elderly people have a higher risk of having a vitamin D deficiency due to a combination of several risk factors, including: decreased sunlight exposure, decreased intake of vitamin D in the diet and decreased skin thickness which leads to further decreased absorption of vitamin D from sunlight [6]. However, young adults are also potentially at high-risk for vitamin D deficiency. This deficiency can cause to them muscle weakness and fractures may ensue [7]. The high prevalence rate of vitamin D insufficiency is a particularly important public health issue because hypovitaminosis D is an independent risk factor for total mortality in the general population [8].

Anemia and vitamin D deficiency are two important public health issues that may accompany many acute and chronic diseases. The association between vitamin D deficiency and anemia is found not only with chronic diseases, such as heart failure, diabetes mellitus and chronic kidney disease but also in the healthy population [9, 10]. Several observational studies have indicated that there is a reverse relationship between vitamin D levels and anemia in adults [11, 12]. Vitamin D has been demonstrated in bone marrow to affect marrow function [13]. Sim and others demonstrate a greater prevalence and risk of anemia in individuals with D25 deficiency compared with those with normal D25 levels [12]. Among Libyan population including young students may has high risk of Vitamin D deficiency. However, there is a paucity of evidence concerning prevalence of vitamin D deficiency and anemia. Therefore, the purpose of the study is to examine vitamin D status among university students and to examine the correlation of vitamin D deficiency with incidence of anemia among them.

Materials and methods

This is a cross-sectional study designed to determine vitamin D status among healthy young pharmacy students studying at the Sebha University. It was carried out between January and March, 2020. All pharmacy students at Sebha University were asked to participate voluntarily in the study. The blood samples were collected randomly from 79 students with ages range between 18 - 25 years. The body mass index (BMI) measurement was done using digital weighing scale without their shoes. Height was measured using a tape measure after asking the students to stand against the wall and take off their shoes. Verbal and written explanation of the study was provided to the students in detail. All the students were informed about the study and were required to read informative brochure to explain the purpose of the survey and the research. The investigator requested patients' verbal and written consent. The ethics approval for the study was obtained from Sebha University Research Ethics Committee (2021).

A venous blood sample (10 ml) was drawn and divided into two tubes:

- A plain tube that does not contain an anticoagulant: the blood sample was placed in it and left for about 30 minutes to clot, then it was placed in a centrifuge to separate the serum. The serum was divided into two eppendorf tubes and then stored at - 20 ^{o}C temperature until the time of analysis.
- A tube containing an EDTA as anticoagulant: 3 ml of blood sample was placed in it and left on the mixing and shaking machine for about 15 minutes, then complete blood count test was performed.

By the automated “Mythic” analyser, the collected specimens were analyzed for complete blood count (CBC) parameters such as hemoglobin (Hb), red blood cells (RBCs), white blood cells (WBCs), platelets count (PLT), hematocrit (HCT), mean corpuscular volume (MCV), mean cell hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Serum 25-hydroxyvitamin D concentration was measured at certified laboratory at Sebha Medical Center, using a radioimmunoassay kit which is the recommended method for vitamin D assessment in epidemiological studies [14]. According to a Society for Adolescent Health

and Medicine [15] and Endocrine Society [16], it is used the following cut-off of the reference ranges for 25[OH]D were as follows: 0 - 20 ng/ml (deficiency), 21 - 29 ng/ml (insufficiency) and 40 - 100 ng/ml (sufficiency). Thus, hypovitaminosis D was defined in the presence of 25-OH-D levels < 30 ng/ml.

Statistical analysis: All data were analyzed by using Microsoft Office Excel - 2013 and SPSS statistical Package. The generated data analyzed into percentage, variant increase and decrease, mean and standard deviation. Paired *t*-test was used to compare between the two groups. A p values < 0.05 was taken as the level of statistical significance difference.

Results

As shown in **Table 1**, this study was conducted on healthy university students of Faculty of Pharmacy, their main age was 20.6 ± 3.2 years, height was 1.6 ± 0.1 meter, weight was 54.7 ± 11.2 Kg and body mass index was 20.4 ± 3.8%. Complete blood counts were studied on 62 students. Their main hemoglobin level was 12.5 ± 1.9 g/dl which was normal according to WHO level (12 g/dl).

Table 1: Anthropometric measurements

Age (years)	Height (meter)	Weight (Kg)	BMI
20.6 ± 3.2	1.6 ± 0.1	54.7 ± 11.2	20.4 ± 3.8%

Data in **Table 2** show that all CBC parameters were normal except for MCV which is less than normal (72.6 ± 7.7).

Table 2: Hematological profile

Parameter	Value	Parameter	Value
Hemoglobin (g/dl)	12.5 ± 1.9	Hematocrit (%)	35.8 ± 4.1
Red blood cells (10^6 cells/mm^3)	04.9 ± 0.5	MCV(fl)	72.6 ± 7.7
White blood cells (10^3 cells/mm^3)	05.6 ± 1.5	MCH (pg)	25.3 ± 3.3
Platelets count (10^3 cells/mm^3)	276.5 ± 77.3	MCHC (%)	34.9 ± 1.6

In order to study the prevalence of anemia in the students, they were divided into two groups according to WHO hemoglobin level: normal Hb concentration (Hb ≥ 12 g/dl) and abnormal Hb concentration (anemic) (Hb < 12 g/dl). The results showed that 36 students (59.1%) were found to have normal hemoglobin concentration (13.7 ± 1.4 g/dl) and 26 students (40.9%) were found to have low hemoglobin concentration (10.8 ± 1.1 g/dl). Also, levels of HCT, MCV, MHC and MCHC on anemic group where statistically significant lower when compared to the normal group. However, there were no statistically significant differences in the number of RBCs, WBC and platelets on anemic group comparing to normal group as shown in **Table 3**.

Data showed that 40.9% of the students were suffering from anemia, this prevalence is considered to be severe according to WHO classification of anemia in population. Normal (04.9% or lower), mild (05.0 - 19.9%), moderate (20.0 - 39.9%) and severe (40% or higher). WHO classified the degree of anemia into: mild (11.0 - 11.9 g/dl), moderate (08.0 - 10.9 g/dl) and severe anemic (< 7.9 g/dl). The results showed that 56.25% was fund to be mild (Hb = 11.5 ± 0.3 g/dl), 40.5% moderate (Hb = 10.2 ± 0.7 g/dl) and 03.25% was severe (Hb = 07.9). The concentration of 25-OH vitamin was studied on 62 students. Their main vitamin D concentration was 17.5 ± 12.5 ng/ml which was abnormal according to the normal range of 25-OH vitamin kit (30 -100 ng/ml). According to the normal range of 25-OH vitamin kit, 8 students (13%) were found to have a normal 25-OH vitamin concentration (51.9 ± 17.5 ng/ml) and 54 students (87%) were found to have abnormal (low) 25-OH vitamin concentration (hypovitaminosis D) (12.3 ± 5.0 ng/ml). Out of the total participants (n = 54) who have low vitamin D, 49 students (79%) of them classified under vitamin D deficiency category while five students (08%) had vitamin D insufficiency.

To study the effect of 25-OH vitamin D concentration on Hb profile, the samples were divided into normal group (30 - 100 ng/ml) and abnormal group (< 30 ng/ml) of 25-OH vitamin D concentration. Data showed there is no significant difference for the Hb profile between both groups as shown in **Table 4**. Studying the correlation between the level of vitamin D concentration and Hb concentration, it is found that there was positive correlation but statistically insignificant (r = 0.573, p = 0.362).

Table 3: Hematological profile of normal and abnormal (anemic) groups

Parameter	Normal group n = 36	Abnormal group n = 26	P value
Haemoglobin (g/dl)	13.7 ±1.4	10.8 ±1.0	0.001
Red blood cells (10^6 cells/mm^3)	5.0 ± 0.5	4.8 ± 0.4	0.223
White blood cells (10^3 cells/mm^3)	5.7 ± 1.5	5.5 ± 1.5	1.00
Platelets count (10^3 cells/mm^3)	261.6 ± 74.5	300.7 ± 73.9	0.175
Hematocrit (%)	38.4 ± 3.2	32.2 ± 2.3	0.001
Mean cell volume (fl)	77.3 ± 6.5	67.0 ± 6.5	0.001
Mean cell haemoglobin (pg)	27.5 ± 2.4	22.7 ± 3.1	0.001
Mean corpuscular HB concentration (%)	35.7 ± 1.2	33.8 ± 1.5	0.001

Table 4: Hematological profile of normal and abnormal vitamin D groups

Parameter	Normal vitamin D n = 8	Abnormal vitamin D n = 54	P value
Hemoglobin (g/dl)	12.4 ± 1.2	12.2 ± 1.6	0.207
Red blood cells (10^6 cells/mm^3)	5.0 ± 0.3	5.0 ± 0.5	0.223
White blood cells (10^3 cells/mm^3)	5.0 ± 1.5	5.9 ± 1.4	1.00
Platelets count (10^3 cells/mm^3)	289.2 ± 86.5	266.6 ± 73.4	0.632
Hematocrit (%)	34.9 ± 3.8	35.9 ± 4.4	0.840
Mean cell volume (fl)	77.4 ± 7.5	71.5 ± 8.0	0.521
Mean cell hemoglobin (pg)	25.7 ± 3.2	25.0 ± 3.5	1.00
Mean corpuscular HB concentration (%)	34.8 ± 1.4	34.9 ± 1.9	0.981

Discussion

The present findings support the hypothesis that vitamin D deficiency is common in pharmacy students and manifest this deficiency by finding of low serum 25(OH)D level in male and female blood donors at Sebha University. Using the definition of serum 25(OH)D concentrations ≤ 30 ng/ml as hypovitaminosis D, of all the screened participants in the present study found that majority of Pharmacy students in male and female blood donors have low vitamin D level which representing 87%. Out the total of participants who have low vitamin D, 79% of them classified under vitamin D deficiency category while only 08% of the students had vitamin D insufficiency. This finding alarmingly, highly prevalent of hypovitaminosis D in the student in Sebha University. People with vitamin D deficiency may develop Osteomalacia [17]. This finding is in line with other published studies conducted in some gulf countries as in Saudi Arabian males which discovered that 90% were deficient and about 10% were insufficient [18]. Furthermore, in a study carried out in Qatar University showed a remarkably high prevalence rate of vitamin D deficiency and insufficiency (97.5%) among healthy college female subjects [19]. Another study conducted on male adolescents in Al Ain in the United Arab Emirates reported that about 20% were deficient and 45% were insufficient [20].

Recently, a study carried out in Libya (medical students at University of Tripoli), a very similar data was reported [21]. Arabia wear the traditional Islamic veil which prevents the penetration of the UVB light needed for the synthesis of vitamin D. The area of study in North Africa and Arabian race with common mild dark skin which could limit the penetration of UVB light and one of risk factors of

vitamin D deficiency [22]. In contrast with our study, Iran's study indicated that the category of insufficiency of vitamin D was more than vitamin D deficiency category which it is found more than half of the female students had vitamin D insufficient compared with half of the same participants had vitamin D deficiency [23]. This variation could be related the difference in sample characteristics and design of the study. This similarity of prevalence rate could be related to the similarity of culture, geographic location and socioeconomic characteristics of these countries with Libyan population. The effect of sunlight on cutaneous vitamin D synthesis can be modified by sunscreen [24]. Previous studies have demonstrated that winter season were associated with lower serum 25(OH)D levels [25]. In consistence with this evidence, the present study conducted and withdrawal of the blood sample of the participants in the winter season from January to March.

The present study found that in addition to high prevalence of vitamin D deficiency, there was a high prevalence rate of anemia. Indeed, almost all anemic participants have hypovitaminosis D level. This finding is in good line with the previous study conducted in Egypt which reported that vitamin D deficiency has a higher incidence rate in Egyptian adolescent females with an iron deficiency anemia compared with healthy controls [26]. A systemic review concluded that vitamin D status has positively been associated with Hb concentrations and inversely associated with risk for anemia, particularly anemia of inflammation [27]. Through these potential mechanisms of action, vitamin D may therefore influence anemia. Almost of all anemia cases in this study were iron deficiency anemia based on MCV of the erythrocytes. Studies have suggested mechanism of action is that vitamin D, by down-regulating pro-inflammatory cytokines and hepcidin, may rise-up iron availability and there is an evidence that vitamin D may support erythropoiesis [27]. A retrospective large study conducted for participants who applied for periodic medical examination to family medicine poly-clinics of training hospital, indicate that vitamin D deficiency is significantly associated with iron deficiency and/or anemia [28]. Still, to examine the association between anemia and vitamin D deficiency was not in the scope of the current study.

Conclusion: This study highlights the prevalence of hypovitaminosis D (25(OH)D) among university students with concomitant high prevalence rate of anemia. The highly prevalence of vitamin D deficiency across Libyan population is significant and supportive despite the abundant sunshine. Vitamin D deficiency at young adult age in Libya represents a public health problem that should be addressed.

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Conflict of interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability statement: The raw data that support the findings of this article are available from the corresponding author upon reasonable request.

Author contributions: All the authors substantially contributed to the conception, compilation of data, checking and approving the final version of the manuscript and agreed to be accountable for its contents.

Ethical issues: Including plagiarism, informed consent, data fabrication or falsification and double publication or submission have completely been observed by authors.

Author declarations: The authors confirm all relevant ethical guidelines have been followed and any necessary IRB and/or ethics committee approvals have been obtained.

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