Original Article

Serum Vitamin D Levels and Associated Risk Factors among Libyan Females living in Tripoli, Libya: A Cross-sectional Study

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Abstract

Background: Vitamin D deficiency is a worldwide health problem and has been associated with religion, faith, caste, education, and socioeconomic status. Scientific evidence indicates that adequate levels of Vitamin D have important implications for the management of health problems. **Aim:** The aim of this study is to investigate the prevalence of Vitamin D deficiency and associated risk factors among Libyan females. **Patients and Methods:** This cross-sectional study included 262 female patients presented with nonspecific musculoskeletal and bone pain at Seoul Hospital in Tripoli, Libya. Vitamin D status was measured and risk factors for Vitamin D deficiency were assessed. **Results:** The mean age ± standard error of mean (SEM) of participants was 40.8 ± 0.9 years (range 18-80 years). In general, approximately 87.7% of the patients (n = 262) have serum Vitamin D concentrations below normal (<30 ng/mL). The mean serum 25-hydroxyvitamin D (25(OH) D) concentration of patients was 13.98 ± 10.2 ng/ml. About 50.8% had 25(OH)D-levels <10 ng/mL which is characterized as severe Vitamin D deficiency, 27.5% had 25(OH)D-levels <20 ng/ml, defined as Vitamin D deficiency. About 9.1% of patients had insufficient Vitamin D, and only 12.6% had sufficient Vitamin D status defined as 25(OH)D-levels ≥30 ng/ml. **Conclusions:** Our data show that patients with generalized body aches, fatigue, and nonspecific bone pains, all have a significant relationship with Vitamin D deficiency strongly related to risk factors such as inadequate exposure to sunlight, wearing covering clothes, niqab, hijab, low dietary Vitamin D intake, and Vitamin D supplementation.

Keywords: Hijab, Niqab, sunlight, Vitamin D

Introduction

Vitamin D was first discovered in 1920 by Mellanby, since then the interest in Vitamin D has been increased considerably among health-care professionals. Studies reported that Vitamin D behaves more like a hormone than a vitamin because it is synthesized in the body when exposed to sunlight, while its active form 1,25(OH)2D circulates throughout the body to influence many other tissues.[1] Ultraviolet (UV) B radiation with a wavelength of 290–320 nanometers penetrates uncovered skin and converts cutaneous 7-dehydrocholesterol to provitamin D3, which in turn becomes Vitamin D3.[2] Maintaining an adequate level of Vitamin D may reduce the burden of chronic diseases and contribute to better health outcomes.[3] Vitamin D is essential to maintain the correct level of calcium in the body by increasing the efficiency of the intestine to absorb dietary calcium and stimulate osteoclast to dissolve the calcium stored in the bone.[4] Vitamin D deficiency occurs when individuals do not get enough exposure to sunlight and do not eat enough foods rich in Vitamin D.

Vitamin D deficiency is associated with an increased risk of fatigue and unexplained bone pain. These, in turn, have a significant negative impact on social life and work performance.[5,6] During the winter season, there is insufficient UV irradiation from sunlight to stimulate the cutaneous synthesis of Vitamin D. Vitamin D levels reach their lowest concentrations during cold seasons, whereas in summer

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seasons, the level of exposure varies according to a number of influences.[7] At latitudes above 37° N and below 37° S, sunlight is insufficient to induce cutaneous Vitamin D synthesis.[8] Obesity also could reduce the serum level of Vitamin D through adipose sequestration of Vitamin D.[9] Darker skin impaired Vitamin D synthesis.[10] Cultural and social lifestyle patterns such as clothing, and the time spent outdoors ultimately affect sun exposure. In Islamic countries, for cultural and religious issues, women wearing clothes that cover their entire body and limited time spent outdoors decreases the amount of sunlight reaching the skin.[11,12] As a Muslim country, wearing hijab and or niqab while going outside is prevalent among the majority of Libyan women. In this study, the relation between Vitamin D deficiency, associated risk factors, and its influence on bone health and muscle fatigue among Libyan females living in Tripoli are addressed.

Patients and Methods

A cross-sectional observational study was conducted among adult female patients aged 18 years and above, presented with generalized body aches, fatigue, and nonspecific bone pains attended both outpatient and inpatient departments of general medicine in Seoul Hospital, during the period of January 2017 until December 2017. Patients, who were not willing to participate in the study, and individuals who suffer from chronic diseases that affect the absorption of vitamin D such as chronic liver disease and kidney disease, and subjects who are taking drugs that could influence Vitamin D like steroid and antiepileptics, were excluded from our selective group study. A total of 262 adult female patients were recruited in the study with a given consent after explanation of the aim of the study. The study was approved by the Institutional Ethics Committee of Libyan Academy.

Data were collected on questionnaires on the day of the visit, and patients were interviewed face to face to gather the necessary data. A questionnaire form was used to collect the information about personal details including age, gender, clothing style, sunlight exposure, dietary habits, and taking Vitamin D supplements as medical advice or self-care.

The amount of sunlight exposure and behaviors were assessed by a questionnaire adapted from a previous study.[13] Participants were asked about their duration and frequency (per week) of doing outdoor activities in five domains, namely activity at work, transportation, home, exercise, and others. In each domain, participants were asked to report their usual outdoor attire and use of sunscreen and umbrellas. In addition, asked for participants' time of direct sun exposure based on three-time segments; morning, afternoon, and late afternoon/early evening. The Rule of Nine is described by Knaysi *et al*.[14] was used to estimate the proportion of body surface area (BSA) exposed to sunlight based on the reported attire during outdoor activity. The sun index was calculated using the following equation:

Sun index = time (hours of sun exposure per week) × BSA exposed to sunlight.

Our study followed the food-based dietary guidelines in the WHO European Region,[15] in designing a questionnaire form that has been used for a dietary section survey, which recommended milk/fish count definitions of portions and servings per week. Patients were asked to describe the amount of milk intake number of consumed milk cups per week (<1 cups/week = low, between 2 and 4 cups/week = moderate, and over 4 cups/week = high).

Seafood, which includes fish and shellfish, the patients were asked to describe the amount of servings time per week (<1 serving time/week = low, between 2 and 4 servings time/week = moderate, and over 4 servings time/week = high).

The serum level of 25-hydroxyvitamin D (25(OH)D) was proposed to be the most reliable indicator of Vitamin D adequacy because it is the major circulating form in the blood with a half-life of 2–3 weeks and reflects, both cutaneous synthesis and dietary sources of Vitamin D metabolites.[16] A blood sample (5 ml) was collected from each subject at the end of the health visit. All tests were performed in the hospital clinical laboratory using available ELISA kit 25-OH Vitamin D Serum Quantification provided by Roche Diagnostics Co., Ltd., Germany, following the American Society for Testing and Materials communication protocol (ASTM communication protocol) on Cobas 411 Automatic Electrochemiluminescence Immunoassay Analyzer (Roche Diagnostics, Mannheim, Germany). The technique has a sensitivity of 3 ng/ml for 25-OH Vitamins D2 and D3. Anti-25-OH vitamin D3 antibodies detect the presence of 25-OH Vitamin D indicated by peroxidase activity and measured as a substrate OD at 450 nm. Results are expressed as ng/ml. Vitamin D can be measured in nanograms per milliliter (ng/mL) or in nanomoles per liter (nmol/L). A measurement of 1 nmol/L equals approximately 0.4 ng/ml. Different threshold levels are used in many studies.[17,18] These have complicated the evaluation of Vitamin D deficiency and insufficiency. In our study, serum 25(OH) D concentration higher than 30 ng/mL is considered as the normal Vitamin D level (sufficient). Vitamin D deficiency described as a Vitamin D level below 20 ng/mL (50 nmol/L), while severe Vitamin D deficiency was defined as 25(OH)D <10 ng/mL (<25 nmol/L) and Vitamin D insufficiency as <30 ng/ml (75 nmol/L).

Statistical analysis was carried out using Statistical Package for the Social Sciences SPSS 16 (IBM SPSS Inc, Chicago, IL, USA) tables are presented for categorical variables. Qualitative variables were presented as numbers and percentages. Quantitative variables were presented as mean ± SEM main different test (*t*-test). Test the significance of difference for quantitative variables, $P < 0.05$ was considered to be statistically significant.

Results

In total, 262 female patients in Tripoli city participated in this study. The mean age ± SEM of all participants was 40.8 ± 0.9 years (range 18–80 years). Table 1 summarizes patients in relation to serum 25OHD. The present study divided participants into five groups according to different age ranges; <30 years, between 30 and 40, 40 and 50, 50 and 60, and older than 60 years. The results showed that there is no significant difference in serum 25(OH)D levels among the five age groups, although it seems that older women getting lower vitamin D levels than other age groups. Regarding the serum calcium level, there was no significant difference in all age groups.

Table 2 shows that the mean serum 25(OH)D concentration of patients (n = 262) was 13.98 ± 10.2 (range 3–70) ng/ml. About 50.8% had 25(OH)D-levels <10 ng/ml which is often expressed as severe vitamin D deficiency and 27.6% had 25(OH)D-levels <20 ng/ml, the threshold often used to define Vitamin D deficiency. In addition, 9.2% of patients had insufficient amount of Vitamin D; only 12.6% had sufficient vitamin D status defined as 25(OH)D-levels ≥30 ng/ml. The increase of serum 25(OH)D concentration in patients with Vitamin D insufficient and Vitamin D sufficient was significant with patients had Vitamin D deficiency (P = 0.001).

Dietary intake, mainly food sources rich in Vitamin D including milk, butter, and oily fish were examined. Table 3 shows that 76 of the participants (29.01%) have a habit of eating foods rich in Vitamin D, whereas (19.08%) of them consume foods low of the vitamin [Table 3]. Patients who reported taking dairy foods rich in Vitamin D had significantly higher 25(OH)D concentrations (23.3 ± 0.17 ng/ml) and calcium 9.1 ± 0.05 mg/ml when compared with the moderate (12 ± 0.9 ng/mL; Ca = 8.9 ± 0.06) and low (8.1 ± 0.9 ng/ ml; Ca = 8.8 ± 0.06), (P < 0.05) consumption of food rich in Vitamin D groups.

Patients who reported taking vitamin D supplementation had significantly higher 25(OH)D concentrations (33.8 ± 2.3 ng/ ml) when compared with nonconsumers (9.7 ± 0.8 ng/ml; P < 0.005) [Table 4]. Humans obtain Vitamin D mainly by cutaneous synthesis through UV radiation. Therefore, the time spent outdoors is an important factor in determining a human's exposure to sunlight, which, in turn, impacts on individual Vitamin D status. In this study, nearly 64.51% of the participants stated that their occupation sun exposure status was mainly indoors, while 35.49% outdoor. There was significantly increase in the concentration level of 25(OH)D outdoor (17.6 ± 0.9 ng/ml) when compared with indoor (12.9 ± 1.4 ng/ml; P = 0.02) [Table 4].

Calculation of the percentage of skin exposed (dress style) was based on the "rules of nines" described by Knaysi *et al.*.[14] According to the rule of nines, the head and neck sun skin exposure accounts as 9%, each arm as 9%, each leg as 18%, and the front and back torso as 18% each. In this study, 70.6% (n = 185) of women were exposing only the face and hands (hijab) to sunlight (9% BSA). Almost 75 of the participants reported that they were fully covered (Niqab) when they were outdoors under the sun (28.6%). The remaining (n = 2, 0.8%) were exposing their face, arms, and hands (27% BSA) [Table 5].

Table 1: Relation of age and sex with 25(OH)D concentration and calcium level

Age group (year)	*n*	25(OH)D ng/ml	Serum Ca
≤30	81	14.3±1.6	8.9±0.08
31-40	72	11.8±1.1	8.9±0.06
41-50	45	15.3±2.1	9.1±0.3
51-60	33	19.5±3.0	9.1±0.1
Above 60	31	9.0±1.3	8.8±0.16

Table 2: Serum 25(OH)D levels in studied patients

Vitamin D state	*n*	%	25(OH)D ng/ml
Sever deficiency	133	50.8	5.3±0.2
Deficiency	72	27.4	14.2±0.3
Insufficient (suboptimal)	24	9.2	22.3±1.5
Sufficient	33	12.6	43.9±2.2

Discussion

Vitamin D deficiency is a worldwide recognized challenging issue. The reasons for the widespread of vitamin D deficiency are not completely understood, but researchers believe that changes in lifestyle may play an important role. In this study, 262 female patients presented with muscle fatigue and nonspecific bone pain at Seoul hospital in Tripoli Libya. All patients were aged above 18 years. After full investigation of the patients to exclude any other diseases that could cause the pain, level of 25(OH) D and calcium serum concentration were measured. Risk

Table 3: Effect of dietary intake on vitamin D concentration

Variables	*n*	%	25(OH)D ng/ml	*P*	Serum Ca mg/ml	*P*
Consume milk or fish?						
High	76	29.01	23.3±0.17	-	9.1±0.05	-
Moderate	136	51.91	12±0.9	0.000	8.9±0.06	0.03
Low	50	19.08	8.1±0.9	0.000	8.8±0.06	0.001

Table 4: Effect of vitamin D supplement and sun exposure on vitamin D concentration

Variables	*n*	%	25(OH)D ng/ml	*P*
Vitamin D supplement				
Yes	49	18.7	33.8±2.3	0.001
No	213	81.3	9.7±0.8	
Sun exposure practices				
Indoor sun exposure	169	64.51	12.9±1.4	0.02
Outdoor sun exposure	93	35.49	17.6±0.9	

factors such as age, dressing style, and the approximate amount of sun exposure were assessed using a designed questionnaire form after obtaining a written permission from all participants.

In terms of age differences, in general, older women appeared to bear a higher burden of Vitamin D deficiency and pain, particularly having pain in more than one location. The findings regarding pain are consistent with other reports in the literature.[19]

As indicated in the present study, serum calcium was significantly decreased in [Table 3]. When related to vitamin D levels, calcium showed a significant positive correlation with Vitamin D. This result is in agreement with previous studies which reported that Vitamin D deficiency is associated with decreased serum calcium.[20] Studies reported a decline in intestinal calcium absorption in both animals and humans with age, resulting in secondary hyperparathyroidism and bone loss.[21] In our study, the level of calcium was correlated to the level of Vitamin D but has not decreased with the age and remained in a similar range (i.e., between 8.7 and 9.5).

Several studies have reported that a concentration of ≤10 ng/mL of serum 25(OH)D is considered as the indicator of severe Vitamin D deficiency.[17,18,22] In our study, severe Vitamin D deficiency is reported; about 50.8% had 25(OH) D-levels <10 ng/mL which is characterized as severe vitamin D deficiency, 27.5% had 25(OH)D-levels <20 ng/ml, defined as Vitamin D deficiency. About 9.1% of patients had insufficient vitamin D, and only 12.6% had sufficient Vitamin D status defined as 25(OH)D-levels ≥30 ng/ml.

The major source of vitamin D for humans is the exposure of the skin to sunlight.[23] It has been estimated that exposure to sunlight for 5–15 min/day (between 10 AM and 3 PM) on the arms and legs or hands, face, and arms, during the spring, the summer, and the fall, provides the body with its required 1000 IU of cholecalciferol.[24] On the other hand, excessive exposure to sunlight may cause many adverse effects, such as eye damage, skin damage leading to wrinkles and freckles, and it accounts as a major risk factor for skin cancer.[25] The majority of our precipitants (64.51%) do not expose themselves enough to the sun.

The larger the skin surface area exposed to sunlight, the more Vitamin D produced. Therefore, clothing is a significant inhibitor of Vitamin D production.[26] The association of 25(OH)D deficiency and female gender is common in Arabian countries due to the outdoor traditional dressing for women, which requires covering all body parts including the face and hands (Niqab) or all body parts except the face and hands (Hijab). In our study population, females dressing Hijab (uncovered face and hands) or Niqab (i.e., covering all their bodies) have less 25(OH)D serum levels than their counterparts' western style-dressed females (face, arm, hands, and legs) living in Libya, 15.3 ± 1 ng/ml, 11.3 ± 1.3 ng/ml, and 46 ± 23.3 ng/ml, respectively. These results agreed with the study performed with Mallah *et al.* in 2011 in Jordan. They have found that most females wearing Hijab or Niqab have 25(OH)D concentrations below the international recommended values (50 nmol/L). They suggested that although sun exposure should be enough in a country such as Jordan, other factors must play a role in these low concentrations.[27] A high prevalence of Vitamin D deficiency has been also noted in adolescent girls who wear a conservative dress in Turkey.[26,28] This is also consistent with a study finding by Meddeb *et al.* that clothing styles are a major cause of Vitamin D deficiency in Tunisia.[29] Hobbs *et al.* documented a very high prevalence of 25(OH)D deficiency attributable to conservative dress in a sample of Arab-American women living in Dearborn, Michigan, the author attributed the deficiency of the vitamin to conservative dress in the region with limited exposure to the sunlight and an adequate intake of vitamin-rich diet.[30]

Conclusions

Based on our study results, it seems that the majority of female patients are suffering from Vitamin D deficiency. Risk factors such as failure to be getting sufficient exposure to sunlight, wearing covering clothes such as Niqab and Hijab by women, dietary Vitamin D intake, and Vitamin D supplementation may

Table 5: The effects of dressing style and the amount of sun exposure on vitamin D concentration serum Ca

Variables	*n*	%	25(OH)D ng/ml	*P*	Serum Ca mg/ml	*P*
Skin exposed						
Face arms hands and legs (western style dressing)	2	0.8	46±23.3	-	10.3±0.5	-
The face and hands (hijab)	185	70.6	15.3±1.0	0.003	9.0±0.1	0.01
Full vague (Nigab)	75	28.6	11.3±1.3	-	8.9±0.1	0.01

have a significant relationship. We are not encouraging anyone to take off their hijab or niqab, we recommend paying special attention to their diet, getting Vitamin D supplementation with enough calcium to go. So, as ever, more work will be needed to finally close the lid on this interaction. It will be appreciated to follow-up some of those patients who have Vitamin D deficiency, normalizing their Vitamin D levels by changing diet, Vitamin D supplementation, and lifestyle to confirm causality.

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Conflicts of interest

There are no conflicts of interest.

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