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Original article

Prevalence of Vitamin D Deficiency and Its Associated Risk Factors among Pregnant Women in Sbea, Libya

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ABSTRACT

Vitamin D deficiency during pregnancy has been linked to various negative health outcomes for mothers, neonates, and children. Sun exposure behaviors and dietary habits are known to impact vitamin D status. However, there is limited observational data on these behaviors among pregnant women in Sbia town. This study aimed to assess the prevalence of vitamin D deficiency among pregnant women in a developed region of Libya and examine the association between sun exposure behaviors and serum vitamin D levels. A total of 144 healthy pregnant women participated in the study, completing a face-to-face questionnaire on knowledge about vitamin D and sunlight exposure behaviors. Serum concentrations of 25-hydroxyvitamin D (25(OH)D) were measured using an enzyme-linked immunoassay. The results showed that 34% of the participants had vitamin D deficiency (25(OH)D < 20 ng/mL), with 43.8% classified as severely deficient (25(OH)D < 10 ng/mL). Women with shorter durations of sun exposure had significantly lower 25(OH)D levels compared to those with longer durations (15 mins: 13.2 ± 6.9 ng/mL vs. 1 hr: 19.2 ± 11 ng/mL; P = 0.03). Additionally, women who covered most of their bodies except for their face and hands had lower 25(OH)D concentrations (13.2 ± 11.3 ng/mL) compared to those who exposed their face, arms, and legs to sunlight (27.6 ± 8.9 ng/mL; P = 0.01). These findings highlight the persistence of maternal vitamin D deficiency and insufficiency despite a sunny environment in a developed region of Libya.

Keywords: *Vitamin D Deficiency, Pregnant Women, Sun Exposure Behaviors, Dietary Behaviors, Sbia Town.*

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من المعروف أن التعرض لأشعة الشمس والعادات الغذائية تؤثر على نسبة فيتامين د. وقد تم ربط نقص فيتامين د أثناء الحمل بسلوكيات الامهات السلبية مما يؤثر نقصه على الام والجنين.ولهذا السبب تم دراسة هده السلوكيات بين النساء الحوامل في بلدة السبيعة .هدفت هذه الدراسة لتحديد مستويات فيتامين (د) في مصل الدم عند النساء الحوامل ومعرفة معدل انتشار نقصه, ومدى التعرض لأشعة الشمس. شاركت في هده الدراسة حوالي 144 امرأة حامل تتمتع بصحة جيدة، وأكملن الاستبيان شخصيا وجهة لوجه وكان الاستبيان حول معرفتهن بفيتامين د و سلوكيات التعرض لأشعة الشمس. تم قياس تراكيز 25-هيدروكسي. فيتامين د (OH)D25)) في المصل باستخدام اختبار الامتصاص الإنزيمي المرتبط بالمناعة. وقد أظهرت النتائج أن 34% من المشاركات يعانين من نقص في فيتامين د اقل من 20 نانوغرام/مل، بينما 43.8% من النساء يعانين من نقص شديد اقل من 10نانوغرام/مل. كما اظهرت النتائج وجود انخفاض معنوي في مستوى فيتامين د لدى النساء الاتي يتعرضن لشمس لفترات قصيرة (15 دقيقة: 13.2 ± 6.9 نانوغرام/مل) مقارنة بالاواتي يتعرضن لفترات اطول (ساعة واحدة: 19.2 ± 11 نانوغرام/مل؛ P = 0.03). بالإضافة إلى ذلك، كانت لدى النساء اللواتي كن يغطين معظم أجسادهن باستثناء الوجه واليدين مستويات OH)D25) (13.2 ± 11.3 نانوغرام / مل) أقل مقارنة بالنساء اللواتي كانوا يعرضن وجوههن وأيديهن وأرجلهن لأشعة الشمس الشمس (27.6 ± 8.9 نانوغرام/ مل؛ P = 0.01) . تبرز هذه النتائج استمرار نقص فيتامين د وعدم كفاية عند النساء الحوامل على الرغم من البيئة المشمسة في منطقة متطورة من ليبيا.

INTRODUCTION

Vitamin D plays a crucial role in skeletal development and maintaining calcium homeostasis in the body. Vitamin D deficiency can lead to decreased mineralization of bones, resulting in conditions like rickets in children and osteomalacia in adults(1). Vitamin D receptors are present in numerous tissues throughout the body, including the immune system, cardiovascular system, brain, and endocrine organs. This widespread distribution of vitamin D receptors suggests that vitamin D may have broader functions and implications for overall health(2, 3). Sunlight is the main source of vitamin D, providing the body with up to 90% of its vitamin D, while other sources such as diet provide only 10% of the body's needs(4, 5). Vitamin D is often referred to as behaving more like a steroid hormone than a vitamin because it can be synthesized in the body through exposure to sunlight(6). The skin produces vitamin D when it is exposed to sunlight, and this synthesized vitamin D then undergoes further metabolism in the liver and kidneys to become the active form, known as calcitriol. Calcitriol acts as a hormone that binds to vitamin D receptors in various tissues throughout the body, influencing gene expression and regulating numerous physiological processes (7, 8). During pregnancy, the mother needs more vitamin D than usual for the fetal development, neonatal outcomes, and the long-term health of the offspring (9, 10). Inadequate vitamin D levels in pregnant women have been associated with various adverse outcomes, including gestational diabetes, preeclampsia, preterm birth, and impaired fetal growth(11). Studies have reported a high incidence of vitamin D deficiency among pregnant women in various countries even in countries with abundant sunlight (12, 13). Many factors affect the VD status of pregnant women, including diet, dietary supplements, time spent outdoors, clothing habits, sunscreen use, weight status, skin color, and medical conditions. Understanding the extent of vitamin D deficiency among pregnant women in Libya, and the contributing factors, is the first step to improve vitamin D levels and improve the health outcomes of pregnant women.

METHODS

Data collection

The cross-sectional observational study took place between September and December in Omer Ali Asker Hospital in Sbea town. The study protocol and procedures were approved by the hospital's ethical committee, ensuring that the study adhered to ethical guidelines. All participants were provided with full information about the study's purpose and they gave informed consent to participate in the study. Totally, 144 pregnant women were recruited to participate in this study from the antenatal clinic during their regular visits. The eligibility criteria specify that the target population comprises pregnant Libyan women in all trimesters of pregnancy residing in Sbia town and the surrounding community. Data were collected on questionnaires on the day of the visit, and patients were interviewed face to face to gather the necessary data. The questionnaire covered various aspects including personal details such as age, height, weight, dietary habits, clothing style and taking Vitamin D supplements as medical advice or self-care. The participants' body mass index (BMI) was calculated using the provided height and weight information. The amount of sunlight exposure and behaviors were assessed by a questionnaire adapted from a previous study. Participants were asked to specify the time segments of their direct sun exposure, such as morning, afternoon, or late afternoon/early evening. In addition, they were asked to quantify their daily time spent in the sun, selecting from four categories: indoor or 15 minutes, 30 minutes, or 1 hour outdoor. They were further requested to indicate which parts of their body were exposed to the sun when outdoors. The sun index was calculated using the following equation: Sun index = time (hours of sun exposure per week) × BSA exposed to sunlight. To gather information on dietary habits, our study followed the food-based dietary guidelines in the WHO European Region. Participants were asked to categorize their fish and milk intake as "never," "yes," or provide the frequency of consumption. The questionnaire also recorded the regular use of multivitamins during pregnancy, specifically focusing on pregnancy-specific multivitamins. The gestational age of the participants was recorded based on the expected date of delivery provided by the obstetrician overseeing their pregnancy.

Analytical Procedures

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(14). For the measurement of serum 25(OH)D (25-hydroxyvitamin D), a fasting blood sample of 5 mL was collected from each subject at the end of their health visit. The tests were conducted in the hospital's clinical laboratory using an available ELISA kit for 25-OH vitamin D serum quantification (EUROIMMUN-Medizinische Labordiagnostika AG). The sensitivity of the technique for 25-OH vitamins D2 and D3 was 1.6 ng/mL. The presence of 25-OH vitamin D was detected using anti-25-OH vitamin D3 antibodies, and the peroxidase activity was measured as substrate optical density (OD) at 450 nm. Results were expressed nanograms per milliliter (ng/mL). 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

The collected data, including the questionnaires and clinical information, were entered into a Microsoft Access Database. Data analysis was performed using SPSS version 16. Quantitative data were presented as means ± SD (standard deviation), while qualitative data were presented as numbers and percentages. Student's T-test was employed to determine the significance of differences for quantitative variables. $P < 0.05$ was considered to be statistically significant.

RESULTS

A total of 144 healthy pregnant women from Sbea town participated in this study. The mean age ± SEM of all participants 30 ± 6.4 years (range: 18-44 years). Table 1 summarizes patients in relation to serum 25OHD. The present study divided participants into four groups according to different age range (≤20, 21-29, 30-39, 40-50). Younger pregnant women (aged ≤20 years) had a significantly higher vitamin D deficiency compared with older women (40–50 years) (*p*-value = 0.05). In terms of skin color, the majority of participants (60.4%) had various shades of brown, while 32.6% had fair skin, and 6.9% had black skin. However, there was no statistically significant difference in 25(OH)D levels based on skin color. Only a small number of participants (18) reported using sunscreen. Those who used sunscreen had lower 25(OH)D concentrations compared to those who did not use sunscreen. However, this difference was not statistically significant. The dietary intake of vitamin D was assessed by identifying the main food sources, including dairy foods and oily fish. Among the participants, 63.9% reported including vitamin D-rich foods in their diet, while 36.1% did not consume these foods. Women who reported taking a vitamin D-rich dairy supplement had significantly higher 25(OH)D concentrations compared to non-users (P < 0.05). Supplemental vitamin D intake: The use of dietary supplements, including vitamin D supplements, was not common among Libyan women. However, during pregnancy, a majority of the women tended to consume the necessary supplements. In this study, approximately 86.8% of the participants did not use any supplements, while 13.2% reported using vitamin D supplements during pregnancy.

Table 1. Demographic characteristics of study participants

Variables	*n*	5(OH)D ng/mL	*p-value*
Age group (year)			
≤20	10	13.9± 4.6	
21- 29	61	13.4± 8.9	
30- 39	59	13.6± 9.6	
40-50	14	17.8± 7.8	
Natural skin color			
Different grade of brown	87	14.2±9.8	
Fair	47	12.09±8.2	
Dark/black	10	10.4 ±5.8	
Do you use sunscreen			
YES:	18	11.1± 7.9	*P* = 0·45
NO:	124	14.3± 11.6	
Consume milk, butter or fish?			
YES:	92	18.5 ± 10.7	*P* < 0·05
NO:	52	11.6 ± 9.5	
Vitamin D supplements?			
YES:	19	11.1 ± 9.9	
NO:	125	14.4 ± 10.2	

The mean serum 25(OH)D concentration of pregnant women (n = 144), was 13.7 ± 10.2 ng/mL, with a range of 2-63 ng/m (Table 2). Among these pregnant women, 43.8% had 25(OH)D levels below 10 ng/ml (severe vitamin D D), 34% had 25(OH)D levels below 20 ng/ml (vitamin D deficiency). Only 9 % of the pregnant women had sufficient vitamin D status, defined as 25(OH)D levels above 30 ng/mL. Serum 25(OH)D concentration in women with Vitamin D insufficient and Vitamin D sufficient was significant with women had Vitamin D deficiency (P < 0.02).

Table 2: Serum 25(OH)D levels in pregnant women.

Vitamin state	n	%	25(OH)D ng/ml
Sever deficiency	63	43.8	5.5±2
Deficiency	49	34%	14.7±2.8
Insufficient	19	13.2%	23±2.9
Sufficient	13	9%	37±4.9

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(15, 16). Nearly 52% of the participants reported that their occupation involved mainly indoor work, while 48% had outdoor occupations (Table 3). The duration of outdoor exposure was recorded, there was a significant increase in the concentration of 25(OH)D at 1 hour of exposure (19.2 ± 11 ng/mL) compared to 15 minutes (13.2 ± 6.9 ng/mL; p = 0.03). Generally, UV radiation levels are highest around the middle of the day, 10 am to 2pm, adapted from Cancer Council NSW. Here, one day was divided into three time periods: morning (9am-12pm), afternoon(12pm-4pm) and late afternoon/early evening (4-7pm). The concentrations of 25(OH)D were similar for participants spending the morning and afternoon under the sun, but lower for those spending the late afternoon/early evening. The percentage of skin exposed to sunlight was calculated using the "rules of nines(17).

Table 3: The effects of sun exposure on vitamin D concentration.

Variables	*n*	VDD %	25(OH)D ng/mL
Sunning practices:			
-Exposure to sun indoor	75	85	12.4±10.8
-Exposure to outdoors sun:	69		
15 minutes	27	85	13.2±6.9
30 minutes	14	78	12.7±10
1hr	28	50	19.2±11
Time of exposure			
Morning	38	73.6	14.2±11
Afternoon	85	80	14.2±10.5
late afternoon/Early evening	21	85.7	10.3±6.3
skin exposed			
The face and hands	134	80	13±11.3
face, arms and hands	7	57	18±9.6
The face, arms and legs	3	66.7	27±8.9

According to this rule, the head and neck account for 9% of the body surface area, each arm for 9%, each leg for 18%, and the front and back torso for 18% each. In this study, 93% of women (n = 134) exposed only their face and hands to sunlight (9% body surface area), while covering their arms with clothing and their head with a scarf. Additionally, 4.8% (n = 7) exposed their face, arms, and hands (27% body surface area), and 2% (n = 3) exposed their face, arms, hands, and legs (63% body surface area). Women who reported exposing their face, hands, arms, and legs had significantly higher 25(OH)D concentrations (27.6 ± 8.9 ng/mL) compared to women who exposed only their face and hands (13.2 ± 11.3 ng/mL; $P = 0.01$).

Of the 144 subjects who were vitamin D deficient, 93 (64.6%) were overweight or obese compared with 51 (35.4%) who were healthy or underweight Table.4. Also, our study included pregnant women at various stages of gestation, ranging from 2 weeks to 40 weeks Nearly 16.6% of the women were in their first trimester of pregnancy (gestational age ≤ 13 weeks), and the rest were in their second and third trimester. The prevalence of vitamin D deficiency was observed across all trimesters. Parity, which refers to the number of times a woman has given birth, The study included women with a parity ranging from one to seven times. Across the three parity groups, no significant statistical difference was found between these groups. The study also recorded the history of miscarriage in the participants. Among the 144 women in the study, 37 (25.8%) had experienced a miscarriage. The impact of miscarriage on vitamin D levels in pregnant women is not further discussed in the provided information.

Table.4 Relation between 25(OH)D levels and Pregnancy Variables

Variable	*n*	VDD %	25(OH)D ng/ml
BMI ≤ 25	51	70	14.7± 10.6
Overweight			
BMI 25.1–30)	48	83	11.1± 7.8
BMI >30	45	78	12.8± 10.7
First trimester	24	79	13.1± 9.4
Second trimester	64	78	14± 10.2
Third trimester	56	80	13.9± 10.1
Number of parity			
First pregnancy	40	82	11.7± 8.6
Second	39	73.6	14.7± 9.9
More than two	65	76.5	13.8± 10.1
Miscarriage history			
0	107	72	14±9.4
≥1	37	79.5	11.5±10.8

DISCUSSION

Vitamin D deficiency is a global health concern, and adequate levels of vitamin D are crucial for both maternal and fetal health. Recent studies showed that VDD is common in Libyan women(18-20) . In line with the finding earlier, in this study, we find that over 90% of pregnant women have insufficient/deficient serum 25(OH)D level. Closely to our result, the high prevalence of VDD in women has also been observed in neighboring countries including Tunisia(21), Morocco (22) and Egypt (23, 24)

Despite the abundant sunlight in Libya throughout the year, indicates that there are other factors contributing to low vitamin D levels in this population. Age is one such factor that may influence vitamin D status. Some studies have reported a higher prevalence of VDD among elderly people(25, 26). whereas, our study and other studies revealed that younger women had a higher prevalence of vitamin D deficiency than older women (27, 28). There could be several potential reasons for this age-related difference in vitamin D deficiency. One possibility is that older women, being more health-conscious, may have healthier eating habits and are more likely to take regular vitamin D supplements. Additionally, older women might have a larger internal vitamin D pool, primarily in the form of adipose reserves of 25(OH)D, which could contribute to their relatively higher.

Other factor skin color, Darker skin contains more melanin, which acts as a natural sunscreen and reduces the skin's ability to produce vitamin D in response to sunlight(29, 30) However, another study found no difference in serum 25(OH)D in dark-skin versus fair-skin(31, 32). In line with the previous study., we did not find a difference in serum 25(OH)D level among all skin type. The use of sunscreens protects the skin from erythematous radiation and skin cancers, but at the same time, it prevents the synthesis of vitamin D3. Some studies have shown decrease in vitamin D with sunscreen use(33, 34), but most have not(35, 36), which consist with this study. This may be because the sunscreen has been applied incorrectly or irregularly or with low SPF.

It has been suggested by vitamin D researchers that approximately 5-30 minutes of sun exposure between 10 AM and 3 PM at least twice a week to the face, arms, legs or back lead to sufficient vitamin D synthesis (2, 37-39). Our results support this notion where we found that the concentration of 25(OH) D is positively associated with the duration of sun exposure and the values were a little higher for participants who exposed themselves to sunlight around 10 am to 4pm. Libya is a sunny country almost all of the year, the high rate of vitamin DD among females might be explained by that they are not receiving enough exposure to sun because of their dressing style(40, 41). Most of the participants who did not mind sun exposure were exposing only their faces and hands to sunlight, and they considered themselves as people who like exposure to sunlight and probably believe it is sufficient. In the present study, there was a significant difference in vitamin D levels between individuals who exposed only their face and hands to sunlight compared to those who exposed their face, arms, and legs(42).

Body mass index (BMI) has been widely used to explore the relationship between obesity and vitamin D levels. The association between obesity and vitamin D deficiency has been observed in various studies, indicating that there may be a link between the two conditions(43, 44). Obese individuals may have lower circulating levels of vitamin D due to factors such as reduced bioavailability of vitamin D stored in adipose tissue, decreased sun exposure, and altered vitamin D metabolism (45). In this study the percentage of vitamin DD in overweight and obese much higher than healthy women.

The previous study found a high occurrence of vitamin D deficiency (90%) among pregnant women in the first trimester (46, 47). This indicates that a significant proportion of pregnant women in the early stages of pregnancy may have insufficient levels of vitamin D. In contrast, the study conducted in India reported a higher prevalence of vitamin D deficiency in the third trimester compared to earlier trimesters (35) . This suggests a possible decline in vitamin D status as pregnancy progresses, with a higher likelihood of deficiency in later stages of pregnancy. In present study, the analysis of vitamin D status according to trimesters revealed deficiency across all trimesters. This suggests that vitamin D insufficiency is a prevalent issue throughout pregnancy, regardless of the specific trimester (48).

Several studies have reported a correlation between parity status and vitamin D levels during pregnancy. It has been suggested that nulliparous women may have higher vitamin D levels(49).On the other hand, the Jordanian study found that women who had delivered five or more children had lower concentrations of 25(OH)D compared to primiparous women (women who gave birth for the first time) (50). Another perspective from a study among Caucasian women, which reported no association between parity and hypovitaminosis (51), which aligns with our study findings. In the medical field, the term "miscarriage" is often used to describe the spontaneous loss of a pregnancy before the fetus reaches a viable stage. A miscarriage typically occurs before 20 weeks of gestation, although some definitions may extend it up to 24 weeks or when the fetus weighs less than 500 grams(52). Some studies have found that women who are vitamin D deficient are at a higher risk of experiencing miscarriage compared to those with sufficient vitamin D levels(53, 54). According to the recent study, which suggest no evidence of a causal relationship between miscarriage and 25-

hydroxyvitamin D levels (55). In our study, we observed a decrease in serum vitamin D levels among women with miscarriage compared to other women, but this decrease was not statistically significant. It suggests that there may be a potential association between miscarriage and lower vitamin D levels, but further data and research are necessary to draw definitive conclusions.

CONCLUSION

According to our results, VDD is a significant public health problem in pregnant women in Libya. Therefore, pregnant women should be supplemented with vitamin D and fortified food to mitigate this public health. Further studies are needed to determine whether untreated vitamin D deficiency during early pregnancy translates to maternal and neonatal complications among Libyan women as well as assist in choosing good treatments.

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