# Vitamin D level Among Female Students in College of Nursing in Saudi Arabia and its Relation to Students' Symptoms

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**Abstract: Objective:** To assess the vitamin D level of a cohort of apparently healthy female students in College of Nursing at the University of Dammam in the Eastern Province of Saudi Arabia. To determine whether there is a relationship between vitamin D levels and students' symptoms. **Subjects and methods:** The study was carried out over 4 months period and included ninety-six Saudi Arabian female students aged between 18- 23 years from the College of Nursing at University of Dammam. They answered a questionnaire about their clinical history, including intake of vitamin D and calcium-rich foods and exposure to sunshine and blood samples were taken for routine biochemistry , serum 25-hydroxyvitamin D [25OHD]**,** calcium, phosphorous, alkaline phosphatase and parathyroid hormone levels**. Results:** Thirty students (31%) were found to have vitamin D insufficiency and 27 students (28%) were found to have vitamin D deficiency. However, only 39 students (41%) were found to have normal level of vitamin D despite adequate exposure to sunlight . No statistically significant difference was found between the three groups regarding the number of food serving of different food items rich in vitamin D and calcium . Moreover, the percentage of students who were complaining from fatigue, insomnia and bone ache with vitamin D deficiency and insufficiency were more than those with normal vitamin D level**. Conclusion:** Even in a sunny country like Saudi Arabia the prevalence of vitamin D deficiency in young female is high. Most likely this is due to a racial difference in vitamin D concentration or a genetic predisposition to vitamin D deficiency among Saudi Arabians.. A policy of vitamin D food fortification should be considered in the future, and in the meantime greater use of vitamin D supplements in this population group should be encouraged.

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## 1. Introduction

Vitamin D is a fat-soluble vitamin that is naturally present in very few foods and produced endogenously when ultraviolet rays from sunlight strike the skin and trigger vitamin D synthesis .[1] Its production in the skin can be limited by a number of factors including type of clothing, using sun screen, the content of skin melanin, and the geographic region of living place.[2,3] Vitamin D promotes calcium absorption in the gut and maintains adequate serum calcium and phosphate concentrations to enable normal mineralization of bone and to prevent hypocalcemic tetany, rickets in children ,and osteomalacia in adults.[4] Serum concentration of 25OHD is the best indicator of vitamin D status. It reflects vitamin D produced cutaneously and that obtained from food and supplements ,[5] and has a fairly long circulating half-life of 15 days.[6]

New research shows that Vitamin D has broader effects for health than was originally suspected. Subclinical vitamin D deficiency not only prevents bone from maximal mineral accumulation but also is a risk factor of serious diseases including hypertension, diabetes, multiple sclerosis, depression, osteoporosis, and cancer colon, prostate and ovary.[7-9] Furthermore, experts have recommended that vitamin D inadequacy should be considered in the differential diagnosis of all patients with bone or joint pain, myalgia, fatigue, fibromyalgia, or chronic fatigue syndrome.[10]

Saudi Arabia, a land with abundant sunshine and exposure to sunlight might be assumed to be sufficient to maintain adequate vitamin D status. However, vitamin D deficiency is common among the Saudi population mainly in females. Since all Saudi women cover themselves in dark veils. We wondered if this practice of avoiding exposure to sunlight might be responsible for vitamin D deficiency, and whether or not the dietary habit is a contributing factor.

As early as 1982 Woodhouse and Norton,[11] reported low vitamin D levels in the ethnic Saudi Arabian population. Later, Sedrani *et al.*[12] confirmed the earlier findings, and also found that low vitamin D levels were not related to one region, sex, age or season.[13]Although recent studies were conducted to evaluate the level of vitamin D in different age group of Saudi Arabia population, none of them address the effects of vitamin D deficiency on heath status. To date, representative data in Saudi Arabia that correlate the prevalence of vitamin D deficiency, the contributing factors, as well as the associated symptoms with hypovitaminosis D are lacking.

This study was conducted to assess the vitamin D level among healthy young female nursing students in the Eastern Province of Saudi Arabia and to establish relationship between vitamin D levels and some common musculoskeletal symptoms like fatigue, muscle ache as well as insomnia.

## 2. Subjects and Methods

This study was carried out between 15 September 2011 and 15 January 2012 at King Fahd Hospital of the University-Al Khobar, located in the Eastern Province of Saudi Arabia. The study was approved by the research and ethical committee, University of Dammam and informed consent was obtained from all the participants .A total of one hundred apparently healthy Saudi Arabian female students from first to fourth year of the college of nursing were initially included in the study, 96 met the inclusion criteria and 4 were excluded from the study due to high level of Alkaline phosphatase that had been shown in their blood tests although there was no history or clinical evidence suggesting the underlying cause of that high level of Alkaline phosphatase. A full clinical examination and clinical history were taken from all students participating in the study. Exclusion criteria included: the presence of illness that affect vitamin D status such as malabsorption, acute or chronic liver or renal disease,, endocrine or autoimmune disease, elevated PTH level ,the use of vitamin D or calcium supplements, or drugs that can affect vitamin D metabolism such as anticonvulsants or corticosteroids, and a family history of hypocalcemia or vitamin D disorders such as osteomalecia. In addition to the clinical examination, participants were given a questionnaire addressing their frequency and duration of daily sun exposure, daily intake of food containing vitamin D and calcium, intake of vitamin D or calcium supplementation, history of fatigue, insomnia and bone ache and intake of any medications that could affect vitamin D metabolism . the size of food serving was determined by adequately containing the daily requirement of vitamin D and calcium as 200 ml of fortified milk, 50 g of cheddar cheese and 1 pot of yoghurt and the number of food serving per week was estimated by the participants. Adequate exposure to sunlight was defined as regular exposure at least twice a week between 10 am - 3 pm for 40 minutes with at least forearms and legs exposed to direct sunlight that represent the duration of sunlight exposure required to synthesis 1000 IU of vitamin D. Weight, height and blood pressure were recorded. Blood samples were collected by venipuncture at 8 am in the morning after an overnight fast and an aliquot of blood was taken on EDTA for haemoglobin measurement. Blood were centrifuged at 1500*g*, and sera were kept frozen at -20ºC until the time of analysis. Laboratory studies included measurement of Serum calcium, phosphorous , alkaline phosphatase , fasting glucose, BUN, creatinine, uric acid, , total protein and albumin and alanine transaminase (ALT). These parameters were measured using a clinical chemistry analyser (Dimension RxL, Siemens Diagnostics).Parathyroid hormone levels were measured by intact PTH electrochemiluminescence immunoassay using a biotinylated monoclonal antibody.[14] Serum levels of 25-hydroxyvitamin D (25OHD) were measured by radioimmunoassay using the Wallac 1470 Gamma Counter (WallacInc, Gaithersburg, MD, USA). 25OHD were considered normal if >30 ng/mL. Vitamin D deficiency was defined as a serum level of 25OHD of ≤20 ng/mL and insufficiency as a serum level of >20 ng/mL and <30 ng/mL.[15] The reference range for other variables were: PTH 1.3-7.6 pmol/L, calcium 8.8-10.3 mg/dL ,phosphorous 2.5–4.5 mg/dL, alkaline phosphatase 51–153 IU/L, haemoglobin 12–15 g/dL, fasting glucose 60 – 110 mg/dL, BUN 7 - 20 mg/dL , creatinine 0.5 - 1.4 mg/dL, uric acid 2.0 - 7.5 mg/dL, ALT <35 IU/L, total protein 64–82 g/L and albumin 34–50 g/L. The data were coded and entered using Microsoft excel 2007 and analyzed using SPSS (Statistical Package for the Social Sciences), version 15.0, Chicago, Illinois. Data were summarized using mean and standard deviation for quantitative variables, percentage and proportion for qualitative variables. Comparison between groups was done using chi-square test for qualitative variables. ANOVA (analysis of variance) test was used to compare between the three groups of different vitamin D level followed by multiple comparison using post-hoc test. *P* values of less than 0.05 ($p<0.05$) was considered statistically significant.

## 3. Results

Ninety-six apparently healthy Saudi Arabian female students between the age of 18 and 23years with the mean age of 20.5 ± 1.94 were assigned in this study. All of them showed no clinical or laboratory evidence of any medical illness and none of them was pregnant , lactating or smoking. Eleven students had dark skin and 85 had white skin. Three students were married and two of the three having one son. Their BMI ranged between 18.0 and 27.8 $kg/m^2$ with a mean of 24.3 ± 2.53. The duration of sun exposure ranged between 3.5 and 7 hour /week with a mean of 4.9 ± 0.5.Their mean fasting blood glucose was 86.5 ± 8.2 and that of haemoglobin was 13.3 ± 0.8 . Their mean BUN, creatinine, uric acid, total protein albumin and ALT were 12.8 ± 2.3 , 0.9 ±0.07 , 4.4 ± 0.9 , 68.8 ± 4.9 , 39.3 ± 3.3 and 28.1 ± 7.0 respectively. (Table 1)

Thirty students (31%) had serum 25OHD level between 20-30 ng/ml, a level that is regarded as vitamin D insufficiency, with a mean of 24.3 ± 2.53, and 27 students (28%) had serum 25OHD level <20 ng/ml, a level that is regarded as vitamin D deficiency, with a mean of 9.7± 4.91. However, only 39 students (41%) was found to have normal level of vitamin D (>30 ng/ml) with a mean of 45.6± 9.64. (Table 2).

Comparing the vitamin D level in the three groups and their dietary intake that obtained from the questionnaire showed no statistically significant difference between the three groups regarding the weekly number of food serving of different food items rich in vitamin D and calcium. (Table 3).

**Table 1:** Clinical and biochemical characteristics of the study group

| **Parameter** | **Mean ± SD** |
|---|---|
| Mean age (years) | 20.5 ± 1.94 |
| Mean BMI (kg/m2)Males (n = 87) | 24.3 ± 2.53 |
| Mean blood pressure (mmHg) | 74 ± 8.3 |
| Duration of sun exposure (hour /week) | 4.9 ± 0.5 |
| **Laboratory parameters** | |
| Fasting glucose (mg/dL) | 86.5 ± 8.2 |
| Haemoglobin (g/dL) | 13.3 ± 0.8 |
| BUN (mg/dL) | 12.8 ± 2.3 |
| Creatinine (mg/dL) | 0.9 ±0.07 |
| Uric acid (mg/dL) | 4.4 ± 0.9 |
| Total protein (g/L) | 68.8 ± 4.9 |
| Albumin (g/L) | 39.3 ± 3.3 |
| ALT (IU/L) | 28.1 ± 7.0 |

**Table 2**: Distribution of Vitamin D level in the study group.

| **Vitamin D level** | **Mean ± SD** | **Number** | **Percent** |
|---|---|---|---|
| > 30 ng/mL* | 45.6 ± 9.64 | 39 | 41% |
| 20-30 ng/mL** | 24.3 ± 2.53 | 30 | 31% |
| < 20 ng/mL*** | 9.7 ± 4.91 | 27 | 28% |

*Normal Vitamin D level, ** Vitamin D insufficiency, *** Vitamin D deficiency

**Table 3:** Number of food serving per week among vitamin D level groups.

| **Food items** | **Normal level of vitamin D Mean ± SD** | **Insufficiency of vitamin D Mean ± SD** | **Deficiency of vitamin D Mean ± SD** | ***P* value** |
|---|---|---|---|---|
| **Red meat** | 2.15±0.96 | 2.27±.1.41 | 2.41±2.00 | <0.78 |
| **Chicken or turkey** | 2.74±1.42 | 2.87±1.83 | 2.93±1.68 | <0.89 |
| **Fish , tuna, salmon, mackerel & shellfish** | 2.26±1.11 | 2.60±1.69 | 2.30±.95 | <0.51 |
| **Whole eggs or egg yolks** | 2.10±1.50 | 2.43±1.77 | 1.81±1.27 | <0.31 |
| **Milk, yogurt or cheese** | 3.05±2.29 | 3.70±2.36 | 3.48±1.98 | <0.47 |
| **Ice cream** | 2.00±1.58 | 2.00±1.68 | 1.96±1.37 | <0.99 |
| **Fruits, fresh or dried** | 3.03±2.14 | 2.83±1.74 | 2.56±1.60 | <0.61 |
| **Raw vegetables** | 3.03±1.94 | 2.87±2.25 | 2.93±1.83 | <0.94 |
| **Cooked vegetables** | 2.64±1.54 | 2.50±1.96 | 2.07±1.59 | <0.40 |
| **Potatoes, rice or Bread** | 3.69±2.10 | 3.57±1.90 | 3.67±2.03 | <0.96 |
| **Biscuits, bakery muffins, croissants, flaky rolls** | 3.08±2.19 | 3.37±1.90 | 2.81±1.64 | <0.57 |
| **Cereals with milk** | 2.33±2.05 | 2.33±2.17 | 2.52±1.64 | <0.91 |
| **Nuts, nut butters** | 2.10±2.08 | 2.67±1.76 | 2.07±1.97 | <0.41 |
| **Chocolate or candy bars** | 2.85±2.20 | 3.33±2.00 | 2.63±2.22 | <0.44 |
| **Soft drinks and fruit drinks** | 2.67±1.67 | 3.30±1.41 | 2.59±1.64 | <0.16 |

No statistically significant difference between the three groups regarding the number of food serving that contains or fortified with vitamin D

When we compared the three groups regarding the serum level of calcium, phosphorus, alkaline phosphates as well as parathormone level, it was found that vitamin D insufficiency and deficiency groups were having statistically significant lower level of serum calcium and statistically significant higher levels of serum phosphorus, alkaline phosphatase as well as parathormone ($p$<0.001) although there level were still within normal limits. (Table 4)

**Table 4**: Blood investigations among three groups of different vitamin D level

| Blood Investigation in different vitamin D groups | Vitamin D Deficiency group (27) **Mean ± SD** | Vitamin D insufficiency group (30) **Mean ± SD** | Vitamin D sufficiency group (39) **Mean ± SD** | F | *P* value |
|---|---|---|---|---|---|
| Calcium level (mg/dl) | **8.97 ± 0.24** | **9.36 ± 0.37** | **10.02 ± 0.31** | **94.99** | **< 0.001*** |
| Phosphorus level (mg/dl) | **4.06 ± 0.40** | **3.65 ± 0.46** | **3.12 ± 0.28** | **50.637** | **< 0.001*** |
| Alkaline Phosphates level (IU/L) | **98.63 ±5.44** | **87.23 ±7.28** | **65.46 ±10.99** | **127.79** | **< 0.001*** |
| Parathormone level (pmol/L) | **6.84 ±0.47** | **5.84 ± 0.56** | **2.73 ± 0.91** | **311.096** | **< 0.001*** |

*p<0.001 is statistically highly significant

Regarding the students' complain in the three groups, 17 students (63 %) were complaining of fatigue in the group of vitamin D deficiency and 12 students (40%) were complaining of fatigue in the group of vitamin D insufficiency while only 8 students (20.5%) were complaining of fatigue in the group of normal vitamin D level with a statistically significant difference between the three groups (*p*<0.002). Regarding the insomnia, 15 students (55.6%) were complaining of insomnia in the group of vitamin D deficiency and 12 students (40%) were complaining of insomnia in the group of vitamin D insufficiency while only 6 students (15.4%) in the group of normal vitamin D level were complaining of insomnia with a statistically significant difference between the three groups (*p*<0.002). Moreover, 16 students (59.3%) were complaining of bone ache in the group of vitamin D deficiency and 13 students (43.3%) in the group of vitamin D insufficiency while only 6 students (15.4%) in the group of normal vitamin D level were complaining of bone ache with a statistically highly significant difference between the three groups ( *p* < 0.001 ) (Table 5).

**Table 5**: Fatigue, insomnia and bone ache among the three groups of different vitamin D level.

| Student's complaints | Normal Vitamin D Level No. (%) | Vitamin D Insufficiency No. (%) | Vitamin D deficiency No. (%) | $X^2$ | *P* value |
|---|---|---|---|---|---|
| Fatigue | | | | | |
| Yes | 8 (20.5) | 12 (40) | 17 (63) | 12.177 | < 0.002* |
| No | 31(79.5) | 18 (60) | 10 (37) | | |
| Insomnia | | | | | |
| Yes | 6 (15.4) | 12 (40) | 15 (55.6) | 12.025 | < 0.002* |
| No | 33 (84.6) | 18 (60) | 12 (44.4) | | |
| Bone Ache | | | | | |
| Yes | 6 (15.4) | 13 (43.3) | 16 (59.3) | 14.148 | < 0.001** |
| No | 33 (84.6) | 17 (56.7) | 11 (40.7) | | |

*p<0.002 is statistically significant, **p<0.001 is statistically highly significant

## 4. Discussion

Humans get vitamin D from exposure to sunlight, from their diet, and from dietary supplements but natural sunlight remains the best and healthiest source of vitamin D.[16] A diet high in oily fish (tuna, salmon, and mackerel) in addition to dairy products, vitamin D fortified foods such as cereals, orange juice, yogurt, and some margarines has been shown to prevent vitamin D deficiency.[17] However, it has been estimated that one billion people worldwide have vitamin D deficiency or insufficiency.[18]

The current study evaluated serum 25OHD and its relationship to students' symptoms and we found that thirty students (31%) had vitamin D insufficiency and 27 students (28%) had vitamin D deficiency, while only 39 students (41%) were found to have normal level of vitamin D, which means that 59 % of our study group had hypovitaminosis D. This finding is in keeping with reports from European countries,[19] and USA. For example, 52% of Hispanic and black adolescents in a study in Boston,[20] and 48% of white preadolescent girls in a study in Maine,[21] had 25-hydroxyvitamin D levels below 20 ng per milliliter. In other studies, at the end of the winter, 42% of black girls and women throughout the United States had 25-hydroxyvitamin D levels below 20 ng/ml,[22] and 32% of healthy students, physicians and residents at a Boston hospital were found to be vitamin D deficient, despite drinking a glass of milk and taking a multivitamin daily and eating salmon at least once a week.[23] Moreover, in studies in Saudi Arabia, the United Arab Emirates, Australia, Turkey, India, and Lebanon, 30 to 50% of children and adults had 25-hydroxyvitamin D levels under 20 ng /ml.[24-26] On the other hand, this prevalence is similar to our results although it is much higher than the previously reported figure of 20% from the Eastern Province of Saudi Arabia by Holick study.[27]

The Eastern Province of Saudi Arabia is located on the coast of the Arabian Gulf, therefore high fish consumption would be expected. the participants in this study reported a sufficient nutritional intake of vitamin D-rich food including fish, tuna, salmon, mackerel and shellfish. The mean number of fish serving was twice weekly and most of them were eaten on a regular base while other vitamin D containing food such as diary product and vitamin D fortified foods such as cereals and orange juice were received by a mean of 2-3 times weekly . However, the estimated dietary vitamin D intake was correspondent to the recommended daily dietary allowance of 600 IU for such age group (Food and Nutrition Board).[28]

In our study, no difference was found between the three groups of different vitamin D level regarding the number of food serving of different food items meaning that dietary intake of vitamin D was not the direct cause of hypovitaminosis D in our study.

The time of year is an important factor in measurement of vitamin D levels in the diagnosis of insufficiency or deficiency. Bolland *et al*[29] found that summer is the ideal time to measure vitamin D levels as there is seasonal variation with a 14% increase 25(OH)D concentrations in men in the summer, while Saadi *et al*.[30] found that mean serum 25OHD was highest in April, which marks the end of the winter season. Despite being the Eastern Province of Saudi Arabia is a sun-rich region all over the year even in winter, most of the residents of this region avoid sunlight exposure in summer because of increasing temperature, for that reason we conducted our study between September and January, a period of college's first semester during which the weather is not so hot and usually people preferred to get outdoor during this period of the year. In our study group, all students living in air conditioned apartments in which direct entry of sunlight is obstructed by walls or glass in addition to the religious and cultural beliefs that encourage wearing of concealing clothing in addition to restriction of outdoor activities. Despite of that . our participants reported adequate duration of sun exposure per week as they should go to the college for five days per week and have some outdoor activities in addition to spending most of their lunch break (which is from 12-1 pm daily) outdoor at College's garden. So, sun exposure is not a contributing factor to the high prevalence of low vitamin D in our study group. These have previously been reported as risk factors for vitamin D deficiency in Saudi Arabian adolescents.[31] In fact Sedrani *et al*[11] found adequate serum 25OHD levels among Saudi female students who were veiled and more extensively clothed. The finding of Sedrani *et al.* stresses the importance of public education about the importance of increasing the dietary intake of vitamin D and of making time for sun exposure for the sake of obtaining vitamin D. Similar to our findings is the result of *Elsammak et al*[32] who found a high prevalence of a vitamin D deficiency in their sample of Saudi Arabians despite > 65% of participants having adequate exposure to sunlight and > 90% reporting adequate intake of dairy products. A possible explanation of this low 25OHD level is a racial difference in vitamin D concentration or a genetic predisposition to vitamin D deficiency among Saudi Arabians .

Skeletal muscles have a vitamin D receptor and may require vitamin D for maximum function and so vitamin D deficiency causes muscle weakness.[5] In one of the cross-sectional studies, Plotnikoff *et al*.[33] included 150 children and adults with chronic non-specific bone pain. They found that 93% of the patients who were admitted to emergency department with muscle and bone pain and who had a wide variety of diagnoses, including fibromyalgia and chronic fatigue syndrome were deficient in vitamin D. Similarly, Al-Faraj *et al*.[34] discovered vitamin D deficiency in 83 % of 299 Saudi patients who had chronic pain for more than six months without a diagnosis. When Al-Faraj normalized the vitamin D of those with low level, the bone pain resolved in all of them. Moreover, two-thirds of those with apparently normal vitamin D level also eliminated their pain by taking vitamin D supplementations.

Our study showed 17 students (63 %) with fatigue in the group of vitamin D deficiency and 12 students (40%) with fatigue in the group of vitamin D insufficiency while only 8 students (20.5%) were complaining of fatigue in the group of normal vitamin D level. Regarding the insomnia, 15 students (55.6%) were complaining of insomnia in the group of vitamin D deficiency and 12 students (40%) were complaining of insomnia in the group of vitamin D insufficiency while only 6 students (15.4%) in the group of normal vitamin D level were complaining of insomnia. Moreover, 16 students (59.3%) were complaining of bone ache in the group of vitamin D deficiency and 13 students (43.3%) in the group of vitamin D insufficiency while only 6 students (15.4%) in the group of normal vitamin D level were complaining of bone ache. Our findings support the results of other studies regarding the role of vitamin D on general health as well as its impact on musculoskeletal symptoms.

**Conclusion**

The present study revealed that even in a sunny climate like Saudi Arabia the prevalence of vitamin D deficiency in young female is high. Furthermore, The low level of 25OHD found in the current study could not be explained by sun exposure and dietary intake of vitamin D and calcium . However, this finding is consistent with other reports suggesting that vitamin D insufficiency is likely a global problem . Given the high prevalence of vitamin D deficiency, vitamin D fortification of foods and/or the use of vitamin D supplements need to be considered to obtain optimal vitamin D status, even in a sunny countries. Moreover ,we suggest to measure vitamin D level in cases with unexplained musculoskeletal symptoms.

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