Prevalence of Vitamin D deficiency in a multiracial female population in KwaZulu-Natal province, South Africa

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Objective: Vitamin D deficiency is a global health issue affecting many countries, especially those in temperate climates. The aim of this study was to determine the prevalence of Vitamin D deficiency and level of 25-hydroxyvitamin D [25(OH)D] in females categorised by age and race.

Methods: The study was performed between January 2015 and January 2016. This study consisted of 1,976 females stratified by age into < 18, reproductive age (18–45) and > 45 years. Demographic variables were recorded and serum 25(OH)D levels measured by chemiluminescent emission.

Results: The predictors of lower 25(OH)D levels included age and race, (p < 0.0001 for each predictor). Approximately 46% of females had < 20 ng/ml 25(OH)D level, the majority of whom were Indian (35%). The 25(OH)D level varied by race (White 27.33 ng/ml; Black 23.43 ng/ml and Indian 15.05 ng/ml; p < 0.0001). In the <18-year age category, White and Black women had significantly higher 25(OH)D levels when compared with Indian women (38.25 ng/ml vs. 37.51 ng/ml vs. 13.68 ng/ml respectively; p < 0.0001). Similarly, in the reproductive age category (18–45 years); White (27.63 ng/ml and Black (20.93 ng/ml) women had a significantly higher 25(OH)D level compared with Indian (13.15 ng/ml) women (p < 0.0001). Moreover, similar data were observed within the >45-year age category, where the White and Black women had higher 25(OH)D levels compared with Indian women (25.46 ng/ml vs. 22.73 ng/ml vs. 17.04 ng/ml; p < 0.0001) respectively. Irrespective of age category, severe vitamin D deficiency was highest amongst Indian females.

Conclusion: This study demonstrates a significant difference in 25(OH)D concentration in healthy females living in Durban, with Indians presenting with the highest vitamin D deficiency. These findings clearly highlight the need for a policy on vitamin D supplementation and/or fortification of food. Further studies are under way to assess the genetic predisposition of women to vitamin D deficiency.

Keywords: Vitamin D, vitamin D deficiency, race, age

Introduction

Vitamin D is an essential nutrient that is required for overall human well-being, and in particular for bone and muscular health. However, vitamin D deficiency (VDD) is a worldwide problem, affecting individuals who are dark skinned, have diets deficient in vitamin D, who live in latitudes far from the equator and those with cultural apparel covering the body because of sociocultural practices. Some 90% of vitamin D is synthesised in the skin via exposure to ultraviolet radiation, therefore apart from a vitamin D deficient diet the commonest cause of vitamin D deficiency (VDD) is lack of exposure of skin to sunlight.1,2

Vitamin D is present in two forms, namely vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Vitamin D3 is derived from 7-dehydrocholesterol by ultraviolet irradiation of the skin. Vitamin D2 is also present in oily fish whilst D3 is found in plants.3 Both precursors are transported to the liver and are synthesised to 25-hydroxyvitamin D [25(OH)D] and 1,25-hydroxyvitamin D [1,25(OH)2D].1,2

Vitamin D deficiency triggers an increase in production of parathyroid hormone (PTH), which is required for calcium metabolism. An elevation of PTH results in an increase in mobilisation of calcium from increased renal production of 1,25(OH)D; however, due to its short half-life, 25(OH)D is the element that is used to measure vitamin D levels.4,5

The circulating levels of 25(OH)D used to define VDD are controversial, with varying reference ranges used to assess serum concentration each based on the type of assay. Nevertheless, internationally a value of < 50 nmol/l (< 20 ng/ml) is generally regarded as an index of VDD.4,5

Vitamin D deficiency has an adverse clinical effect on children and adults. In children, VDD causes rickets and abnormal growth development, whilst in adults it is associated with a range of conditions such as multiple sclerosis, osteoporosis and cardiovascular diseases.6,7 VDD is also linked to a host of chronic diseases such as auto-immune disease, cancer and insulin resistance.8,9 Apart from a poor diet, risk factors for VDD include age, obesity, dark skin pigmentation, race, cultural/religious attire and a lack of adequate exposure to sunlight.9

The prevalence of VDD is reported to be increased amongst adolescents.11 In a study carried out in Boston, young adults (24%; n = 307) were shown to be vitamin D deficient with the highest prevalence occurring in adults of African descent.12 Vitamin D deficiency is also reported to be more common in females.13,14 A study conducted in Brazil showed that the majority of women with VDD were of reproductive age whilst an Iranian study with a large sample size of 1111 participants also reported a higher prevalence of VDD in females.15,16
Studies on VDD have been conducted in high-income countries, on both native and migrant populations, in Middle Eastern countries and in various geographical regions of the Indian sub-continent. There is a scarcity of data on VDD from countries in sub-Saharan Africa. A number of studies report that dark-skinned individuals who have migrated to countries at higher latitudes have VDD in comparison with the native population. In addition, a study conducted within the nine provinces of South Africa showed that VDD varies according to race and gender. However, the sample size in the different population groups in all nine provinces was small and failed to show statistical significant correlations based on gender, race and reproductive age.

The aim of the current study therefore was to assess the prevalence of VDD amongst the female population group of the different racial groups in the province of KwaZulu-Natal (KZN), South Africa.

Methods

Study population
Ethical permission was obtained from the biomedical research ethical committee at the University of KwaZulu-Natal (BREC reference number BE448/16).

A retrospective population-based study was conducted from January 2015 to January 2016 in Durban and surrounding areas. The study population consisted of a total of 1,976 females born in South Africa. Only participants defined as Black, White and Indian South African population groups were included in the study. Each group was further stratified by age into: less than 18 years, within 18–45 years old (reproductive age) and older than 45 years old. The province of KwaZulu-Natal has a subtropical climate with hot and humid summers and warm and dry winters that are snow and frost free.

All blood samples were sent from general practitioners and were non-hospitalised females. The general practitioners requested vitamin D levels as a general test and not for specific reasons. Clinical, demographic and biochemical data were obtained from a large private laboratory service within the province.

Measurement of serum vitamin D
Initially, serum samples (15 µl) were incubated with dithiothreitol (1 g/l, pH 5.5) and sodium hydroxide 55 g/l to release the bound vitamin D (25-OH) from the vitamin D binding protein. Thereafter, the pre-treated samples were incubated in ruthenium labelled vitamin D binding protein to form the complex between vitamin D (25-OH) and ruthenylated vitamin D binding protein. Subsequently, streptavidin-coated micro particles were incubated with vitamin D (25-OH) labelled biotin forming a ruthenium vitamin D binding protein and biotinylated vitamin D (25-OH) complex. The reaction mixture was then aspirated into the measuring cell where the micro particles were magnetically captured onto the surface of the electrode. Unbound substances were removed. Chemiluminescent emission was measured on the Cobas machine (E411; Roche Diagnostics, Japan) and the level of 25-hydroxyvitamin D was extrapolated from a calibration curve.

We used the following range for vitamin D status:
- Severe vitamin D deficiency: 5–10 ng/ml;
- Suboptimal vitamin D: 20–30 ng/ml;
- Optimal vitamin D: 30–50 ng/ml.

Statistical analysis
Statistical analysis was performed using GraphPad Prism version 5.01 (https://www.graphpad.com/scientific-software/prism/). A normality test was used to determine data distribution. Analysis of variance (ANOVA) was performed to evaluate differences in 25(OH)D for non-parametric data (median ± interquartile range). Dunn’s multiple comparison test was used for comparisons across the groups. A two tailed p-value where p < 0.0001 was considered significant.

Results

Study population

Patient demographics
The study population consisted of a total of 1,976 participants, the demographics of whom are described in Table 1.

Females were stratified by age and ethnicity as follows:
- Less than 18 years: Black (0.8%), White (0.5%) and Indian (2.3%);
- 18–45 years: Black (15%), White (8.7%) and Indian (24%);
- > 45 years: Black (11.7%), White (10.5%) and Indian (26%).

Figure 1 shows that the median interquartile of 25(OH)D levels in the study population were as follows: Black (23.43 ng/ml), White (27.33 ng/ml) and Indian females (15.05 ng/ml).

Figure 2 represents 25(OH)D levels among females who are less than 18 years old and is categorised according to ethnic group. The 25(OH)D median was Black (38.25 ng/ml), White (37.51 ng/ml) and Indian (13.68 ng/ml) females.

Figure 3 represents 25(OH)D levels among females of reproductive age categorised according to ethnicity. The 25(OH)D median was Black (20.93 ng/ml), White (27.63 ng/ml) and Indian (13.15 ng/ml) females.

Table 1: Age category, ethnicity of study population and median 25(OH)D

| Characteristics                      | No. (%) | Median 25(OH)D |
|--------------------------------------|---------|---------------|
| Total number of females              | 1,976   |               |
| Age and ethnicity:                   |         |               |
| Less than 18 years                   | 72 (3.6)| 38.25         |
| Black                                | 17 (0.8)|              |
| White                                | 10 (0.5)| 37.51         |
| Indian                               | 45 (2.3)| 13.68         |
| Reproductive age 18–45 years old     | 947 (47)|              |
| Black                                | 299 (15)| 20.93         |
| White                                | 173 (8.7)| 27.63        |
| Indian                               | 475 (24)| 13.51         |
| Older than 45 years old              | 957 (48)|              |
| Black                                | 232 (11.7)| 22.73        |
| White                                | 208 (10.5)| 25.46        |
| Indian                               | 517 (26)| 17.04         |
Figure 1: 25(OH)D level and ethnicity of study participants.

Figure 2: 25(OH)D levels among < 18-year-olds.

Figure 3: 25(OH)D among 18–45-year-olds.

Figure 4: 25(OH)D levels among > 45 year-olds.

Table 2: Frequency of 25(OH)D deficiencies among the female population

| Age and ethnicity | No. % | 5–10 ng/ml | 10–20 ng/ml |
|-------------------|-------|------------|------------|
| Less than 18 years| 72 (3.6) | 1 (0.05) | 0 |
| Black             | 17 (0.8) | 0 | 0 |
| White             | 10 (0.5) | 0 | 0 |
| Indian            | 45 (2.3) | 12 (0.6) | 21 (1.06) |
| Reproductive age 18–45 years | 947 (47) | 10 (0.5) | 97 (4.9) |
| Black             | 299 (15) | 10 (0.5) | 22 (1.1) |
| White             | 173 (8.7) | 5 (0.25) | 194 (9.8) |
| Indian            | 475 (24) | 154 (7.8) | 103 (5.2) |
| Older than 45 years| 957 (48) | 103 (5.2) | 211 (10.6) |
| Black             | 232 (11.7) | 22 (1.1) | 70 (3.5) |
| White             | 208 (10.5) | 16 (0.8) | 41 (2.0) |
| Indian            | 517 (26) | 103 (5.2) | 211 (10.6) |

Table 3: Vitamin D deficiency, ethnicity and age categories

| Vitamin D deficiency and age | Ethnic groups |
|-----------------------------|---------------|
| Black | White | Indian | p-value |
| Less than 18 years | n = 72: | | |
| 5–10 ng/ml (severe vitamin D deficiency) | 1 (0.05) | 5 (0.25) | 103 (5.2) | 0.0001 |
| 10–20 ng/ml (vitamin D deficiency) | 0 | 0 | 21 (10.6) | 0.0001 |
| Reproductive age (18–45 years) n = 947: | | | |
| 5–10 ng/ml (severe vitamin D deficiency) | 10 (0.5) | 5 (0.25) | 154 (7.8) | 0.0001 |
| 10–20 ng/ml (vitamin D deficiency) | 97 (4.9) | 22 (1.1) | 194 (9.8) | 0.0001 |
| Older than 45 years | n = 957: | | |
| 5–10 ng/ml (severe vitamin D deficiency) | 22 (1.1) | 16 (0.8) | 103 (5.2) | 0.0001 |
| 10–20 ng/ml (vitamin D deficiency) | 70 (3.5) | 41 (2.0) | 211 (10.6) | 0.0001 |

25(OH)D levels
25(OH)D levels varied within the racial groups (Figure 1). The White group had significantly higher 25(OH)D levels compared with the Black and Indian groups (27.33 ng/ml; 23.43 and 15.05 ng/ml respectively; p < 0.0001). Histograms depicting 25(OH)D level in each population group by age are outlined in

Prevalence of Vitamin D deficiency in a multiracial female population in KwaZulu-Natal province, South Africa
The mean age (range) per 25 (OH)D thresholds per age category in each population group is outlined in Table 4.

The Indian group demonstrated severe vitamin D deficiency (< 10 ng/ml) and vitamin D deficiency (10–20 ng/ml) respectively. The Black and White population group had a significantly higher 25(OH)D level when compared with Indian women (38.25 ng/ml vs. 37.51 ng/ml vs. 13.68 ng/ml; p < 0.0001) respectively.

The Indian group demonstrated severe vitamin D deficiency (n = 154[7.8%]) and vitamin D deficient (n = 194 [9.8%]) 25 (OH)D levels.

Similarly, in the > 45-year age category, White and Black women had higher 25(OH)D levels compared with both Black and Indian women (27.63 ng/ml vs. 20.93 ng/ml vs. 13.15 ng/ml; p < 0.0001) respectively.

The Indian group demonstrated severe vitamin D deficiency (n = 103[5.2%]) and vitamin D deficient (n = 211 [10.6%]) levels.

An overall comparison among all three groups indicate that the White population group had a significantly higher 25(OH)D level compared with the Black and Indian groups (p < 0.0001). However, the Black and White population group were severe vitamin D deficiency (1.1%; 0.8%) and vitamin D deficient (3.5%; 2%).

Discussion

The present study investigated the circulating level of 25(OH)D in a fairly large sample (n = 1 976) of female Black, Indian and White South Africans. An overall comparison across all three racial groups indicated that 25(OH)D levels were significantly higher in White compared with Black and Indian women (p < 0.0001). We also report that Indian women have significantly higher levels of VDD compared with the White and Black groups irrespective of age (see Figure 1; p < 0.0001). These results of high VDD in Indian compared with White and Black women within South Africa are corroborated by another study.20 Associations between VDD and racial groups have also been reported in various other studies.12,18,19,21,22 More recently, George et al. (2013) found that the prevalence of VDD was higher among the Indian (15%) compared with the African (3%) population group in Johannesburg, irrespective of gender. These researchers have implicated the influence of sun exposure, season, dietary intake of calcium and vitamin D, total body fat and body fat distribution on 25(OH)D concentration and found levels of < 12 ng/ml in 28.6% of the Indian group in comparison with 5.1% of the Black group.20 These results correlate to both the Indian reproductive age (18–45 years) and > 45-year age groups in the present study, which report severe VDD (13.0%) and VDD (20.4%) respectively.

Factors that may have contributed to the difference in 25(OH)D levels among the population groups enrolled in the current study include season, time of day, latitude, skin phenotype, sun exposure duration, type of clothing and use of sunscreens.11,19,23 Skin pigmentation is considered a determinant of 25(OH)D production. However, Bogh et al. (2010) examined baseline 25(OH)D in fair and dark skinned participants and found that post short-wave ultraviolet B ray exposure (UVB), skin phenotype was not a determinant of 25(OH)D production whilst cholesterol varied with the duration of UVB exposure.21 Despite sunlight being mandatory for 25(OH)D synthesis, countries receiving high levels of sunlight such as Saudi Arabia, Qatar, United Arab Emirates, Turkey, Pakistan and Iran all report high VDD.16 Notably in the latter countries, socio-religious and cultural practices do not facilitate sufficient sun exposure, which is required for 25(OH)D synthesis. The use of traditional clothing that blocks ambient sunlight decreases cutaneous vitamin D production hence contributing to lower levels of 25(OH)D.24 In a study assessing awareness of VDD (18–25 years) in women using the burqa, niqab, hijab and chador, many were unaware of the negative effect of this type of clothing. In Pakistan similar attire is used and VDD occurs in the majority of women of reproductive age (73%).25

A high prevalence of VDD has been reported from various geographical areas in India among children, adolescents, young adults, pregnant women and lactating mothers and those > 50 years old.26 Also, VDD is highly prevalent in Pakistan, Bangladesh, Nepal, Sri Lanka, Myanmar and Bhutan, countries with similar geographic and socioeconomic cultures.26,27 Similar to our study, Bandeira et al. (2006) report that an Indian group demonstrated a higher prevalence of VDD regardless of skin colour and sun exposure.18 Additionally, our results are corroborated by Balasubramanian et al. (2013) who reported a 50–90% prevalence of VDD in the Indian subcontinent, attributable to low dietary calcium along with skin colour and changing lifestyle.24 Nonetheless, the findings of our study show that, despite the westernised lifestyle in South Africa, Indian women have a higher degree of 25(OH)D deficiency. Also supporting this finding was the observation of vitamin D deficiency in South Indians residing in the UK, where a low calcium intake diet, clothing and sun exposure were the influencing factors.28 Irrespective of migration, Indians worldwide adhere to similar cooking methods such as slow cooking and deep-frying. Vitamin D is degradable at temperatures above 200°C, gas cooking reaches a temperature of 1900°C and coal stove 300–700°C. When foods are deep fried, 25(OH)D is thermally degraded as it comes out of the cooking medium.29,30 This

### Table 4: Mean age and 25(OH)D deficiency

| Age and ethnicity | Mean age (5–10 ng/ml and 10–20 ng/ml) |
|-------------------|--------------------------------------|
| Less than 18 years old: | |
| Indian | 11.3 |
| Reproductive age: | |
| Indian | 32.3 |
| Black | 35.0 |
| White | 37.5 |
| Older than 45 years old: | |
| Indian | 58.8 |
| Black | 57.3 |

Figure 2 (< 18 years), Figure 3 (18–45 years) and Figure 4 (> 45 years).

The mean age (range) per 25 (OH)D thresholds per age category in each population group is outlined in Table 4.
Vitamin D deficiency among women of reproductive age has many clinical complications. The reproductive organs including the cervical epithelia, endometrial and epithelial cells of the fallopian tubes, ovaries, and pituitary glands contain receptors and enzymes that are involved in vitamin D metabolism. Vitamin D deficiency may also lead to infertility associated with chronic anovulation and endometriosis, pre-eclampsia and breast cancer. Anovulation and endometriosis, pre-eclampsia and breast cancer have been associated with vitamin D deficiency.34–36 In the present study, Indian women demonstrated severe and deficient 25(OH)D levels compared with other racial groups. These results are corroborated by Capatina et al. (2014) who reported 25(OH)D insufficiency in postmenopausal women. The latter study emphasises the role of vitamin D in women irrespective of their age and highlights the need for vitamin D supplementation in post-menopausal women.

In the < 18-year age group, 41.1% of the Indian participants had severe vitamin D deficiency and VDD levels with the mean age reported at 11.3 years old. Only 1.35% of the Black group was VDD whilst none was reported in the White group (Figure 2). However, when stratified by race, a limitation of the study was the small sample size of < 18-year age group. In the present study 41.1% of Indian children had 25(OH)D < 13 ng/ml, therefore there is a need to assess the 25(OH)D levels in South African children, with a focus on the Indian group. Nonetheless, it is well documented that vitamin D levels in children of Black race are associated with vitamin D deficiency, and dark skin is a risk factor for vitamin D deficiency.12,26 Despite previous studies showing that those of Black ethnicity have lower levels of vitamin D, the results in the present study were dissimilar. Interestingly, a study conducted by Poopedi et al. (2009) reported vitamin D deficiency among children in Johannesburg, SA. The latter study correlates with our study in that 74% of the Black population aged 10 years had adequate vitamin D status.

The strengths of the present study include the large sample size, the standard methodology and the multi-ethnic nature of the study participants. Limitations of the study include variability in age, absence of calcium and PTH levels and the absence of a pregnant cohort. Additionally, pre-treatment and post-treatment comparison of vitamin D supplementation was not performed. Nonetheless, future research should include diet, body mass index, supplementation data and socioeconomic background as well as daily UVB exposure during different seasons within KwaZulu-Natal province, South Africa.

In conclusion, this study has shown that there are major differences in 25(OH)D concentrations in healthy adults living in Durban, with Indians showing the highest 25(OH)D deficiencies. The findings clearly demonstrate that a policy on vitamin D supplementation and or fortification of food should be strongly considered locally. Further studies are under way to assess the genetic predisposition of women to vitamin D deficiency.
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