ABSTRACT
Context: Despite the increasing knowledge about the potential effect of vitamin D deficiency on ovarian reserve of premenopausal women, the burden of this disorder among ‘at risk’ women in sub-Saharan Africa is notably scanty.
Aims:
- To determine the prevalence of vitamin D deficiency among premenopausal Nigerian women
- To assess the relationship between serum 25-hydroxyvitamin D [25(OH)D] and serum anti-mullerian hormone (AMH).
Settings and Design: A prospective cross-sectional study of 218 premenopausal Nigerian women, attending a tertiary hospital in Lagos.
Materials and Methods: Serum levels of 25(OH)D and AMH were assayed using ELIZA technique, for each eligible participant.
Statistical Analysis Used: To determine the association between serum vitamin D and serum AMH were Kruskal-Wallis test and Pearson’s correlation coefficient. Data analysis was performed on 211 participants with complete data.
Results: The mean (± SD) concentrations of serum 25(OH)D and AMH were 37.8 (± 21.4) ng/ml and 1.6 (± 0.6) ng/ml, respectively. The proportion of study participants with serum vitamin D deficiency, insufficiency and sufficiency were 18.5%, 24.6%, and 56.9%, respectively. There was no statistically significant difference in the mean serum AMH among participants with deficient, insufficient, and sufficient vitamin D levels (1.41 ng/ml versus 1.56 ng/ml versus 1.59 ng/ml, \( P \) value = 0.539). Overall, there was no correlation between serum 25(OH)D, and serum AMH (\( r = 0.056, \ P > 0.05 \)).
Conclusion: Although the proportion of women with subnormal levels of serum vitamin D was relatively high, there was no association between serum levels of vitamin D and AMH.
Key words: Anti-Mullerian hormone; Nigerian women; ovarian reserve; premenopausal women; Vitamin D.

Introduction
Vitamin D is an essential steroid hormone, synthesized mainly by the skin on exposure to ultraviolet B radiation. The 25-hydroxyvitamin D [25(OH)D] is the circulating form of vitamin D, and more than 80% is bound to vitamin D binding protein.\(^1\) The assay of 25(OH)D reflects the total body store of vitamin D, and indicates an individual’s vitamin D status. In the human body, 25(OH)D is converted to the physiologically active form, 1,25-dihydroxyvitamin D, by the action of 1-\(\alpha\)-hydroxylase enzyme.\(^2\)
Vitamin D plays an important role in the maintenance of calcium and phosphate homeostasis. In addition to skeletal health, vitamin D has several other important biological effects and functions including normal cell division and differentiation. Vitamin D also contributes to the normal function of the immune system and nervous system, and normal function of other hormones such as parathyroid hormone and insulin.\(^3\,^4\)

The biological action of vitamin D is mediated through vitamin D receptor (VDR), which is found in calcium regulating tissues such as the intestine, bone, and parathyroid glands.\(^5\) Interestingly, VDR has also been identified in the female reproductive organs such as the uterus and the granulosa cells of the ovary. The identification of VDR in the female reproductive system suggests that vitamin D plays an important role in female reproduction.\(^5\,^6\) In animal studies, VDR knockout mice showed reproductive dysfunctions such as uterine hypoplasia, impaired folliculogenesis, and hypergonadotrophic hypogonadism.\(^7\) In human ovarian cells, studies have shown that vitamin D increases the production of ovarian hormones such as progesterone, estradiol and estrone.\(^8\,^9\) In addition, the recognition of functional VDR element in the promoter region of the anti-mullerian hormone (AMH) gene suggests that vitamin D modulates AMH secretion.\(^10\) AMH is secreted by granulosa cells of primary, preantral, and small antral follicles undergoing gonadotropin-independent development. It inhibits recruitment of primordial follicles into folliculogenesis.\(^11\) In female reproductive physiology, AMH is a reliable marker of ovarian reserve.

Several studies have shown an association between serum vitamin D and markers of ovarian reserve. A study by Merhi \textit{et al.}, reported a positive correlation between serum vitamin D and AMH in women aged 40 years or older.\(^12\) Furthermore, Dennis \textit{et al.}, showed that the seasonal changes in AMH level correlated with the vitamin D level in women, and that cholecalciferol supplementation prevented the seasonal AMH change.\(^13\) A study by Jukic \textit{et al.}, showed an inverse relationship between serum vitamin D and serum follicular stimulating hormone (FSH) – a marker of ovarian reserve.\(^14\) These studies suggest a relationship exists between vitamin D and ovarian reserve. In contrast, some studies have shown no relationship between serum vitamin D and AMH in premenopausal women.\(^15\,^17\) Furthermore, vitamin D supplementation studies reported inconsistent effect on serum AMH level in premenopausal women.\(^18\,^19\) Interestingly, none of these studies were conduct among dark-skinned women in sub-Saharan Africa at risk of vitamin D deficiency.

The global picture of vitamin D deficiency shows a major unrecognized epidemic in women of reproductive age, particularly in women with dark skin pigmentation.\(^20\) Paradoxically, little is known of the potential effect of vitamin D deficiency on the ovarian reserve of dark-skinned women in sub-Saharan Africa. This study aims to determine the prevalence of vitamin D deficiency among premenopausal Nigerian women and to assess the relationship between serum 25(OH)D and AMH.

**Materials and Methods**

This was a prospective cross-sectional study performed at the gynecological outpatient clinic of the Lagos University Teaching Hospital (LUTH) Lagos, from September to December 2016.

The study participants were women aged between 18 and 49 years, who presented at the clinic with benign gynecological conditions such as infertility, uterine fibroids, and menstrual irregularity. Excluded from the study were women who had undergone ovarian surgery, pelvic radiation, cancer chemotherapy, and women who had systemic medical conditions such as diabetes, hypertension, parathyroid and kidney diseases. Also, women on exogenous steroid, hormonal therapy, calcium and/or vitamin D supplementation were excluded.

After counselling potential participants on the study objectives and study procedures, consecutive eligible participants who signed written informed consent were recruited into the study. A total of 218 women was recruited for the study. Each participant was interviewed using a structure questionnaire. Information obtained during the interview included socio-demographic data, medical history, medication history including hormonal therapy, calcium and vitamin D supplementation, the average number of daytime hours spent outdoor per day, and dressing style. The subject’s skin complexion was recorded as evident from the face, neck, hands and feet. Each participant was also weighed and height measured using a weighing scale and stadiometer (SECA 220, Hamburg, Germany). Five milliliters of venous blood was collected in plain bottle from each participant. The blood sample was allowed to clot and serum separated within 2 hours of collection. Serum samples were divided and stored in aliquots at –20°C.

The study was commenced after obtaining ethical approval from the Human Research and Ethics Committee of the LUTH (HREC number ADM/DCST/HREC/APP/940).
Laboratory assay
The laboratory analysis was performed at the Central Research Laboratory. The samples were run in duplicate by a single operator. Serum vitamin D and serum AMH assays were performed by an automated ELISA analyser machine (DNM-9602 Microplate Reader) by Madell Technology Corporation (California, USA).

The 25-Hydroxy Vitamin D ELISA reagent kit manufactured by Calbiotech (California, USA) was used for serum vitamin D assay. The analytical sensitivity of the assay is 2.5 ng/ml with a range of detection of 2.5 to 150 ng/ml. The enzyme immunoassay AMH/MIS kit manufactured by Immunotech, Beckman Coulter Laboratories (Marseille, France) was used for the AMH assay. The intra- and inter-assay coefficients of variations were less than 12.3% and 14.2%, respectively. The analytical sensitivity of this assay is 0.14 ng/ml, with a range of detection of 0.14 to 21 ng/ml. Serum AMH values below 0.14 ng/ml were treated as a zero value of analysis.

Data management
Data analysis was done using SPSS version 20 (IBM Corp., Armonk, NY, USA, 2011, version 20.0). Test of normality was performed for continuous data using one-way Kolmogorov-Smirnov test. Categorical data were presented as count (and percentages) and continuous variables were presented as mean (standard deviation) or median (interquartile range), as appropriate. The Kruskal-Wallis test was used to compare the mean serum concentration of AMH between subgroups of vitamin D status. Serum vitamin D status was classified as deficient (<20 ng/ml), insufficient (20‑29.9 ng/ml), and normal status (≥30 ng/ml). Pearson’s correlation coefficient was used to determine the linear relationship between serum 25(OH)D and AMH levels. Partial correlation was adjusted for age and BMI. The level of statistical significance was set at 0.05.

Results
Data were complete for 211 (96.8%) of the 218 women recruited into the study. Of the 211 participants, 101 (47.8%) had infertility, 49 (23.2%) had uterine fibroids, 39 (18.6%) had irregular menstrual cycle, and 22 (10.4%) had other benign gynecological conditions. Table 1 shows the baseline characteristics of study participants. The mean (± SD) age of the participants was 33.8 (± 7.0) years and about half of the participants were aged 30 to 39 years. The mean body mass index (BMI) of participants was 25.8 (± 5.2) kg/m² and over half of them were either overweight or obese. All the participants had dark-skinned pigmentation. None of the participants used sunscreen agents. Most (69.2%) of them had uncovered dressing style. The median outdoor stay per day was 7 hours with a range of 1 hour to 15 hours. Most of the participants neither smoked cigarette nor drank alcoholic beverages.

Overall, the mean (± SD) serum 25(OH)D level for the study participants was 37.8 (± 21.4) ng/ml with a range of 8.0 to 105.3 ng/ml and the mean (± SD) serum AMH concentration was 1.6 ng/ml (± 0.6) ng/ml with a range of 0.58 to 3.7 ng/ml. Of the 211 participants, 39 (18.5%) had vitamin D deficiency and 52 (24.6%) had vitamin D insufficiency.

Figure 1 shows the boxplot of serum AMH levels among study participants with deficient, insufficient and sufficient vitamin D status. The mean differences in serum AMH levels among subgroups of participants with deficient, insufficient and sufficient vitamin D status (1.41 ng/ml versus 1.56 ng/ml versus 1.59 ng/ml) were not statistically
significant ($P$-value = 0.539). Figure 2 shows the Pearson correlation between serum AMH levels and serum 25(OH)D levels. Overall, there was no correlation between serum AMH and serum vitamin D ($r = 0.056$, $P$-value = 0.452); even after adjustment for known confounders such as age and BMI ($r = 0.048$, $P$-value = 0.490).

Discussion

This study showed that the proportion of premenopausal Nigerian women with vitamin D deficiency was 18.5% based on the Endocrine Society Clinical Practice Guideline. In addition, about one-quarter of the women also had vitamin D insufficiency. The prevalence rate of vitamin D deficiency in this study was lower than the 42.4% reported by Nesby-O’Dell et al., among African-Americans in the United States.\[22\] Similarly, Durazo-Arvizu et al., reported a higher proportion of vitamin D deficiency in African-American women when compared to Nigerian women in a study conducted among dark-skinned women.\[23\] Available data suggest that vitamin D deficiency is common in geographical locations with seasonal change in weather conditions, due to inadequate skin exposure to ultraviolet light.\[20,24-26\] In addition, the poor absorption of ultraviolet light by melanin in dark-skinned individuals reduces the synthesis of vitamin D.\[20,25,26\] A meta-analysis by Martin et al., reported a high prevalence rate (56%) of vitamin D deficiency among immigrants from sub-Saharan Africa.\[24\]

In our study, the relatively lower prevalence rate of vitamin D deficiency among premenopausal Nigerian women may be due to the abundant sunshine in the tropics as well as adequate skin exposure to ultraviolet B radiation. Nigeria (4° N and 14° N) is located in the tropics, and most women recruited in our study had lifestyle that assured adequate skin exposure to sunlight such as “uncovered” dressing style and extended outdoor activities during the daytime.
In this study, there was no difference in the mean serum AMH levels among women with different levels of serum vitamin D based on established cut-off values. Furthermore, there was no correlation between serum concentrations of AMH and 25(OH)D. These findings are consistent with similar studies by several authors, which showed no relationship between serum 25(OH)D, and AMH levels in premenopausal women.[12,13,19] In contrast, this study failed to demonstrate the findings by some authors that showed a correlation between serum vitamin D and serum AMH.[12,13,18] Interestingly, the studies that reported a significant correlation between serum vitamin D and serum AMH were not conducted in sub-Saharan Africa. It is plausible that the serum levels of vitamin D in dark-skinned women resident in sub-Saharan Africa have little or no effect on the serum AMH.

The strength of this study lies on its prospective design and study participants, who were dark-skinned women ‘at risk’ of vitamin D deficiency. The potential limitations of this study include the possibility of selection bias because the study was hospital-based.

Conclusion

Even though vitamin D deficiency was relatively common among premenopausal Nigerian women, there was no relationship between serum vitamin D and serum AMH. Thus, it can be inferred that serum vitamin D status has no effect on the ovarian reserve of premenopausal Nigerian women.

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Author’s contributions
MCC and AZ conceptualized the study. AZ participated in data collection. MCC and AZ involved in data analysis and interpretation. AZ and MCC drafted and reviewed the manuscript. The authors read and approved the final version of the manuscript.

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Conflicts of interest
There are no conflicts of interest.

References


