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Serum Vitamin D and Calcium Status in Pregnant Women in Calabar, Southern Nigeria

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ABSTRACT

Background: Vitamin D_3 plays a vital role in calcium homeostasis, which is especially important during pregnancy due to increased foeto-maternal demand. However, in many developing countries, vitamin D and calcium assessments are not routinely conducted during antenatal visits. This study evaluated maternal serum vitamin D and calcium levels among antenatal care attendees.

Methods: An analytical cross-sectional study was conducted using systematic random sampling to recruit participants from an antenatal clinic. Serum vitamin D_3 was measured by enzyme-linked immunosorbent assay (ELISA), while calcium levels were determined using an automated spectrophotometer enzymatic method. Data were analyzed with SPSS version 26.0, using Fisher's Exact test, independent t-test, and Pearson correlation. Statistical significance was set at $p<0.05$.

Results: A total of 224 pregnant women participated, with a mean age of 29.0 ± 4.8 years. The median (IQR) serum vitamin D level was 91.5 (92.8) nmol/L, with 37.5% showing insufficiency (17.9%) or deficiency (19.6%). The mean serum calcium level was 1.78 ± 0.49 mmol/L, and 76.4% were hypocalcaemic. Serum vitamin D correlated positively with gestational age and inversely with systolic blood pressure ($p<0.05$).

Conclusions: The high prevalence of hypovitaminosis D and hypocalcaemia among pregnant women highlights the need for routine nutritional education on vitamin D and calcium intake during antenatal care. Community-based awareness and further research to explore causal relationships are recommended to guide potential policies on screening and supplementation.

Keywords: Serum Levels, Vitamin D, Calcium, Pregnant women, Antenatal Care

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INTRODUCTION

Vitamin D is a micronutrient and fat-soluble vitamin that is essential for calcium and phosphate homeostasis, especially during pregnancy when there is high foeto-maternal physiologic demand.[1]

Vitamin D can be obtained by skin exposure to sunlight, dietary sources (ingestion of oily fish, eggs, cheese, and fortification of dietary products), and intake of nutritional supplements.[2]

Vitamin D exists in two forms: vitamin D2 / ergocalciferol (found in plants, fungi, and fortified foods) and vitamin D3 / cholecalciferol (obtained through skin exposure to the sun's ultraviolet B rays, animal sources, and fortified foods). Ultraviolet B (UVB) radiation from the sun causes the photolytic non-enzymatic conversion of 7-dehydrocholesterol in the skin to pre-vitamin D3, which is subsequently thermally isomerized into vitamin D3.[3] Vitamin D3 is biologically inactive and a prohormone which requires its first hydroxylation in the liver by the enzyme Vitamin D 25-hydroxylase to become 25-hydroxyvitamin D [25(OH)D] or calcidiol. This form is biologically inactive, but it is the main circulatory form of vitamin D and is used as the determinant of the body's Vitamin D status.[4] However, 25-Hydroxyvitamin D 25(OH)D] requires a second hydroxylation in the kidney by 1&-hydroxylase enzyme (1,25 OHD-1-OHase) to form 1, 25-dihydroxyvitamin D [1,25(OH)2D] or calcitriol, which is its biologically active form. This biologically active form of vitamin D stimulates the absorption of dietary calcium and phosphorus. [4,5]

Vitamin D deficiency (VDD) in pregnancy is a global public health problem and has been reported in many low, middle-, and industrialized countries.[6,7] VDD has been linked to a higher risk of several adverse maternal and fetal outcomes, such as pre-eclampsia, gestational diabetes mellitus, preterm delivery, low birth weight, nutritional rickets, and childhood autoimmune diseases. [2,4,5,8-10]

The prevalence of VDD in pregnancy is said to be higher in developing countries such as Nigeria. [11,12] The risk factors associated with vitamin D deficiency include darker skin pigmentation, regular use of sunscreen, obesity, spending little time under the sun, winter/rainy season, highly polluted geological locations, smoking, and extensive body coverage by clothing (for religious, cultural, or protective reasons).[13,14]

The Endocrine Society's 2011 clinical practice guidelines and World Medical Association 2015 and 2025 Statement defined Vitamin D deficiency as serum levels of 25(OH)D <50 nmol/l, insufficiency as serum levels of 52.5-72.5 nmol/l, and optimal levels as >75 nmol/l.[15,16]

In this study, the vitamin D was determined at gestational ages below 20 weeks. This was to enable us to determine the baseline levels of vitamin D early enough in pregnancy, before the effects of potential complications such as pre-eclampsia and gestational diabetes in the second half of pregnancy.

In this study, calcium levels were assayed in addition to Vitamin D levels. This is because both of them are intricately connected in the maintenance of bone health. The levels of both vitamin D and calcium directly affect the efficiency of the other. Vitamin D is required for intestinal reabsorption of calcium. In the presence of vitamin D deficiency, only 10 – 15 % of dietary calcium is absorbed from the intestine compared to the absorption of 30 – 40% in the presence of sufficient vitamin D levels. In addition, pregnancy is associated with significant physiological changes to maintain calcium homeostasis as well as support adequate foetal growth and development. Most of foetal calcium is transferred from the mother through the placenta. Calcium is required by the foetus in the early embryonic stages, particularly for control of cell division and cell signaling. It is also required in the third trimester for the support of foetal skeletal mineralization. [17-19] Thus, the pregnant woman has a higher risk of developing hypocalcaemia than the general population; this is further worsened in the presence of vitamin D deficiency. This underscores the importance of the measurement of both vitamin D and calcium levels, which have been done in this study.

There is a paucity of studies evaluating both Vitamin D and calcium status in pregnancy. Presently, no study on this has been done in our geographical location. This is the research gap that this study seeks to fill. This study has been done among pregnant women to determine the baseline serum vitamin D and calcium levels among antenatal clinic attendees and factors associated with low serum levels of vitamin D.

MATERIALS AND METHODS

Study Design, Population Setting: Pregnant women receiving antenatal care (ANC) in the University of Calabar Teaching Hospital, Calabar, Nigeria, were studied for 10 weeks using an analytical cross-sectional design. The ANC is run each weekday by the Department of Obstetrics and Gynaecology, which

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receives an estimated 420 pregnant women, drawn from diverse settings including referrals from primary and secondary health facilities. Booking of pregnancy at first ANC visit typically occurs during early gestation, with frequent follow-up visits as pregnancy progresses. Routine testing for clinic visits includes vital signs check, urinalysis, haematocrit, and retroviral testing, but excludes assays for vitamin D and calcium.

Sample Size Calculation: A minimum sample size of two hundred and nineteen (219) was calculated using the Cochrane formula for cross-sectional studies ($z^2{*}p\ q/d^2$) with a 95% level of confidence, 5% margin of error, 14.1% prevalence of maternal vitamin D deficiency obtained from a previous study, and an assumption of 15% non-response rate.[20,21]

Eligibility: Consenting singleton pregnant women who were below twenty (20) weeks of gestation were eligible to participate, irrespective of their booking status. Those who were acutely ill and had diabetes mellitus, sickle cell disease, and chronic kidney disease were ineligible to participate. Pregnant women receiving vitamin D and/or calcium supplementation were also excluded.

Sampling Technique: Recruitment of subjects was done using systematic random sampling via the ANC clinic attendance register as the sampling frame. A sampling interval of four (4) was calculated based on estimated client uptake and allocated daily sample size, given the study duration of 10 weeks. Balloting for the first subject was done among the initial four attendees on the register; then subsequent recruitment was carried out using the calculated sampling interval. If a client did not give consent or was not eligible to participate, then the next attendee on the register was recruited, and sampling continued from that point until completed.

Instrument, Data Collection, and Biochemical Assay: A structured questionnaire comprising sociodemographic and obstetric characteristics (section 1) and serum levels of vitamin D and calcium (section 2) was used to obtain quantitative data. The questionnaires were administered to consenting and eligible participants by trained researchers. After consenting participants had had their vital signs checked at the nursing station and seen by their doctor, they were asked to complete section 1 of the questionnaire before having the blood drawn and assayed for vitamin D and calcium. A senior laboratory scientist was engaged to draw blood and perform the biochemical assay. An assay for vitamin D (25-hydroxy vitamin D3) was done using 8 ml of maternal overnight fast venous blood sample. The blood draw was done using aseptic precautions and placed in labeled serum separator test tubes. Following clotting, there was 4,200 rpm centrifuging for 10 minutes, serum separation, and storage at -20 degrees Celsius. The Enzyme-Linked Immunosorbent Assay (ELISA) method was used to assay for vitamin D3. Mindray MR96A microplate reader (Shenzen, China) was used to read the optical density at 450nm, with the calculation of 25(OH)D levels via a standardized plotted curve. Precision studies were carried out using quality control materials. Two levels of quality control materials (high and low control sera) were assayed with each batch of 10 samples analysed simultaneously to ensure analytical accuracy and precision for serum vitamin D analysis. Between – run and within – run precision were carried out. An automated spectrophotometer enzymatic method (Biolis 24i, North Carolina, USA) was used to estimate serum calcium. The normal range values of calcium for this instrument are 2.1 – 2.6 mmol /l. Hypocalcaemia is defined as serum calcium levels below 2.1 mmol /l and hypercalcaemia as serum calcium levels above 2.6 mmol/l.

The ELIZA method assays 25 – hydroxyvitamin D3, which is the standard and best indicator of vitamin D status. This is because it is the major circulating storage form of vitamin D, which has a long half-life of two to three weeks and best reflects total body stores and dietary/supplement intake. It is the best determinant of vitamin D sufficiency, insufficiency, and deficiency. The active form of vitamin D, I,25 – dihydroxyvitamin D has a short half – life of four to six hours and can remain normal or high (due to secondary hypoparathyroidism) when there is vitamin D deficiency. Thus, it is not a reliable test of vitamin D status. Measurement of total vitamin D (D2 + D3) also does not reflect functional storage of vitamin D accurately because it also measures inactive parent compounds Thus, it is also not a reliable marker of vitamin D status. [4,5] The most reliable and standard marker of vitamin D status is 25 – hydroxyvitamin D, which was assayed in this study.

Data Analysis: Field and laboratory data were entered and analyzed using SPSS version 26.0. Sociodemographic and obstetric characteristics were presented using frequency tables. Normality testing indicated a non-normal distribution of serum vitamin D values. Hence, the median and interquartile range were used as measures of central tendency and dispersion, respectively. However, serum calcium was normally distributed; hence, the mean and standard deviation

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were used as means of central tendency and dispersion, respectively. Vitamin D level was categorized as normal (≥30ng/ml), insufficient (20-29 ng/ml), and deficient (<20 ng/ml). Serum calcium was categorized as hypercalcemia (<2.12 mmol/l), normal (2.12-2.62 mmol/l), and hypercalcemia (>2.62 mmol/l). A frequency table was used to present the categories of vitamin D and calcium. Chi-square, Fisher's Exact, independent t-test, and Pearson correlation analysis were used as inferential statistics. P-value was set at 0.05.

Ethical Approval: Ethical approval was obtained from the Institutional Research Ethics Committee before conducting the study (Reference number: UCTH/HREC/33/Vol. 111/026. 1st April 2022). Informed and written consent were also obtained before data collection. Test results were communicated to subjects, and those with abnormal values received counseling and referral for specialist care. The study was conducted with the standards as per the Helsinki Declaration of 1975, as revised in 2000.

RESULTS

Table 1: Sociodemographic and Obstetric Characteristics (N=224)

Variable	Frequency	Percent (%)	95% CI
Age group (years)			
<20	11	4.9	[2.1, 7.7]
21–30	131	58.5	[52.1, 64.9]
31–40	80	35.7	[29.4, 42.0]
>40	2	0.9	[0.0, 2.1]
Educational level			
Primary	2	0.9	[0.0, 2.1]
Secondary	57	25.4	[19.7, 31.1]
Tertiary	165	73.7	[67.9, 79.5]
Marital status			
Married	220	98.2	[96.5, 99.9]
Unmarried	4	1.8	[0.1, 3.5]
Parity			
Nulliparous	60	26.8	[21.0, 32.6]
Multiparous	158	70.5	[64.5, 76.5]
Grand multiparous	6	2.7	[0.6, 4.8]
Gestational age			
First trimester	77	34.4	[28.2, 40.6]
Second trimester	147	65.6	[59.4, 71.8]

Data were analyzed from 224 subjects (96.4% response rate). The cohort was characterized by a mean age of 29.0 ± 4.8years (range: 16–43). Most participants were under 31 years of age (63.4%), married (98.2%), and had attained a tertiary level of education (73.7%). Regarding obstetric history, 70.5% were multiparous, and 65.6% were in their second trimester. Clinical history revealed abnormal blood pressure (hypertension) in 5.4% (95% CI [2.5%, 8.3%]) and a history of miscarriage in 13.4% (95% CI [9.0%, 17.8%]).

Table 2: Serum Levels of Vitamin D and Calcium (N=224)

Variable	Category		%	95% CI
Vitamin D	Normal (>75 nmol/l)	140	62.5	[56.1, 68.9]
	Insufficient (50-75nmol/l)	40	17.9	[12.9, 22.9]
	Deficient (<50 nmol/l)	44	19.6	[14.4, 24.8]
Calcium	Normal (2.1-2.6 mmol/l)	44	19.6	[14.4, 24.8]
	Hypocalcemia (<2.1 mmol/l)	171	76.4	[70.8, 82.0]
	Hypercalcemia (>2.6mmol/l)	9	4.0	[1.4, 6.6]

Serum levels of vitamin D were not normally distributed, with median (IQR) values of 91.5 (92.8) nmol/l. Approximately one-fifth each had insufficient (17.9%) and deficient (19.6%) vitamin D levels (table 2). Hence, 37.5% of subjects had insufficient or deficient levels of vitamin D. Serum calcium was normally distributed with a mean of 1.78 ± 0.49 mmol/l, ranging from 0.44 to 3.66 mmol/l. Approximately three-quarters (76.4%) of subjects had (subclinical) hypocalcemia

Table 3: Factors Associated with Vitamin D and Calcium Status (N=224)

Variable	Vitamin D: Inadequate n (%) [95% CI]	Vitamin D: Normal n (%) [95% CI]	p-value	Hypocalcemia n (%) [95% CI]	Normal Calcium n (%) [95% CI]	p-value
Age group			0.28			0.13
< 30 years	57 (40.1) [32.0, 48.2]	85 (59.9) [51.8, 68.0]		113 (79.6) [73.0, 86.2]	29 (20.4) [13.8, 27.0]	
> 30 years	27 (32.9) [22.7, 43.1]	55 (67.1) [56.9, 77.3]		58 (70.7) [60.8, 80.6]	24 (29.3) [19.4, 39.2]	
Education			0.56			0.98
Sec. or less	24 (40.7) [28.2, 53.2]	35 (59.3) [46.8, 71.8]		45 (76.3) [65.5, 87.1]	14 (23.7) [12.9, 34.5]	
Tertiary	60 (36.4) [29.1, 43.7]	105 (63.6) [56.3, 70.9]		126 (76.4) [69.9, 82.9]	39 (23.6) [17.1, 30.1]	
Marital Status			0.60			0.94
Married	83 (37.7) [31.3, 44.1]	137 (62.3) [55.9, 68.7]		168 (76.4) [70.8, 82.0]	52 (23.6) [18.0, 29.2]	
Unmarried	1 (25.0) [0.0, 67.4]	3 (75.0) [32.6, 100]		3 (75.0) [32.6, 100]	1 (25.0) [0.0, 67.4]	
Parity			0.28			0.14
Nulliparous	19 (31.7) [19.9, 43.5]	41 (68.3) [56.5, 80.1]		50 (83.3) [73.9, 92.7]	10 (16.7) [7.3, 26.1]	
Parous	65 (39.6) [32.1, 47.1]	99 (60.4) [52.9, 67.9]		121 (73.8) [67.0, 80.6]	43 (26.2) [19.4, 33.0]	
Gestational Age			0.80			0.21
1st Trimester	28 (36.4) [25.7, 47.1]	49 (63.6) [52.9, 74.3]		55 (71.4) [61.3, 81.5]	22 (28.6) [18.5, 38.7]	
2nd Trimester	56 (38.1) [30.2, 46.0]	91 (61.9) [54.0, 69.8]		116 (78.9) [72.3, 85.5]	31 (21.1) [14.5, 27.7]	
Miscarriage			0.36			0.96
Yes	9 (30.0) [13.6, 46.4]	21 (70.0) [53.6, 86.4]		23 (76.7) [61.5, 91.9]	7 (23.3) [8.1, 38.5]	
No	75 (38.7) [31.8, 45.6]	119 (61.3) [54.4, 68.2]		148 (76.3) [70.3, 82.3]	46 (23.7) [17.7, 29.7]	
Blood Pressure			0.76			0.56
Normal	79 (37.3) [30.8, 43.8]	133 (62.7) [56.2, 69.2]		161 (75.9) [70.1, 81.7]	51 (24.1) [18.3, 29.9]	
Hypertension	5 (41.7) [13.8, 69.6]	7 (58.3) [30.4, 86.2]		10 (83.3) [62.2, 100]	2 (16.7) [0.0, 37.8]	
Calcium Status			0.18			
Hypocalcemia	60 (35.1) [27.9, 42.3]	111 (64.9) [57.7, 72.1]		—	—	—
Normal	24 (45.3) [31.9, 58.7]	29 (54.7) [41.3, 68.1]		—	—	—

Note: Vitamin D Inadequate includes Deficient and Insufficient levels (<75 nmol/L). No sociodemographic or obstetric variable was associated with serum vitamin D or calcium status (Table 3).

Table 4: Correlation and Comparison of Mean Serum Vitamin D, Calcium, and Continuous Variables (N=224)

Variable	Mean ± SD	SEM	95% CI for Mean	Correlation with Vit D (r)	Correlation with Calcium (r)
Age (years)	29.0 ± 4.8	0.32	[28.4, 29.6]	-0.03 (p=0.66)	0.18 (p=0.01)
Gestational Age (wks)	14.2 ± 4.1	0.27	[13.7, 14.7]	0.15 (p=0.03)	-0.07 (p=0.33)
Systolic BP (mmHg)	114.5 ± 8.0	0.53	[113.5, 115.5]	-0.11 (p=0.09)	-0.09 (p=0.16)
Diastolic BP (mmHg)	71.6 ± 9.4	0.63	[70.4, 72.8]	-0.07 (p=0.73)	-0.04 (p=0.52)
Parity (Number)	1.52 ±0.8	0.05	[1.42, 1.62]	0.01 (p=0.87)	0.01 (p=0.90)
Serum Calcium (mmol/l)	**1.78 ± 0.49**	0.03	[1.72, 1.84]	-0.12 (p=0.08)	--
Serum Vitamin D (nmol/l)	**91.5 ± 12.8**	0.85	[89.8, 93.2]	--	-0.12 (p=0.08)

However, there was a significant positive correlation between gestational age and serum vitamin D (Table 4). There was an inverse correlation between systolic blood pressure (SBP) and serum vitamin D levels, with significantly lower SBP

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among subjects with deficient or insufficient compared with normal vitamin D levels (113 vs 117 mmHg, p=0.03). Also, there was an inverse correlation between serum levels of calcium and vitamin D, with significantly higher levels of serum calcium among subjects with vitamin D deficiency or insufficiency (1.84 vs 1.69 mmol/l, p=0.02).

DISCUSSION

The prevalence of vitamin D deficiency (VDD) in pregnancy in this study is 19.6%, and that of vitamin D insufficiency is 17.9%. Thus, 37.5% of the participants in this study had either vitamin D deficiency or insufficiency. This is relatively high because the study location has a tropical climate with sunshine almost all year round.

VDD in pregnancy from studies done in the Western part of Nigeria reported prevalence rates of 4.8%,[22] 14.1% [23,] and 29%. [24] This prevalence reported in this study is higher than that reported in the first study but comparable to the results in the other 2 Nigerian studies. However, none of these studies assessed the calcium levels of the participants in addition.

The prevalence of VDD in pregnancy reported here is comparable to that obtained in a study in Bangladesh (17.3%) [25] but lower than that obtained in studies in Ghana (81.7%)[26,] Saudi Arabia (50%) [27], and Indonesia (82.8%). [13] These studies also did not measure calcium levels of the participants in addition. This wide variation in the prevalence rates of VDD in pregnancy obtained from different studies may be influenced by the different study designs, sample sizes, socio-demographic characteristics, and climatic/seasonal variations of the locations of the participants in the different studies.

This wide variation can also be influenced by the slightly different definitions of hypovitaminosis D by different international bodies, such as the Endocrine Society [15], World Medical Association [16,] and Institute of Medicine (now National Academy of Medicine) [28.] Both the Endocrine Society and the World Medical Association have similar definitions of Vitamin D deficiency, insufficiency, and sufficiency as previously stated. However, Endocrine Society has currently withdrawn its previous definitions and advocates for additional research for thresholds in healthy individuals who would benefit from vitamin D supplementation. [29] The Institute of Medicine (now National Academy of Medicine) defines VDD as serum levels of 25(OH) < 30nmol/l, Vitamin D insufficiency are levels of 30 – 50 nmol/l and 50 nmol/l and above as being sufficiency.[28]

The normal range of serum calcium is 2.1- 2.6 mmol / l as determined by the instrument used in this research. Hypocalcaemia is defined as a serum calcium level less than 2.1mmol /l, and hypercalcemia when serum calcium levels are higher than 2.6 mmol /l.

The prevalence of VDD in this study is 17.9 %, and vitamin D insufficiency is 19.6%, which is relatively high because the study location has abundant sunshine almost all year round. This may be due to the fact that the black skin of the inhabitants has lower absorption of vitamin D from sunlight. In addition, despite the abundant sunshine, there may be limited exposure to sunshine by the participants due to urban lifestyle and indoor living. [13,14] A recent study done in this location revealed poor knowledge of the importance/sources of vitamin D among pregnant women, a poor perception of the need for sun exposure, as well as intake of vitamin D-rich food and supplements.[30] These factors may also contribute to the level of vitamin D deficiency in the participants.

The prevalence of hypocalcemia reported in this study is 76.4%, which is about four times higher than the reported prevalence of VDD in pregnancy (19.6%). Vitamin D is required for the absorption of calcium from the intestines. Vitamin D deficiency usually leads to hypocalcaemia. In the presence of VDD, 10-15% of calcium is absorbed from the intestines, but in the presence of optimal vitamin D levels, calcium absorption increases to 30-40%.[17-19] In addition, there is a physiological increase in calcium demand in pregnancy because foetal calcium is mostly transferred from the maternal circulation.[17-19,31] However, the relatively high prevalence of hypocalcaemia is a surprising finding because the study location is in Southern Nigeria, a coastal city with abundance of sunshine almost all year round; as well as availability of fatty fish (sardines, mackerel) and other seafood (prawns, shrimps, oysters, crayfish) as well as green leafy vegetables which are naturally rich in vitamin D and calcium. This surprisingly high level of hypocalcemia suggests that this may not be caused by vitamin D deficiency alone but also by physiologic increased calcium demand of the growing foetus. In addition, the high level of hypocalcaemia relative to VDD may also be caused by dietary deficiency because the participants may not be consuming sufficient quantities of vitamin D and calcium – rich food due to financial constraints / prevailing high cost of the items. It may also be due to

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ignorance of the importance of these foods to their health, as well as that of their growing fetuses. In addition, most of the routine iron supplements used in our geographical location do not contain vitamin D or calcium. Assay of vitamin D and/or calcium is not routinely done during their antenatal care, and these supplements are not routinely taken. Further studies in a wider population need to be carried out to confirm this finding and delineate the causes/risk factors of VDD and hypocalcemia in this population.

This study revealed that the participants had higher vitamin D levels at higher gestational ages. The higher prevalence of hypovitaminosis D in the first trimester of pregnancy is probably due to the effects of emesis gravidarum, poor appetite, limited outdoor activities, and non-supplementation of vitamin D.[13,27,31] However, the relative prevalences of hypovitaminosis D in different trimesters of pregnancy showed inconsistent trends in different studies.[25,32]

This study showed an inverse correlation between systolic blood pressure and vitamin D levels. Thus, the participants who had higher systolic blood pressures also had significantly lower vitamin D levels and vice versa. This is consistent with findings in some studies that found an association between low serum levels of vitamin D and raised blood pressure/pre-eclampsia.[26,33,34] Since the participants in this study were not above the gestational age of 20 weeks, this association cannot be reported in the population until further studies are done at later gestational ages.

In our study, no other socio-demographic or obstetric variable (such as age, parity, educational status, or marital status) was found to be associated with vitamin D or calcium levels. This finding is consistent with that obtained in some studies but at variance with reports from other studies. [21,35]

One of the strengths of this study is that it assessed the levels of both vitamin D and calcium in the participants, which has not been done in many of the studies on vitamin D in pregnancy in our locality, given that both of them are closely and directly linked in maintaining bone health in both the mother and foetus. This study was also done in pregnant women below the gestational age of 20 weeks, which enabled us to determine baseline levels before potential complications (such as pre-eclampsia, gestational diabetes mellitus) that can occur in the second half of pregnancy. This study has helped to measure baseline values of both vitamin D and calcium in our locality, upon which further research can be built.

The limitations of this study include that some of the confounders of Vitamin D status (such as body mass index, socioeconomic status, diet, and sunshine exposure were not assessed. In addition, this is an analytical cross–sectional study, thus direct causal relationships cannot be established. Thus, further and larger studies, such as longitudinal cohort studies and randomized controlled trials, are required to establish direct causal relationships between different variables.

The implication of the findings of this study is that there is inadequate utilization of abundant sunshine and consumption of vitamin D and calcium - rich food despite their natural availability in the study location.

We recommend that widespread health enlightenment campaigns should be carried out to educate pregnant women and their families on the importance of vitamin D and calcium to the pregnant woman and her growing foetus. They should also be educated on the need for adequate consumption of the available vitamin D and calcium – rich foods in the locality. These campaigns should be done in the antenatal clinics and in the community with the help of community / religious leaders, as well as mass / social media. There is also a need to identify pregnant women at risk of vitamin D deficiency and hypocalcaemia to receive drug supplementation.

CONCLUSION

This study has revealed that 37.5% and 76.4% of the participants had suboptimal levels of vitamin D and hypocalcaemia, respectively. We recommend widespread health enlightenment campaigns for all pregnant women and their families on the importance and sources of vitamin D and calcium-rich foods. We recommend further studies in a wider population of participants to confirm the findings and delineate possible causes/risk factors and potential foeto-maternal consequences of hypovitaminosis D and hypocalcemia in the population. This may help to inform the policy guide on vitamin D and calcium assay and/or supplementation, at least for high-risk pregnant women in the population.

Declarations

Ethics: Ethical approval was obtained from the Institutional Research Ethics Committee before conducting the study (Reference number: UCTH/HREC/33/Vol. 111/026. 1st April 2022).

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Informed and written consent were also obtained before data collection. Test results were communicated to subjects, and those with abnormal values received counseling and referral for specialist care. The study was conducted with the standards as per the Helsinki Declaration of 1975, as revised in 2000.

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