Serum 25-Hydroxyvitamin D Levels of Apparently Healthy Nigerian Children Aged 1-24 Months

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Abstract

Introduction: The role of vitamin D in promoting health and contributing to disease is an emerging area of research interest that has important health care and public health implications. As the health relevance of vitamin D outside bone health is now being explored globally, scanty data is available about the vitamin D status of healthy Nigerian children. Because infants and young children have a relatively low supply of foods rich in vitamin D and may also have inadequate exposure to sunlight, they are at risk of vitamin D deficiency. We measured and described 25-hydroxyvitamin D levels of apparently healthy young Nigerian children.

Subjects and methods: This was a cross sectional, descriptive study involving 112 apparently healthy children living in Zaria, North-West Nigeria which has adequate sunshine all through the year. Serum 25(OH)D concentrations were determined using a vitamin D ELISA kit. A level above 75 nmol/l was used to define vitamin D sufficiency in accordance with the Endocrine Society recommendations.

Results: The mean 25-Hydroxyvitamin D level was 58.6 ± 30.5 nmol/l (range: 6.5-146 nmol/l). Only 31 (27.7%) of the children had 25-hydroxyvitamin D levels above 75 nmol/l, while 35 (31.3%) had insufficient (50-75 nmol/l) and 46 (41.0%) had deficient (< 50 nmol/l) serum vitamin D levels. There was no significant difference with regard to sex, age, nutritional and socioeconomical status between the groups.

Conclusion: Apparently healthy young Northern Nigerian children had high prevalence of 25-hydroxyvitamin D insufficiency and deficiency. It is recommended that population studies be carried out across Nigeria to define the 25-hydroxyvitamin D levels of Nigerian children and establish predictors or determinants of vitamin D levels in them. This will assist in the development of governmental policies and strategies to prevent, detect, and treat vitamin D deficiency in Nigeria.

Keywords

Vitamin D, 25-hydroxyvitamin D, Young children, Nigeria

Introduction

Vitamin D is essential for maintaining bone health in children and adults and its deficiency is an established cause of nutritional rickets and osteomalacia. More recently, vitamin D is proposed to have extra-skeletal activities including protecting against many chronic conditions like autoimmune diseases, diabetes, cardiovascular diseases, and cancer [1-7]. It may also facilitate the ability of immune cells to defend against infections like respiratory tract infections including pneumonia [8-12]. The health relevance of vitamin D outside bone health is now being explored globally and it is speculated that vitamin D may have a role to play in the management of the new coronavirus disease (Covid-19) [11-14].

Cutaneous synthesis is the most important source of vitamin D from sunlight. Nutritional sources become important in the absence of sunlight [15]. Breast milk has a low vitamin D content (approximately 12-60 IU/L) even in a mother with sufficient vitamin D [15,16]. Animal products constitute the bulk source of vitamin D that occurs naturally in unfortified foods. Liver, organ meats, fatty fish such as herring, salmon, sardines, tuna, mackerel, and fish liver oils are good sources of vitamin D [15-17].

Serum or plasma 25-Hydroxy Cholecalciferol (25(OH) D) is the most commonly used and appropriate bio-
chemical marker of vitamin D status [15,18]. There is no absolute consensus on the definition of normal or healthy 25-hydroxyvitamin D levels. While The Institute of Medicine of the United States National Academies [19] defined vitamin D deficiency as serum 25(OH)D concentrations < 12 ng/ml (< 30 nmol/l), insufficiency as serum 25(OH)D concentrations from 12-20 ng/ml (30-50 nmol/l) and sufficiency as serum 25(OH)D concentrations > 20 ng/ml (> 50 nmol/l), some researchers have argued that this concentration may not be sufficient to confer the extra-skeletal benefits of vitamin D. Thus a higher serum 25(OH)D concentration between 50 and 125 nmol/l was proposed to be adequate [20-23].

Vitamin D deficiency or insufficiency is a global health problem and its prevalence varies from one country or region to another because of difference in risk factors [24-27]. Although there is abundant sunshine all through the year in Nigeria, the practice of inadequate sun exposure for cultural, religious or personal reasons (staying in door, excessive clothing when outdoors) might put infants and young toddlers at high risk of hypovitaminosis D. Other factors like maternal vitamin D deficiency during pregnancy, exclusive breastfeeding, consumption of family diet with low vitamin D [16,28], may predispose to hypovitaminosis D in children. Most foods that are naturally rich in vitamin D are not easily affordable as part of weaning foods for young Nigerian children.

Little is known about vitamin D status of apparently healthy Nigerian children. A study done over twenty years ago by Pfitzner, et al. [29] reported that there was no vitamin D deficiency in young Nigerian children (aged 6-35 months); whereas three other studies done later which involved Newborns [30] older children [31] and adolescents [32] reported subnormal 25-hydroxyvitamin D levels.

In order to prevent vitamin D deficiency in breast feeding infants, vitamin D supplementation of 400 IU/day is recommended [28,33]. This recommendation may be appropriate in temperate climates but this may not be necessary in Nigeria because of its abundant sunshine all the year round. Hence, data on vitamin D status of Nigerian young children is needed for decision making as whether to adopt or reject this recommendation.

This study described the levels of 25-hydroxyvitamin D levels in apparently healthy young Nigerian children. We hope that this study will contribute to the body of evidence in defining the vitamin D status of Nigerian children. This will also be relevant in formulating appropriate national and local policies and strategies aimed at reducing hypovitaminosis D and its related diseases among children in Nigeria.

Subjects and Methods

Study location

This study was carried out in the Department of Paediatrics of Ahmadu Bello University Teaching Hospital (ABUTH), Zaria. The city is located in the North-Western Nigeria on latitude 11.09’N and longitude 7.72’E [34,35] and it receives abundant sunshine (average of 7 to 8 hours per day) [35] all through the year. ABUTH is a multi-specialty health care provider which provides both secondary and tertiary care to the people of Kaduna state and its environs.

Ethical clearance

Approval was obtained from the Human Research and Ethical Committee of ABUTH, Zaria. A written informed consent was obtained from parents/guardians before enrollment of children into the study.

Study design

The study was part of a larger prospective cross sectional comparative study conducted over a 12-month period from August 2015 to July 2016 in order to determine association between vitamin D status and acute pneumonia. The data of apparently healthy children aged 1 to 24 months were extracted and reported.

Subjects

The subjects were 112 apparently healthy children (with no known chronic medical conditions like HIV, sickle cell anaemia, severe malnutrition etc.) aged 1-24 months who were brought for immunization, minor surgery (such as circumcision and herniorrhaphy) or who accompanied sick siblings; and whose parents consented to participate. All the participants were living in Zaria and its environs which have abundant sunshine all the year round. Children who were taking drugs known to affect vitamin D metabolism (such as phenytoin, phenobarbitalne, carbamazepine, clotrimazole, rifampicin and dexamethasone) or who had received vitamin D supplements or cod liver oil in the six weeks preceding enrolment into the study were excluded.

Sample collection

A structured interviewer-administered questionnaire was used to obtain relevant information about the recruited children. Socio-economic index scores were awarded to each child based on the occupations and educational attainment of the parents or their substitutes using Oyedeji’s classification [36]. Detailed Physical and systemic examinations were carried out on each child. The measured weight and length/height were plotted on the WHO growth chart to determine the nutritional status of the child using the Z score cut-off points defined by WHO [37].

Three milliliters of peripheral venous blood was collected from each of the recruited children into a plain tube and allowed to clot at room temperature. The clotted blood sample was then centrifuged and the serum separated into another plain tube using a Pasteur
pipette. The serum was transported on dry ice packs to the chemical pathology laboratory of ABUTH, Zaria where samples were stored at -20 °C until analysis.

Serum 25(OH)D (vitamin D) was assayed using commercial vitamin D ELISA kit (AccuDiag™ 25-OH Vitamin D (total) ELISA Kit, Diagnostic Automation/ Cortes Diagnostics Inc., Woodland Hills, USA) [38]. The kit has a range of assay between 2.89-130 ng/ml; specificity of antibodies: 25-OH Vitamin D3 - 100%, 25-OH Vitamin D2 - 74%, 1,25 -OH Vitamin D3 < 0.1%; sensitivity < 2.98 ng/ml; Intra assay variability (CV) of 3.0-6.6% and interassay variability (CV) of 8.6-10%.

Data analysis

Data was analyzed using SPSS (version 20: IBM Corporation). We determined the prevalence for each vitamin D groups: Deficiency (serum 25(OH)D concentration < 50 nmol/l), insufficiency (serum 25(OH)D concentration 50-75 nmol/l), and sufficiency (serum 25(OH)D concentration > 75 nmol/l) as recommended by Endocrine Society [23]. Any relationship between 25-hydroxyvitamin D levels and the determinant variable was tested using appropriate statistical tools. A p value of less than 0.05 was considered statistically significant.

Results

A total number of 112 children aged 1-24 months were studied, consisting 72 (64.3%) males and 40 (35.7%) females with mean age 10.8 ± 6.8 months.

Vitamin D status of participants
The mean 25-Hydroxyvitamin D level was 58.6 ± 30.5 nmol/l with the range between 6.5 and 146 nmol/l. Only 31 (27.7%) of the children had 25-hydroxyvitamin D levels above 75 nmol/l (Vitamin D sufficiency) while 35 (31.3%) and 46 (41.0%) children had vitamin D insufficiency and deficiency respectively (Table 1).

Predictors of 25-Hydroxyvitamin D Levels

Table 2 shows that the mean 25-hydroxyvitamin D levels for males and females were similar (p = 0.977). Children in the first 6 months had the lowest mean serum 25-hydroxyvitamin D level compared to others; this was not statistically significant (p = 0.094).

Nutritional status appeared to predict 25-hydroxyvitamin D levels; the mean 25-OH vitamin D levels followed a decreasing pattern from underweight, through normal weight to overweight (p = 0.730).

Children with high socioeconomic class parents had highest mean 25-hydroxyvitamin D level while those with low socioeconomic class parents had the lowest. This difference was not statistically significant (p = 0.284).

Serum 25(OH)D was dichotomized into normal vitamin D group (serum 25(OH)D level above 75 nmol/l) and low vitamin D group (serum 25(OH)D level of

| Variable                  | Frequency (%) | Mean serum 25(OH)D nmol/l | Test statistics | P value |
|---------------------------|---------------|---------------------------|-----------------|---------|
| Sex                       |               |                           |                 |         |
| Male                      | 72 (64.3)     | 58.6 ± 30.4               | t = -0.028      | 0.977   |
| Female                    | 40 (35.7)     | 58.8 ± 30.9               |                 |         |
| Total                     | 112 (100)     |                           |                 |         |
| Age group                 |               |                           | F = 2.183       | 0.094   |
| 1-6 months                | 37 (33.0)     | 49.1 ± 30.7               |                 |         |
| 7-12 months               | 38 (33.9)     | 62.1 ± 33.5               |                 |         |
| 13-18 months              | 20 (17.9)     | 60.6 ± 21.5               |                 |         |
| 19-24 months              | 17 (15.2)     | 69.5 ± 29.2               |                 |         |
| Total                     | 112 (100)     |                           |                 |         |
| Nutritional status        |               |                           | F = 0.316       | 0.730   |
| Normal weight             | 84 (75.0)     | 58.8 ± 31.8               |                 |         |
| Under weight              | 26 (23.2)     | 59.5 ± 25.3               |                 |         |
| Overweight                | 02 (1.8)      | 41.8 ± 30.5               |                 |         |
| Total                     | 112 (100)     |                           |                 |         |
(25 nmol/l) to define normal 25-hydroxyvitamin D levels at that time compared to the cut off points recently proposed by the Endocrine Society of below 50nmol/l for deficiency and 50-75 nmol/l for insufficiency. We reported a slightly lower mean of 58.6 ± 30.5 nmol/l than their own report of 66.8 ± 24.2 nmol/l, but both means were in the category of vitamin D insufficiency. Also, more than half of the children studied by Pfitzner, et al. had serum 25(OH)D below 30 ng/ml (75 nmol/l) - this would have been classified as insufficiency vitamin D level now based on the recent classification.

Vitamin D deficiency and insufficiency is a global health problem and the prevalence varies across countries and subpopulations depending on the risk factors and the serum 25(OH)D cut off points used. Using a cut-off serum level of less than 50 nmol/l, we reported a low vitamin D prevalence of 41%. This is lower compared to findings from studies on 25-hydroxyvitamin D among young children from Iran (66.7%, cut-off of less than 50 nmol/L) [24], Mexico (54% cut-off of less than 50 nmol/L) [25], Saudi Arabia (58.8%, cut-off of less than 75 nmol/l or less).

We compared the children with low vitamin D level against those that had normal vitamin D level with regard to sex, age, nutritional and socioeconomical status. There was no statistically significant difference between the two groups (Table 3).

**Discussion**

This study described the 25-hydroxyvitamin D levels in apparently healthy young Nigerian children aged 1-24 months. Based on the 25-hydroxyvitamin D levels more than 7 out of every 10 children had a low level of 25-hydroxyvitamin D while less than 3 out of every 10 children were vitamin D sufficient.

These findings are quite important as it appears that, despite the location of Nigeria and the intensity of UV rays it receives throughout the year, Nigerian children have insufficient levels of vitamin D. Pfitzner, et al. [29] had earlier reported over two decades ago, that vitamin D deficiency was absent in young Nigerian children (aged 6-35 months) living in Jos, North-Central Nigeria. However, they used a lower cut off point of 10 ng/ml (25 nmol/l) to define normal 25-hydroxyvitamin D levels at that time compared to the cut off points recently proposed by the Endocrine Society of below 50nmol/l for deficiency and 50-75 nmol/l for insufficiency. We reported a slightly lower mean of 58.6 ± 30.5 nmol/l than their own report of 66.8 ± 24.2 nmol/l, but both means were in the category of vitamin D insufficiency. Also, more than half of the children studied by Pfitzner, et al. had serum 25(OH)D below 30 ng/ml (75 nmol/l) - this would have been classified as insufficiency vitamin D level now based on the recent classification.

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| Socioeconomic status     | F = 1.273 | 0.284 |
|--------------------------|-----------|-------|
| High (Classes 1 & 11)    | 38 (33.9) | 65.0 ± 27.2 |
| Middle (Class 111)       | 51 (45.6) | 55.8 ± 34.6 |
| Low (Classes 1V & V)     | 23 (20.5) | 54.4 ± 24.8 |
| Total                    | 112 (100) |       |

F: ANOVA; t: Independent samples T-test

| Table 3: Association between sex, age, nutritional, socioeconomical and vitamin D status. |
|----------------------------------|----------------------------------|-----------------|-----------------|
| Variable                         | Normal vitamin D level n = 31    | Low vitamin D level n = 81 | Total | Test statistics | P value |
| Sex                              |                                  |                              |       |                |        |
| Male                             | 20 (64.5%)                       | 52 (64.2%)                  | 72 (64.3%) | χ² = 0.001    | 0.975   |
| Female                           | 11 (35.5%)                       | 29 (35.8%)                  | 40 (35.7%) |                |        |
| Total                            | 31 (100%)                        | 81 (100%)                   | 112 (100%) |                |        |
| Age group                        |                                  |                              |       |                |        |
| 1-6 months                       | 07 (22.6%)                       | 30 (37.1%)                  | 37 (33.0%) | χ² = 4.710    | 0.194   |
| 7-12 months                      | 11 (35.5%)                       | 27 (33.3%)                  | 38 (33.9%) |                |        |
| 13-18 months                     | 05 (16.1%)                       | 15 (18.5%)                  | 20 (17.9%) |                |        |
| 19-24 months                     | 08 (25.8%)                       | 09 (11.1%)                  | 17 (15.2%) |                |        |
| Total                            | 31 (100%)                        | 81 (100%)                   | 112 (100%) |                |        |
| Nutritional status               |                                  |                              |       |                |        |
| Normal weight                    | 23 (74.2%)                       | 61 (75.3%)                  | 84 (75.0%) | χ² = 0.509    | 0.775   |
| Under weight                     | 07 (22.6%)                       | 19 (23.5%)                  | 26 (23.2%) |                |        |
| Overweight                       | 01 (03.2%)                       | 01 (01.2%)                  | 02 (1.8%)  |                |        |
| Total                            | 31 (100%)                        | 81 (100%)                   | 112 (100%) |                |        |
| Socioeconomic status             |                                  |                              |       |                |        |
| High (Classes 1 & 11)           | 12 (38.7%)                       | 26 (32.1%)                  | 38 (33.9%) | χ² = 0.440    | 0.803   |
| Middle (Class 111)               | 13 (41.9%)                       | 38 (46.9%)                  | 51 (45.5%) |                |        |
| Low (Classes 1V & V)            | 06 (19.4%)                       | 17 (21.0%)                  | 23 (20.6%) |                |        |
| Total                            | 31 (100%)                        | 81 (100%)                   | 112 (100%) |                |        |

χ² = Pearson Chi-Square
The reasons for the high prevalence of low vitamin D levels in these children is not clear but some speculations that need further research may be proffered. The Northern region of Nigeria is predominantly Muslims and the use of hijab is a common practice among mothers. They usually back their infants and young toddlers covered with their hijab whenever they are going out. Also, some of the women are usually in door with their young children as they are not gainfully employed or doing some petty trading at home. These practices may interfere with adequate exposure of these infants and young toddlers to sunlight and may put them at risk of low vitamin D status.

To the best of our knowledge, this is the first published work on serum 25-hydroxyvitamin D levels among apparently healthy young children living in the North-West region of Nigeria and one of the few studies on this subject from Sub-Saharan Africa. The implication of our findings is that that population studies need to be carried out across the country to define the 25-hydroxyvitamin D levels of Nigerian children and establish predictors or determinants of vitamin D levels in them. However, health professionals, policy makers, and the general public in Nigeria should be aware of the prevalence of vitamin D deficiency and insufficiency despite the abundant sunshine in Nigeria. Also, given the growing understanding of the incidence of some disease conditions like respiratory tract infections, autoimmune disorders, etc. [4,6,8,12] efforts to reduce the burden of diseases in Nigeria should also incorporate strategies to prevent, detect, and treat hypovitaminosis D.

Our study is not without limitations. We did not evaluate for effects of seasons, sunlight exposure practices and diets; as our goal was not to verify the causes or predictors of low levels of 25-hydroxyvitamin D, but rather to describe the 25-hydroxyvitamin D levels in these apparently healthy children. Although we only included studies with healthy participants in this hospital-based study, population-based studies are better at inferring the 25-hydroxyvitamin D levels of the general population.

In conclusion, despite the location of Nigeria and the intensity of UV rays it receives throughout the year, apparently healthy young Northern Nigerian children had a high prevalence of 25-hydroxyvitamin D insufficiency and deficiency. It is recommended that population studies be carried out across the country to define the 25-hydroxyvitamin D levels of Nigerian children and establish predictors or determinants of vitamin D levels in them. These will assist in the development of governmental policies and strategies to prevent, detect, and treat vitamin D deficiency in Nigeria.

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