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Vitamin D Levels in Malnourished Children under 5 Years in a Tertiary Care Center at Muhimbili National Hospital, Dar es Salaam, Tanzania—A Cross-sectional Study

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ABSTRACT

Objective: To evaluate vitamin D levels/deficiency among malnourished children <5 years admitted at a tertiary care center, the Muhimbili National Hospital, Dar es Salaam, Tanzania. Children with malnutrition may have co-existing vitamin D deficiency (VDD), which may be severe.
Methods: Serum vitamin D and alkaline phosphatase were evaluated, and X-ray of the wrist was carried out on 134 children.
Results: VDD was found in 41 of 134 children (30.6%). The mean vitamin D level was 74.8 nmol/l. The mean alkaline phosphatase level was 176.6 U/l. Sixty-four (48%) children were found to have severe stunting, of whom 20 (31.2%) were vitamin D deficient. Marasmic children had higher odds of VDD compared with other forms of malnutrition.
Conclusion: The high prevalence of VDD in malnourished children underlines the need for active surveillance and aggressive management.

KEYWORDS: vitamin D deficiency, malnourished children, Tanzania.

INTRODUCTION

Vitamin D deficiency (VDD) is prevalent in children worldwide and is recognized to be a major public health problem [1].

Studies on children and adolescents in low- and middle-income countries show VDD ranging from 28 to 62% [2, 3]. A study by Ejaz *et al.* [4] in Pakistan found that 33.6% of the severely malnourished children had rickets. Malnutrition is a major preventable public-health problem especially in developing world and contributes over 50% of childhood mortality among children <5 years of age [5–7].

A survey of VDD among malnourished children in sub-Saharan Africa from 2012 to 2014 showed a prevalence of 28% [8, 9].

The association of vitamin D and malnutrition is well recognized, but data from sub-Saharan Africa are scarce [1].

A study done by Msomekela *et al.* [10] in Tanzania concluded that metabolic bone disease is

common in very-low-birth-weight (VLBW), exclusively breast-fed, preterm infants with a rate of 33% and this was identified radiographically[]. A prospective cohort study in HIV-infected and HIV-exposed children in Dar es Salaam, Tanzania, revealed low level of vitamin D in both groups, an average of 18 ng/ml, indicating probable VDD in Tanzanian children, which persisted in 34.6% of uninfected children at the age of 6 months [11, 12].

The specific objective of this study was to identify vitamin D levels among malnourished children and correlate with the alkaline phosphatase and X-ray findings.

METHODS

A cross-sectional descriptive study of malnourished children admitted at Muhimbili National Hospital was conducted from May 2014 to January 2015. This is a tertiary referral hospital.

All children <5 years whose weight for height/length was < -2 SD using the Standard World Health Organization (WHO) growth charts [13, 14], a mid-upper arm circumference (MUAC) <115 mm (for children aged 6–59 months) or had edema of both feet and whose parents/caretaker gave consent were included in the study. Children with clinical signs of hepatic disease or renal disease as defined by presence of jaundice, dark urine, pale stools, itchy skin and/or easy bruising and abnormal laboratory results when applicable or by reduced urine output (less than estimated 1 ml/kg/day), swelling beginning from the face, confusion and/or coma were excluded.

The primary outcome was the serum level of vitamin D, and secondary outcomes were its correlation with serum alkaline phosphatase, nutritional status and baseline factors such as breast-feeding, birth weight and weaning. This information was collected in a structured questionnaire.

Using an assumed prevalence of 33.6% [4, 13] and the formula for cross-sectional studies, with a confidence level of 95%, the sample was estimated to be 134.

The sample size was estimated from the following formula:

$$n = \frac{z^2 p(100 - p)}{\varepsilon^2},$$

where

z=level of confidence (1.96 for 95% confidence level); p=assumed proportion of malnourished children with rickets (33.6%) [4, 13]; and ε=margin of error (8%).

$$n = \frac{1.96^2 * \ 33.6 \ (100 - 33.6)}{8^2}$$

$$= 133.9 = 134$$

All admitted and eligible children were recruited consecutively, until sample size was reached. Detailed clinical examinations, anthropometric measurements and laboratory tests were recorded. All anthropometric measurements were done in a standard method as per the recommendations by WHO [13, 14].

Nutritional status was as per WHO guidelines [13–16]. Mild stunting was defined as height/length for age between −1 SD and −2 SD; moderate stunting was defined as height/length for age between −2 SD and −3 SD; and

severe stunting was defined as height/length for age < -3 SD.Severe acute malnutrition was defined as weight for height/length < -3SD (<70%) or MUAC of <115 mm (for children between 6 and 59 months) or edema of both feet (with or without severe wasting) for children <5 years of age.

Laboratory investigations

Alkaline phosphatase was evaluated using the ARCHITECT system from Abbott Laboratories and expressed in U/l as part of the routine workout of children in the ward. Blood for vitamin D was centrifuged and serum separated in three aliquots and stored at a temperature of -86°C for batch testing. In every batch, calibration and levels of controls were included. This laboratory participates in the external quality assurance program called Vitamin D External Quality Assessment Scheme (DEQAS) to ensure accuracy and reliability of results. Each sample was run in duplicates. Serum vitamin D levels were determined using 25(OH) enzyme-linked immunoassay (Immunodiagnostics, UK). Children with vitamin D levels of <50 nmol/l were categorized as vitamin D deficient, while those who had insufficient and sufficient levels were designated as no deficiency (≥50 nmol/l). An alkaline phosphatase

level >350 U/l was considered to be abnormally high.

Lateral and anteroposterior views of wrist X-ray, either left or right wrist, were reviewed by an experienced radiologist, who identified changes using the conventional criteria for rickets [17].

Analysis

Data were analyzed using the SPSS version 20.0. Proportions, χ^2, Fisher's exact tests, odds ratios (ORs) and confidence intervals (CIs) were used where appropriate. Sensitivity and specificity of alkaline phosphatase levels and wrist X-ray findings were determined using the receiver operating characteristic (ROC) curve as a parameter of robustness of the test.

Univariate association was found to identify relationship between each variable among malnutrition and X-ray features with VDD.

Correlation coefficient was used to correlate the relationship of vitamin D with alkaline phosphatase levels. A *p*-value of <0.05 was regarded as statistically significant.

Ethical issues

Ethical clearance was granted by the institutional review board of MUHAS (www.muhas.ac.tz) and Muhimbili National Hospital. The mother/caretaker provided written consent, and the children whose tests showed deficiency were treated with supplements and followed up at the Malnutrition Clinic. Refusal of consent did not alter attention and care given to child.

RESULTS

A total of 134 children were recruited into the study, of which 101 (75.4%) were <2 years of age with a median age of 16 months. More than half (56.7%) of all malnourished children were males. In total, 101 children (75.4%) were born with a birth weight of >2.5 kg. Early weaning (<6 months) was practiced in 71 (58.2%) children regardless of age, while among those >6 months of age ($n = 118$), 66 children (55.9%) were weaned early (not shown in Table 1). Among those >1 year of age ($n = 83$), 63 children (75.9%) were breast-fed for a period of >1 year (not shown in Table 1). Of all the children who had stopped breast-feeding regardless of age, 54 (61.4%) children had breast-fed for >1 year (Table 1). None of the children had received any vitamin D supplementation or multivitamins before admission. The dietary intake data were limited to breast-feeding practices only. Exposure to sunlight was variable and not part of the data analyzed.

The median interquartile range (IQR) of vitamin D level was found to be 74.8 nmol/l (46.9–100.3). The mean (±SD) alkaline phosphatase level was 176.6 U/l (±133.1; Table 1).

Seven (5.2%) of these children had moderate acute malnutrition, and the rest had severe acute malnutrition (SAM). Among those with SAM, 74 (58.3%) had marasmus, 37 (29.1%) had marasmic kwashiorkor and the rest had kwashiorkor. This study found that almost half (64 of 134) children had severe stunting and 27.6% were moderately stunted. Only 10 children (7.4%) had no stunting.

Among 134 children, 41 (30.6%) had VDD (Table 2).

Of 74 children with marasmus, 31 (41.9%) had VDD and 43 (58.1%) had no VDD. Among those with kwashiorkor, one (6.2%) child was vitamin D deficient, and among marasmic kwashiorkor, six children (16.2%) had VDD. Among the seven children with moderate acute malnutrition, three (42.9%) had VDD.

About 22% (30 of 134) of the children had one or more X-ray features of VDD (Table 3).

In total, 8 of 10 (80%) children with cupping in the wrist X-ray had VDD as compared with 33 (26.6%) who had no cupping. Among all those who had fraying, 17 (85%) children were found to have VDD, and among those who had demineralization, five (83.3%) had VDD. Twenty-four children (21.1%) with VDD did not have fraying. About 36 children (28.1%) of those who did not have demineralization were not vitamin D deficient. All the three features of wrist X-ray were significantly associated with VDD. A sensitivity of 61% and a high specificity of 95% for X-ray findings vs. vitamin D were found in this study (Table 3).

VDD varied significantly between the three forms of SAM ($p = 0.01$; Table 4).

VDD was prevalent in children with stunting. Age <2 years and low birth weight had no significant differences for vitamin D status (Table 5). Likewise,

Table 1. Sociodemographic characteristics of malnourished children

Characteristic	*N*	%
Age—months, median (IQR)	16, 9.0–24.3	
Age groups, months		
0–24	101	75.4
24–59	33	24.6
Sex		
Male	76	56.7
Female	58	43.3
Birth weight, g		
<1500	12	9.0
1500–2400	21	15.7
≥2500	101	75.4
Still breast-feeding (n =134)		
Yes	46	34.3
No	88	65.7
If no, duration of breast-feeding, months (n =88)		
0–5	11	12.5
6–12	23	26.1
>12	54	61.4
Still exclusively breast-feeding (n =134)		
Yes	12	9.0
No	122	91.0
If no, age at weaning, months (n =122)		
0–5	71	58.2
≥6	51	41.8
Weight (kg) (mean±SD)	5.9±2.0	
Vitamin D level (nmol/l) median, IQR*	74.8, 46.9–100.3	
Alkaline phosphatase (U/l) (mean±SD)	176.6±133.1	

*Median vitamin D level was determined instead of mean because of extreme vitamin D levels in one participant.

Table 2. Proportion ofVDD among malnourished children <5 years

Vitamin D status	*N*	%
No deficiency (≥50 nmol/l)	93	69.4
Deficiency (<50 nmol/l)	41	30.6
Total	134	100

Table 3. Relationship between vitamin D levels and radiographic features

Feature	VDD	No VDD	*p* value*
Cupping			<0.01
Yes	8 (80%)	2 (20%)	
No	33 (26.6%)	91 (73.4%)	
Fraying			<0.01
Yes	17 (85%)	3 (15%)	
No	24 (21.1%)	90 (78.9%)	
Demineralization			0.01
Yes	5 (83.3%)	1 (16.7%)	
No	36 (28.1%)	92 (71.9%)	

*Fisher's exact test.

eight children had raised alkaline phosphatase of which only two were associated with VDD. Thirty-nine (31%) of VDD children had normal alkaline phosphatase levels (p = 1.0). The area under the ROC curve was 0.501 (p = 0.992, CI = 0.387–0.614).

A logistic regression analysis was carried out using X-ray features and categories of SAM. Children with cupping in the wrist X-ray were more likely to have VDD than those without cupping, and this was statistically significant (OR 11, 95% CI = 2.23–54.63, $p < 0.01$). Children who had fraying were more likely to have VDD (OR 23, 95% CI = 5.75–78.55, $p < 0.01$). Children with demineralization were 12.8 times more likely to have VDD (OR 12.8, 95% CI = 1.44–113.2, $p < 0.02$). Those with marasmus were 10.8 times more likely to have VDD than those with kwashiorkor, and this was statistically significant (OR 10.8, CI = 1.356–86.236, p = 0.03). Also, those who had marasmus were more likely to have VDD than those with marasmic kwashiorkor, and this was statistically significant (OR 3.7, 95% CI = 1.396–10.0, $p < 0.01$; Table 5).

The correlation coefficient for vitamin D with alkaline phosphatase was poor (r =−0.065).

DISCUSSION

The prevalence of VDD among the 134 malnourished children was 30.6%, with 80% of these being

Table 4. Relationship between vitamin D levels and malnutrition

Diagnosis	VDD	No VDD	*p* value*
Severe acute malnutrition			
Marasmus	31 (41.9%)	43 (58.1%)	
Kwashiorkor	1 (6.2%)	15 (93.8%)	<0.01
Marasmic kwashiorkor	6 (16.2%)	31 (83.8%)	
Chronic malnutrition			
Severe stunting	20 (31.2%)	44 (68.8%)	0.34
Moderate stunting	8 (21.6%)	29 (78.4%)	
Mild stunting	8 (34.8%)	15 (65.2%)	
No stunting	5 (50%)	5 (50%)	

*χ^2 test.

<2 years of age. The median vitamin D level was found to be 74.8 nmol/l. These findings are similar to those seen in Pakistan (33.6%) [4]. VDD in children from other countries shows variable prevalence, for instance the prevalence among 12–24-month-olds in China was 65.3%; non-malnourished children in Qatar was 68.8%, urban children aged 1–6 years in Saudi Arabia was 63%, in rural Ethiopia among school-aged children in the community was 49% and hospitalized malnourished children in Uganda was 43.6% [18–22]. Except for the similarity of the cohort between Uganda and this study, all other studies had different groups of children. Nevertheless, these data indicate that VDD is prevalent. This could be related to decrease nutritional intake, poor sunlight exposure and possibly impaired absorption because of enteric dysfunction or a disease process [13].

A study on genetic predisposition and fish intake among indigenous population in Tanzania showed no significant deficiency in that population [23]. This study is the first one among hospitalized children with malnutrition in our setup.

A study in Qatar found higher rates in girls [19], while our study did not find any difference between boys and girls ($p = 0.64$). This may be possibly because of cultural reasons of dress code.

Although studies have shown that breast-feeding duration without supplementation is associated with VDD [10, 22], our study did not show this association with breast-feeding duration ($p = 0.96$). This could be because of selection bias of our cohort.

Among those who had low birth weight (≤2 500 g), 36.4% had VDD and 33.3% had radiographic features of rickets. A study among exclusively breast-fed VLBW infants at post-natal age of 12 weeks found that 33% of those had metabolic bone disease radiographically identified [10].

The mean (±SD) alkaline phosphatase in this study was found to be lower than that found in another study done by Carpenter *et al.* (10,27) and among the VLBW in our center. Previous studies have found that biochemistry is of little diagnostic value in malnourished children, as malnutrition could alter their biochemical changes [13, 24].

Stunted and marasmic children had higher prevalence and could be because of an adaptation of their body mechanisms to poor nutrition [4, 13, 24]. Interestingly however, in the Uganda study of malnourished children, there was no difference in the prevalence of VDD levels between malnourished and non-malnourished children [25]. Raghuramulu *et al.* (26) from India found that most of the children with malnutrition had VDD.

It is still not clear whether malnutrition and rickets co-exist because of poor economic status or whether malnutrition is causally related to rickets. Autier *et al.* [13] in their systematic review showed that VDD arises from diseases and not vice versa[].

Those with radiological features of rickets had a sensitivity of 61% and a specificity of 95%, and this can arguably be used as a tool to aid measure VDD in resource-limited settings. Radiological evidence of rickets as a surrogate for diagnosing VDD is not

Table 5. Relationship ofVDD and radiological appearance and type of malnutrition

Parameter	Comparison	OR	95% lower limit	95% upper limit	*p* value
Cupping	No cupping	11.030	2.227	54.625	<0.01
Fraying	No fraying	21.250	5.749	78.549	<0.01
Demineralization	No demineralization	12.778	1.442	113.187	0.02
Marasmus	Kwashiorkor	10.814	1.356	86.236	0.03
Marasmus	Marasmic kwashiorkor	3.725	1.386	10.011	<0.01
Kwashiorkor	Marasmic kwashiorkor	0.344	0.038	3.124	0.343

Note: This was logistic regression analysis, in which factors associated with a p value of < 0.2 were added for analysis. They remained statistically significant.

scientifically sound, as radiological features will take time to develop and might not be present in many cases with low to moderate VDD.

The WHO guidelines for treatment of malnourished children in resource-poor settings only elude on potential VDD along with other vitamins and provide a recommended dose for supplementation, but do not emphasize the need to identify and treat severe VDD and rickets [19].

The limitations of this study are as follows: it was a hospital-based study, calcium and phosphate levels were not checked and detailed nutritional analysis was not performed.

In conclusion, the high prevalence of VDD in malnourished children underlines the need for active surveillance and aggressive management.

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