Scientific Research Publishing

Open Journal of Pediatrics, 2021, 11, 421-437
https://www.scirp.org/journal/ojped
ISSN Online: 2160-8776
ISSN Print: 2160-8741

Vitamin D Status among Infants Attending a Reproductive and Child Health Clinic in Arusha, Tanzania: A Cross-Sectional Study

Victor Urio[1*], Victor Musiime[1,2], Thereza Piloya[1], Phillip Kasirye[3], Stephen Swanson[4,5]

[1]Department of Paediatrics and Child Health, School of Medicine, College of Health Sciences, Makerere University, Kampala, Uganda
[2]Research Department, Joint Clinical Research Centre, Kampala, Uganda
[3]Department of Paediatrics and Child Health, Mulago National Referral Hospital, Kampala, Uganda
[4]Global Pediatrics Program, Department of Pediatrics, University of Minnesota Medical School, Minneapolis, Minnesota, USA
[5]Department of Paediatrics, Arusha Lutheran Medical Centre, Arusha, Tanzania
Email: *victororio21@yahoo.com, musiimev@gmail.com, tpiloa@yahoo.com, kasiryep@yahoo.com, swan0027@umn.edu

How to cite this paper: Urio, V., Musiime, V., Piloya, T., Kasirye, P. and Swanson, S. (2021) Vitamin D Status among Infants Attending a Reproductive and Child Health Clinic in Arusha, Tanzania: A Cross-Sectional Study. *Open Journal of Pediatrics*, **11**, 421-437.
https://doi.org/10.4236/ojped.2021.113040

Received: June 28, 2021
Accepted: September 10, 2021
Published: September 13, 2021

Abstract

Background: Globally there is a high burden of low serum vitamin D deficiency (VDD) with children being acknowledged at risk due to low vitamin D content in both breastmilk and available foods and inadequate cutaneous synthesis of vitamin D. Even in countries with abundant sunshine, vitamin D deficiency (VDD) remains a problem. There is little characterization of the status of vitamin D among infants in East Africa. This study aimed to determine the prevalence and factors associated with vitamin D deficiency among infants attending the Reproductive and Child Health (RCH) Clinic in Arusha, Tanzania. **Methods:** A cross-sectional study of 304 infants aged 6 weeks to 12 months was conducted at Arusha Lutheran Medical Centre (ALMC). Infants were enrolled during the warm season between November 2018 and January 2019. A pre-coded questionnaire was used to collect data on sociodemographic characteristics of the infant with consent from their caretakers. Physical examination was done for anthropometric measures and signs of rickets. Blood was drawn for assessment of serum 25-hydroxyvitamin D 25(OH)D, calcium, phosphorus and alkaline phosphate. Vitamin D deficiency was defined as 25(OH)D level below 20 ng/ml (<50 nmol/L) and Vitamin D insufficiency defined as a 25(OH)D level 20 - 30 ng/ml (50 - 75 nmol/L). Statistical analysis was performed using STATA 14 version and factors associated with VDD explored with multivariate analysis. **Results:** The mean serum 25(OH)D among infants was 34.51 ng/ml (±15.53). Vitamin D deficiency was found in 67/304 (22%) infants and Vitamin D insufficiency in 50 (16.5%) infants. Hy-

pocalcemia was observed in 33 (10.9%) infants and clinical findings of rickets were found in 11 infants (3.6%). Factors independently associated with VDD included age < 6 months (Adjusted Odds Ratio (AOR) 1.56, 95% CI 1.19 - 4.0, p value < 0.026), serum signs of rickets and serum hypocalcemia (p-value < 0.001 and <0.002, respectively). **Conclusion and Recommendation:** A high prevalence of Vitamin D deficiency (22%) and insufficiency (16.5%) was observed among infants attending RCH Clinic in Arusha, Tanzania. Age < 6 months, a single serum measurement of hypocalcemia and the presence of the clinical sign of rickets were independently associated with VDD. Clinicians should actively assess for VDD and supplement with vitamin D as indicated, especially among infants < 6 months.

Keywords

Vitamin D Deficiency, Rickets, Infants, RCH Clinic, Tanzania

1. Background

Vitamin D deficiency is a major public health problem worldwide in all age groups, even in those residing in countries with low latitude, where it is generally assumed that ultraviolet radiation is adequate to prevent this deficiency [1]. Humans obtain vitamin D from exposure to sunlight, diet, and dietary supplements, with sunlight exposure providing the commonest source [2]. Globally, approximately 1 billion people have low vitamin D levels (serum 25(OH)D < 30 ng/ml), with infants being at increased risk [3]. A review and meta-analysis of 129 studies in Africa involving 23 countries found the overall prevalence of vitamin D deficiency among 21,472 participants was 34.22% using a serum 25(OH)D cut off of less than 20 ng/mL (50 nmol/L) and 59.54% using a cut of <30 ng/mL (75 nmol/L) [4].

A Tanzanian study among infants along the coastal area of Dar es Salaam observed an increased prevalence of VDD among younger infants, with 76.4% of 6-week-old infants having VDD compared to 21.2% of 6-month-old infants [5]. Factors associated with vitamin D deficiency include inadequate sun exposure, dark skin pigmentation, exclusive breastfeeding, prematurity, chronic illnesses (*i.e.*, liver and kidney disease) malabsorption, selective anticonvulsant medications and malnutrition [2] [3] [5] [6] [7].

This study was conducted at a hospital clinic in Arusha, Tanzania, where the weather is cooler for much of the year than in more tropical regions of Tanzania. Arusha city in northern Tanzania (altitude 1400 meters) experiences a wide temperature range (12˚C - 28˚C), in contrast to other areas in Tanzania where climate temperatures remain above 23˚C [8]. It is hypothesized that cooler weather exposure necessitates the common local practice of swaddling infants in ways that limit direct sun exposure, potentially contributing to higher levels of Vitamin D deficiency [8] [9]. We had also observed that breastfeeding mothers

and infants are not routinely supplemented with Vitamin D, and infants undergoing supplemental feeding are fed locally available grain-based foods without Vitamin D fortification. Despite these observations, the prevalence of Vitamin D deficiency had not previously been assessed in this region of northern Tanzania.

This study seeks to describe the prevalence of Vitamin D deficiency and associated risk factors among infants attending a Reproductive and Child Health Clinic in Arusha, Tanzania. Findings may offer further evidence for the role of early intervention and Vitamin D supplementation amongst infants who reside in Tanzania.

2. Methods

2.1. Study Design and Setting

A cross-sectional study was employed to recruit a total of 304 infants attending Reproductive and Child Health Clinic (RCH) at Arusha Lutheran Medical Centre (ALMC) in Arusha, Tanzania. The study was conducted during the months of November 2018 to January 2019 which is the warm season.

Arusha lies in northern Tanzania at 1400 meters (4600') altitude, at −3.386˚ latitude and 36.68˚ longitude. The average annual temperature in Arusha is about 19.2˚ Celsius [10]. The rainy season is generally during the months of March through May and November and December. The major tribes in this region are the Maasai and Wameru peoples and during cold season infants and children are dressed in sweaters and Maasai Shuka, which prevent arms and legs from exposure to sunlight.

ALMC is a faith-based hospital and serves as a zonal referral hospital for Northern Zone of Tanzania. The RCH clinic is located directly within ALMC hospital. Clinic is conducted twice a week for children under five years of age. Infants and children living far away from the hospital may receive services at RCH irrespective of weekday. A monthly average of 320 infants and toddlers are cared for at ALMC RCH clinic, and 25% (80) are typically new patients. Vitamin D supplementation is not routinely offered in clinic to either infants or pregnant mothers.

2.2. Study Participants

The study was powered at 80%, at an absolute error between the estimated and true value of 5% with a 95% confidence interval. We used a prevalence of vitamin D deficiency 76.4% among infants as reported by Sudfeld CR *et al*. in a study entitled *Vitamin D deficiency is not associated with growth or the incidence of common morbidities among Tanzanian infants* [5]. The sample size calculated was 304.

Infants aged 6 weeks to 12 months that attended RCH at ALMC during the study period whose parents gave informed consent were recruited into the study.

Infants with known chronic renal disease, liver disease and those who already started vitamin D supplementation were excluded. The study was conducted in partial fulfillment of thesis requirements for a Master of Medicine in Paediatrics

and Child Health at Makerere University. Ethics approval was obtained from the School of Medicine Research and Ethics Committee (SOMREC), College of Health Science, Makerere University and the study site, Arusha Lutheran Medical Centre Ethics Committee, prior to study commencement.

2.3. Study Procedure

Study participants who included infants aged 6 weeks to 12 months attending the RCH clinic identified during the study period were consecutively enrolled as they presented to the clinic on Mondays and Thursdays, after obtaining consent from their caretakers. The research team comprised of 4 research assistants (2 nurses, 2 physicians) and the principal investigator. Systematic sampling was used to enroll the infants in this study and written informed consent obtained from the caretakers of the children. During the reviews, a clinical history was taken with information obtained through questionnaire on family socio-demographics, gestational age at birth, growth and developmental history, diet and mode of feeding, dressing habits, assessment of infant's level of sun exposure, HIV-exposure or infection status, and presence of known chronic illnesses. Further information was obtained on maternal use of chronic medications and maternal HIV status. Adequate sun exposure was defined as having at least 30 minutes of sun exposure for a minimum of 5 days per week involving face, back of the hand, forearm, and part of the trunk, with exposure occurring between the daylight hours of 10 am and 4 pm [11].

A detailed physical examination was performed by study team physicians to assess for clinical signs of rickets, including craniotabes, rachitic rosary, widened/thickened wrists or ankles, bowlegs, and Harrison groove. Anthropometric measurements included weight in kilograms and length in centimeters that were taken using weight scale and stadiometer respectively.

2.4. Laboratory Investigation

A volume of 3 ml of blood was drawn and analyzed for serum 25(OH)D, calcium, phosphorus, alkaline phosphate, albumin, alanine transaminase and creatinine. The biochemical tests were also measured using ALMC laboratory. A Eurolyser machine was used for renal function tests, electrolytes, liver function tests and serum 25(OH)D were obtained using COBAS machine at Kilimanjaro Christian Medical Centre (KCMC) in Moshi, Tanzania. KCMC laboratory participates in both internal and external quality control audits and is accredited by Southern African Development Community Accreditation Services; ISO15189-2012.

Serum samples for the measurement of 25(OH)D were processed and stored at −20 degree Celsius as they waited to be measured later using the Elecsys immunoassay technique.

This is an electrochemiluminescence immunoassay supplied by Roche Diagnostics, Germany. It measures the vitamin D concentrations in the range of 4 -

100 ng/ml. The 25(OH)D level above >30 ng/mL (>75 nmol/L) was considered normal, >20 - 30 ng/mL (50 - 75 nmol/L) as insufficient and value below 20 ng/mL (<50 nmol/L) as deficient. A total corrected calcium level of 8.5 - 10.5 mg/dl (2.1 -2.6 mmol/L) was considered normal and a level of corrected calcium below 8.5 mg/dL as hypocalcemia. Phosphorus level of 4.8 - 7.5 mg/dL (1.55 - 2.42 mmol/L) was considered normal. Alkaline phosphate level > 410 IU/dL was considered raised. Albumin less than 3 g/dL was determined low. Creatinine level between 0.2 - 0.4 mg/d L and an alanine transaminase between 13 - 45 IU was considered normal.

All infants with vitamin D deficiency were treated as per the standard guidelines and linked to Paediatrics clinic.

2.5. Statistical Analysis

Statistical analysis was performed using the Stata 14.0 statistical package. Continuous variables were summarized using median (interquartile range) and means (standard deviations). The prevalence of vitamin D deficiency was calculated as the proportion of infants with vitamin D deficiency among all those enrolled in the study. Categorical variables were compared between infants with 25(OH)D less than 20 ng/mL as deficiency versus ≥20 ng/mL. To determine the factors associated with vitamin D deficiency, logistic regression was used to determine the factors independently associated with vitamin D deficiency. Multivariate logistic regression was used to perform all variables found to have a p-value ≤ 0.2 at bivariate analysis and all variables that are biologically plausible that did meet the criteria were entered in the model. Weight-for-length and weight-for-age were calculated from the WHO standard chart, 0 - 24 months. A p-value less than 0.05 was considered significant.

3. Results

This study was conducted between 15th November 2018 and 31st January 2019. Study enrollment profile is shown in Figure 1.

4. Study Profile

4.1. Characteristics of the Study Participants and Mothers/Caretakers

Table 1 shows the characteristics of the participants. The median age of the study participants was 6-months (IQR 7), with 159 (53.3%) of the infants aged ≥ 6-months. Male infants were 155 (51%). Term gestation with a birth weight > 2.5 kilograms was reported in 278 (91.4%) participants. Exclusive breastfeeding was common among this population. Twenty-five infants (8.2%) were considered HIV-exposed due to maternal status, and eleven (3.6%) infants were previously confirmed to be HIV-infected. Sun exposure was reported as good among 77% of infant participants, with mothers reporting at least 30 minutes of sun exposure to skin for 5 days/week. Fifteen of the mothers (4.9%) were on Vitamin D

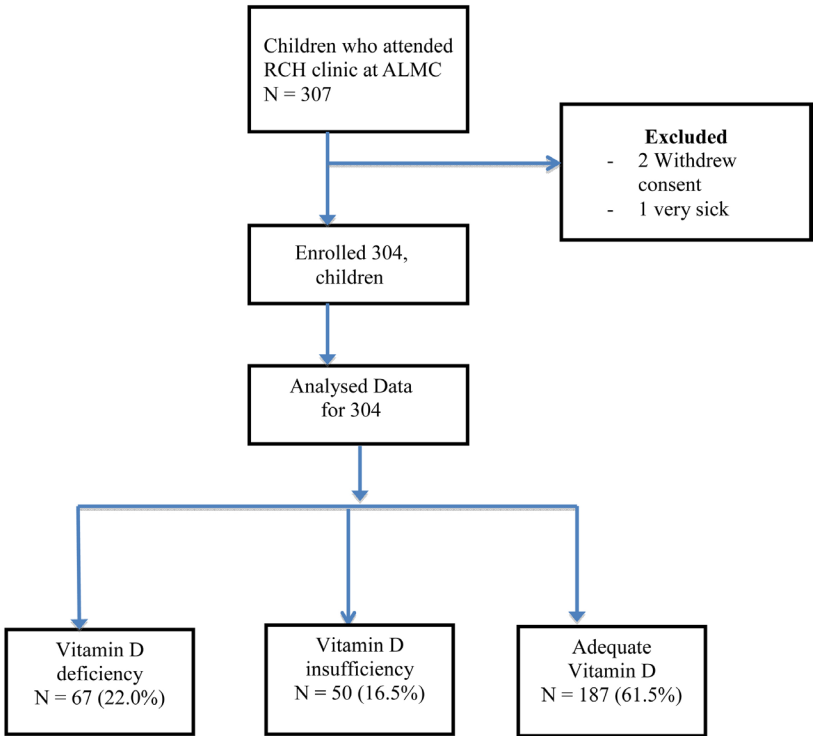

Figure 1. Showing study profile of the infants.

containing supplement.

4.2. Prevalence of Vitamin D Deficiency

The prevalence of vitamin D deficiency was 67/304 (22%, **Figure 2**). The mean serum 25(OH)D level among all infants was 34.51 (±15.53) ng/mL and the mean calcium level was 10.24 mg/dL (±2.44). Mean vitamin D deficiency level among infants was 15.7 ng/mL (±1.99) and the mean calcium level VDD infants was 8.5 ng/mL (±1.53).

Most of the infants (40/67) with Vitamin D deficiency were <6 months. Among all infants, 10.9% had low calcium (<8.5 mg/dl), 7.6% had low phosphorus (<4.2 mg/dl), and 7.9% had raised alkaline phosphatase (ALP, >400 IU). Among the 67 infants with VDD, 10 (15%) infants had 25(OH)D levels less than 12 ng/ml (30 nmol/L), with a mean serum calcium of 8.45 (±1.24). One VDD infant had severe acute malnutrition.

Eleven infants (3.6%) out 304 infants were determined by clinical exam to have rickets. Infants with rickets had a mean (SD) serum 25(OH)D level of 24.23 ng/mL (±14.5), and mean serum values of calcium 8.4 (±1.24) mg/mL, phosphorus 5.12 (±0.93) mg/dL and ALP 380 (±74.5) IU. Among the 11 infants with rickets, 4 (36%) infants had raised ALP > 400 IU with 7/11 (63.6%) demonstrating hypocalcemia and 5/11 (45%) hypophosphatemia.

4.3. Factors Associated with Vitamin D Deficiency

Table 2 shows factors associated with vitamin D deficiency at bivariate analysis

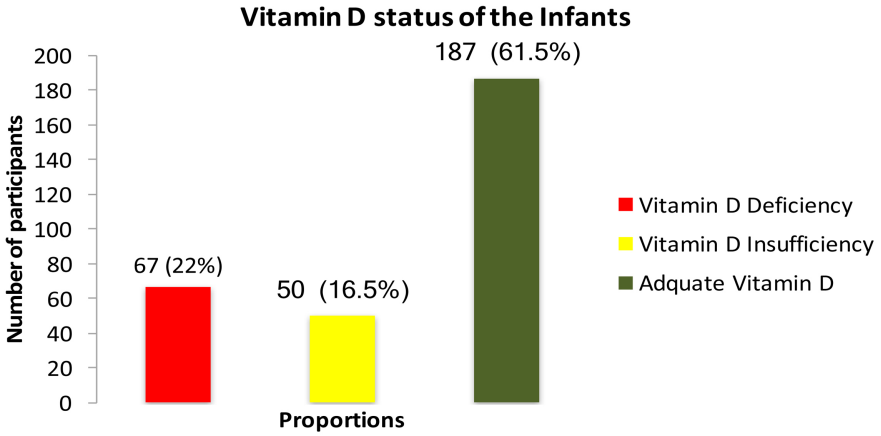

Figure 2. Bar graph showing vitamin D status of the infants.

Table 1. Baseline characteristics of infants and mothers/caretakers.

	Characteristics	Frequency	Percentage %
Age	<6 month	145	47.7
	≥6 months	159	53.3
Sex	Male	155	51
	Female	149	49
Religion	Christian	263	86.5
	Muslim	41	13.5
Gestational age	Term	278	91.4
	Preterm	26	8.6
Infants HIV status	Negative	268	88.2
	Positive	11	3.6
	HIV-exposed, uninfected	25	8.2
Type of feeding	Exclusive Breastfeed	142	46.7
	Mixed/Complimentary feeding	159	52.3
	Formula feeding	3	1
Other Chronic illness*	No	291	95.7
	Yes	13	4.3
Season of the month	Rainy Season	64	21.1
	Sunny/Dry Season	240	78.9
Sun exposure**	Yes	234	77
	No	70	23
Milestone	Up to date	289	95.1
	Delayed	15	4.9
Maternal religion	Christian	265	87.2
	Muslim	39	12.8
Maternal HIV status	Negative	268	88.2
	Positive	36	11.8

*Chronic illness: epilepsy, malabsorption, liver disease, kidney disease and congenital heart disease. **Sun Exposure: was defined as at least 30 min daily of exposure to sun from between 10 am - 4 pm.

Table 2. Factors associated with vitamin D deficiency at bivariate and multivariate analysis.

Variable		Not deficient	25(OH)D Deficient	OR (95% CI)	p-value	AOR (95% CI)	p-value
Age	≥6 month	132 (83)	27 (17)	1		1	
	<6 month	105 (72.4)	40 (27.6)	**1.86 (1.07, 3.23)**	**0.026**	**1.56 (1.19, 4.0)**	**0.026**
Sex	Male	119 (76.8)	36 (23.2)	1			
	Female	118 (79.2)	31 (20.8)	0.87 (0.50, 1.50)	0.611		
Gestational age	Term	217 (78.1)	61 (21.9)	1		1	
	Preterm	20 (76.9)	6 (23.1)	1.07 (0.41, 2.77)	0.894	0.93 (0.3, 2.9)	
HIV status	Negative	209 (78)	59 (22)	1		1	
	Positive	8 (72.7)	3 (27.3)	1.33 (0.34, 5.17)	0.682	1.11 (0.38, 3.29)	0.845
	Exposed, uninfected	20 (80)	5 (20)	0.89 (0.32, 2.46)	0.816	1.13 (0.27, 4.69)	0.866
Type of feeding	Breastfeeding only	104 (73.2)	38 (26.8)	1			
	Mixed or Complimentary feeding	133 (82.1)	29 (17.9)	0.60 (0.35, 1.03)	0.064		
Sunlight Exposure	No	48 (68.6)	22 (31.4)	1		1	
	Yes	189 (80.8)	45 (19.2)	**0.52 (0.29, 0.95)**	**0.032**	0.6 (0.31, 1.16)	0.129
Religion	Christian	205 (77.4)	60 (22.6)	1			
	Muslim	32 (82.1)	7 (17.9)	0.75 (0.31, 1.78)	0.51		
Maternal vitamin D Supplement	No	225 (77.9)	64 (22.1)	1			
	Yes	12 (80)	3 (20)	0.88 (0.24, 3.21)	0.845		
Mother HIV status	Negative	204 (78.2)	57 (21.8)	1			
	Positive	33 (76.7)	10 (23.3)	1.08 (0.5, 2.33)	0.836		
Clinical signs of Rickets	No	233 (79.5)	60 (20.5)	1		1	
	Yes	4 (36.4)	7 (63.6)	**6.8 (1.93, 23.98)**	**0.003**	**15.63 (2.9, 84.33)**	**0.001**
Corrected Hypocalcemia	No	233 (79.5)	60 (20.5)	1		1	
	Yes	4 (36.4)	7 (63.6)	**2.25 (1.01, 5.01)**	**0.002**	**2.34 (1.99, 5.54)**	**0.002**
Alkaline Phosphatase (ALP)	Normal	212 (76.0)	67 (24.0)	1			
	Low ALP	4 (80)	1 (20)	0.84 (0.09, 7.66)	0.878		
	High ALP	3 (12.5)	21 (87.5)	0.48 (0.14, 1.66)	0.248		

and multivariate analysis. Infants aged <6 months were 86% more likely to develop VDD at bivariate analysis with p value < 0.026. Factors such as hypocalcemia and having had sign of rickets were significantly associated with VDD, OR: 2.25 (95% CI: 1.01, 5.01) p value = 0.002, and OR: 6.8 (95% CI: 1.93, 23.98), p value = 0.003 respectively at bivariate analysis. Other factors such as sun ex-

posure was protective at bivariate analysis OR 0.52 (0.29, 0.95) at p value of 0.032 but was not significant on multivariate analysis.

Biologically plausible factors such as prematurity, breastfeeding and HIV status underwent multivariate analysis.

Factors that were independently associated with VDD at multivariate analysis including; infant age < 6 month (AOR: 1.56 CI: 1.19, 4.0) p value < 0.026, Rickets feature (AOR: 15.35 CI: 2.86, 82.3) P value < 0.001, and hypocalcemia (AOR 2.34 C.I 1.99, 5.54) p < 0.048.

5. Discussion

The prevalence of vitamin D deficiency (<20 ng/mL) in this study of 304 Tanzanian infants was 22%, with an additional 16.5% determined to be vitamin D insufficient (20 - 30 ng/mL). Only 61.5% of infants had optimal levels of serum Vitamin D. Infants who were <6 months old were significantly more likely to be VDD (27.6%) than infants ≥ 6 months (17%). The prevalence of VDD among infants in our study was consistent with a previous study conducted in Nairobi, Kenya by Said *et al.*, where VDD was noted in 23.5% of exclusively breastfed infants [5] [12]. Both Nairobi and Arusha share similar weather characteristics and altitudes [13] [14].

More than 1/3 of the infants in our study population were vitamin D insufficient or deficient. These findings can be partially explained by weather in Arusha, which is considered cooler, with average daily temperatures ranging from 16.8˚C to 22.7˚C throughout the year and having temperature lows of 12.1˚C during the coolest months [8]. With cooler climate infants are often kept indoors and swaddled, allowing less sun opportunity for exposure. Susanna *et al.* were able to associate cold weather with vitamin D deficiency [15]. While most parents reported adequate sun exposure, we were unable to independently verify the actual degree of sun exposure occurring in our study population. Most of the infants in our study population were dark skinned, and pigmentation can hinder the conversion of vitamin D by ultraviolet light in the skin. Ann Prentice *et al.* were able to associate Vitamin D deficiency and dark skin pigmentation [16].

Despite our study's climate and location, other studies carried out in Uganda and Tanzania have suggested an even higher prevalence of VDD exists among young infants. A VDD prevalence has been reported as high as 76.4% at 6 weeks and between 34.6% - 83.7% among infants < 6 months [5] [6] [12] [17]. The discrepancy in VDD prevalence in these studies with our results may be explained by age and socioeconomic differences of study participants. Our study had infants aged 6 weeks to one year, while the other studies evaluated only infants < 6 months. Furthermore, our study site (ALMC, Arusha) serves a more middle-income, urban population with potentially lower rates of both child and maternal malnutrition and broader available food sources containing Vitamin D.

In our study, VDD was present in 3.5%, 22% or 38.5% of infants, depending

on whether a 25(OH)D level of <12 ng/ml, <20 ng/ml or <30 ng/ml was utilized, respectively. In comparison, Mogire *et al*'s meta-analysis of 23 countries noted a higher prevalence of 18.46%, 34% and 59.59% at 25(OH)D levels of <12 ng/mL, <20 ng/mL and <30 ng/mL, respectively [4]. This difference from our findings could also be explained by variations of age, population groups, socio-economic status, nutrition, and climate (northern/southern Africa vs Arusha, Tanzania) in Mogire *et al*.'s meta-analysis.

Our study demonstrated that age of the infants, clinical signs of rickets and presence of hypocalcemia were significantly associated with VDD. Infants who are older than 6 months were less likely to have VDD. Increases in physical activity above 6 months of age, such as crawling, may increase opportunities sun exposure. Older infants will have initiated foods which are also likely sources of vitamin D. Ziegler *et al.* observed low rates of vitamin D deficiency among older children [13]. The commonly used infant foods in middle-income Tanzanians include eggs and porridge with vitamin D-containing margarines and fish oils. Locally available powdered formula milk (Nestle® Lactogen and NAN) also contain Vitamin D [14]. As such, older infants may have less VDD through introduction of these non-breastmilk feedings, including vitamin-D enriched formula. Younger bottle fed infants appear more likely to have less vitamin D deficiency than exclusively breastfed infants [13]. Breast milk has low level of vitamin D and mothers in Arusha are not routinely supplemented with Vitamin D. Gordon CM *et al*., in a study of infants, noted that exclusively breastfed infants had a >10-fold increase in VDD compared to those who were exclusively formula-fed babies [15]. Said *et al.* observed vitamin D deficiency among <6 months with exclusive breastfeeding [12]. Gordon C, Sudfeld C, and Nalunkuma Cissy were also able to associate vitamin D deficiency with age [5] [6] [15]. In our study, only 2 participants less than 6 months were on formula milk. This made it difficult to assess if formula milk reduces the risk of vitamin D deficiency because of the inadequate sample size. However, Gordon et al reported infants on fortified formula milk to have adequate vitamin D level, thereby highlighting its protective effect against VDD [15].

In our study, only 11 infants had demonstrated signs of rickets. Clinical rickets were determined by examination only, and imaging was not obtained as part of this study. Those patients who were found to have vitamin D deficiency were referred to the Paediatric Outpatient Clinic, where imaging was later done. The finding of few patients with features of rickets is similar to a study done by Piloya *et al.* [18]. Infants with signs of rickets frequently have a level of 25(OH)D < 12 ng/ml (<30 nmol/L) [19] [20]. Our study's finding that 7 infants with clinical signs of rickets also had VDD is not surprising. However, 4/7 infants with clinical rickets had serum 25(OH)D levels > 12 ng/dL. This is consistent with findings in the WHO nutritional rickets report of 2020 whereby rickets can occur at vitamin D > 12 ng/ml [21]. Furthermore, 4/11 infants with clinical signs of rickets did not demonstrate any VDD, which was unexpected. Signs of rickets likely arise as evidence rather than a cause of VDD. Holick MF *et al.* made similar ob-

servations [19]. Unlike for adults, serum vitamin D deficiency thresholds in infants remain an area of research.

Laboratory investigations of serum calcium concentration, serum phosphate and serum alkaline phosphate were also performed. Only serum calcium level was associated with VDD in our study. This can be explained by the role of vitamin D in the metabolism of calcium, as vitamin D helps maintain calcium homeostasis by increasing absorption of ionized calcium in the intestines and through bone resorption. Thus, the major physiologic function of vitamin D is to maintain serum calcium and phosphorus levels within the normal physiologic range. Therefore low 25(OH)D level can result in hypocalcemia. A study by Sukru Hatun *et al*. also made similar observations between vitamin D deficiency and hypocalcemia among a cohort of Turkish children [22].

Alkaline phosphatase levels were normal in majority of our infants with VDD. This finding is contrary to studies which have examined ALP in relation with VDD. Nalunkuma *et al*. were able to associate vitamin D with elevated ALP [6]. However, finding no association between 25(OH)D and ALP levels is similar to the study done by Basu *et al*., where VDD was not correlated with ALP [23]. In our study, the mean 25(OH)D level among VDD infants was 15.7 ng/mL, whereas the development of abnormal bone markers like ALP may begin at 25(OH)D levels < 14 ng/mL [24].

5.1. Strength of the Study

This is the first study in Tanzania that measured serum 25(OH)D level among infants aged 6 weeks to 12 months who attended an RCH clinic, providing a prevalence of vitamin D deficiency. This study was conducted at the RCH clinic in which services are free. The laboratory analysis used (electrochemiluminescence) has a good sensitivity and a broad dynamic range [25].

5.2. Limitations of the Study

We obtained information from the caregivers, which may be subject to recall and information bias. The cross-sectional study design applied may not establish causality. We were unable to objectively assess the infant's true degree of exposure to sunshine, and caregiver responses were subjective. Furthermore, we were unable to assess the level of dietary sources of calcium and vitamin D among study participants, nor able to measure the parathyroid hormone (PTH) levels.

6. Conclusion

There is a high prevalence of Vitamin D deficiency (22%) among infants attending RCH clinic at ALMC in Arusha, Tanzania. Infants less than 6 months of age, and with evidence of rickets or hypocalcaemia were more likely to have VDD.

Recommendation

Active assessment for Vitamin D deficiency and health education should be rou-

tinely provided for all infants presenting at RCH clinic, where growth assessment and vaccine administration are taking place. Tanzania's Ministry of Health should consider formulating a policy on vitamin D supplementation for infants below 6 months.

Acknowledgements

We acknowledge all the caretakers and those who participated in this study as well as the staff at ALMC Reproductive and Child Health clinic.

Ethics Approval and Consent to Participate Statement

Approval to carry out this study was obtained from the Arusha Lutheran Medical Centre Institutional Review Board (Rec Number N/A) Arusha, Tanzania. Written Informed consent was obtained from the mothers of the participants. All study procedures were done in accordance with institutional guidelines of ALMC.

Consent for Publication

There are no images, videos and details relating to individual persons in this Manuscript.

Funding

ALMC NICU Fund funded this study. The funders were not involved in the design of the study, collection of data, analysis, and interpretation of results and in the writing of the manuscript.

Authors' Contributions

VU participated in the conception and design of the study, data collection, participated in the statistical analysis and drafting the manuscripts. PK participated in the design of the study, contributed to the interpretation of data and helped to draft the manuscript. VM participated in the design of the study, statistical analysis and helped to draft the manuscript. TP participated in the design of the study, contributed to the interpretation of data and helped to draft the manuscript. SS participated in the design of the study, contributed to the interpretation of data and helped to draft the manuscript. All authors read and approved the final manuscript.

Availability of Data and Material

The original data set will be made available by the corresponding author upon request.

Conflicts of Interest

The authors declare they have no competing interests.

References

[1] Palacios, C. and Gonzalez, L. (2014) Is Vitamin D Deficiency a Major Global Public Health Problem? *The Journal of Steroid Biochemistry and Molecular Biology*, **144**, 138-145. https://doi.org/10.1016/j.jsbmb.2013.11.003

[2] Holick, M.F. (2003) Vitamin D: A Millennium Perspective. *Journal of Cellular Biochemistry*, **88**, 296-307. https://doi.org/10.1002/jcb.10338

[3] Holick, M.F. and Chen, T.C. (2008) Vitamin D Deficiency: A Worldwide Problem with Health Consequences. *The American Journal of Clinical Nutrition*, **87**, 1080S-1086S. https://doi.org/10.1093/ajcn/87.4.1080S

[4] Mogire, R.M., Mutua, A., Kimita, W., Kamau, A., Bejon, P., Pettifor, J.M., *et al*. (2020) Prevalence of Vitamin D Deficiency in Africa: A Systematic Review and Meta-Analysis. *The Lancet Global Health*, **8**, e134-e142. https://doi.org/10.1016/S2214-109X(19)30457-7

[5] Sudfeld, C.R., Manji, K.P., Smith, E.R., Aboud, S., Kisenge, R., Fawzi, W.W., *et al*. (2017) Vitamin D Deficiency Is Not Associated with Growth or the Incidence of Common Morbidities among Tanzanian Infants. *Journal of Pediatric Gastroenterology and Nutrition*, **65**, 467-474. https://doi.org/10.1097/MPG.0000000000001658

[6] Cissy, N., Paul, L., Edison, M. and Fox, L. (Eds.) (2014) Survey of Vitamin D Status in Breastfeeding Infants under the Age of 6 Months Attending the Growth Monitoring Clinic at Rubaga Hospital. *European Society for Paediatric Endocrinology* (*ESPE*) 53*rd Annual Meeting*, Dublin, 18-20 September, ESPE Abstract (2014) P-D-3-1-665.

[7] Voortman, T., van den Hooven, E.H., Heijboer, A.C., Hofman, A., Jaddoe, V.W. and Franco, O.H. (2015) Vitamin D Deficiency in School-Age Children Is Associated with Sociodemographic and Lifestyle Factors. *The Journal of Nutrition*, **145**, 791-798. https://doi.org/10.3945/jn.114.208280

[8] WeatherBase (2019) Arusha Weather 2019. http://www.weatherbase.com/weather/weather.php3?s=98736

[9] Climates to Travel (2018) Climate to Travel in Arusha 2018. http://www.climatestotravel.com/climate/tanzania

[10] Tanzania Meterological Agency (2019) Statement on the Status of Tanzania Climate in 2019. http://www.meteo.go.tz/uploads/publications/sw1626329327-Climate_Statement2019.pdf

[11] Tangpricha, V. (2015) What Is the Recommended Sun Exposure for Production of Vitamin D? http://www.medscape.com/answers/128762-54304/what-is-the-recommended-sun-exposure-for-production-of-vitamin-d

[12] Said, N.A., Kamenwa, R.W., Limbe, M.S., Okumu, M.O. and Macharia, W.M. (2020) Prevalence of Vitamin D Deficiency in Exclusively Breastfed Infants at a Tertiary Healthcare Facility in Nairobi, Kenya. *Archives of Endocrinology and Metabolism*, **64**, 726-734. https://doi.org/10.20945/2359-3997000000281

[13] Ziegler, E.E., Hollis, B.W., Nelson, S.E. and Jeter, J.M. (2006) Vitamin D Deficiency in Breastfed Infants in Iowa. *Pediatrics*, **118**, 603-610. https://doi.org/10.1542/peds.2006-0108

[14] Nestle Lactogen 1 Formula Ingredients (2020). https://eshop.tesco.com.my/groceries/en-GB/products/7074044729

[15] Huh, S.Y. and Gordon, C.M. (2008) Vitamin D Deficiency in Children and Adolescents: Epidemiology, Impact and Treatment. *Reviews in Endocrine and Metabolic*

Disorders, **9**, 161-170. https://doi.org/10.1007/s11154-007-9072-y

[16] Prentice, A. (2008) Vitamin D Deficiency: A Global Perspective. *Nutrition Reviews*, **66**, S153-S164. https://doi.org/10.1111/j.1753-4887.2008.00100.x

[17] Rwebembera, A., Sudfeld, C.R., Manji, K.P., Duggan, C., Aboud, S. and Fawzi, W.W. (2013) Prevalence and Risk Factors for Vitamin D Deficiency among Tanzanian HIV-Exposed Uninfected Infants. *Journal of Tropical Pediatrics*, **59**, 426-429. https://doi.org/10.1093/tropej/fmt028

[18] Piloya, T., Odongkara, B., Were, E.M., Ameda, F., Mworozi, E. and Laigong, P. (2018) Nutritional Rickets among Children Admitted with Severe Pneumonia at Mulago Hospital, Uganda: A Cross-Sectional Study. *BMC Pediatrics*, **18**, Article No. 336. https://doi.org/10.1186/s12887-018-1310-9

[19] Holick, M.F. (2006) Resurrection of Vitamin D Deficiency and Rickets. *The Journal of Clinical Investigation*, **116**, 2062-2072. https://doi.org/10.1172/JCI29449

[20] Vitamin D Deficiency (Beyond the Basic) (2020). https://www.uptodate.com/contents/vitamin-d-deficiency-beyond-the-basics

[21] WHO (World Health Organization) (2020) Nutritional Rickets: A Review of Disease Burden, Causes, Diagnosis, Prevention and Treatment. World Health Organization, Geneva.

[22] Hatun, S., Ozkan, B., Orbak, Z., Doneray, H., Cizmecioglu, F., Toprak, D., *et al.* (2005) Vitamin D Deficiency in Early Infancy. *The Journal of Nutrition*, **135**, 279-282. https://doi.org/10.1093/jn/135.2.279

[23] Basu, S., Gupta, R., Mitra, M. and Ghosh, A. (2015) Prevalence of Vitamin D Deficiency in a Pediatric Hospital of Eastern India. *Indian Journal of Clinical Biochemistry*, **30**, 167-173. https://doi.org/10.1007/s12291-014-0428-2

[24] Atapattu, N., Shaw, N. and Högler, W. (2013) Relationship between Serum 25-Hydroxyvitamin D and Parathyroid Hormone in the Search for a Biochemical Definition of Vitamin D Deficiency in Children. *Pediatric Research*, **74**, 552-556. https://doi.org/10.1038/pr.2013.139

[25] Arneson, W.L. and Arneson, D.L. (2013) Current Methods for Routine Clinical Laboratory Testing of Vitamin D Levels. *Laboratory Medicine*, **44**, e38-e42. https://doi.org/10.1309/LMONQZQ27TIN7XFS

Abbreviations

25(OH)D, 25 hydroxyvitamin D; ALMC, Arusha Lutheran Medical Centre; ALP, Alkaline Phosphatase; ALT, Alanine Transaminase; HIV, Human Immunodeficiency Virus; KCMC, Kilimanjaro Christian Medical Centre; NICU, Neonatal Intensive Care Unit; RCH clinic, Reproductive and Child Health clinic; WHO, World Health Organization.

Appendix

Study ID No ____ Date of enrollment …./…./….

ENGLISH QUESTIONNAIRE/CASE RECORD FORM

CHILD FACTORS	
1) Date of birth	_ / _/ _
2) Age in months	…..……..
3) Sex	1. Male (…….) 2. Female (……) Tick at appropriate box
4) Religion	1. Muslim ( ) 2. Christian ( ) Tick at appropriate box
5) Birth weight of your child	……. (kgs)
6) Your child was born Term or Preterm	1. Term ( ) 2. Preterm( ) Tick at appropriate box
7) HIV status of your child	1. Positive (…..) 2. Negative (….) Tick at appropriate box
8) Type of the feeding	1. Breastfeed only( ) 2. Mixed feeding ( ) 3. Formula milk only ( ) 4. Cow milk only ( ) 5. If Others specify ( )
9) Duration of exclusive breastfeeding	1. <6 month (……..) 2. 6 month (……..) 3. >6 month (…….)
10) Is the child suffered from any chronic illness? **If the answer is No is question 10, skip question 11**	1. Yes ( ) 2. No ( )
11) If the answer is yes from question 10, which one among this?	1. Sickle Cell Disease ( ) 2. Heart Disease ( ) 3. If any other specify ……
12) Is your child on any chronic medication (s)? **If the answer is No in question 12, skip question 13**	1. Yes ( ) 2. No ( )
13) If the answer is yes from question 12, which one among this?	1. Antiepileptic (……) 2. AntiTB (……..) 3. ARV (……..) 4. Specify if any other ……..
14) Milestone	1. Up-to-date milestones ( ) 2. Delayed milestones ( )

ENVIRONMENTAL FACTORS

15) Are you expose your baby on Sunlight? **If the answer in question 15 is No, skip question 16 ,17 and 18**	1. Yes ( ) 2. No ( )
16) If the answer is Yes in question 15, How many days in a week you exposed your child on sunlight?	1. less than 4 days in a week ( ) 2. four days or more than for days in a week ( ) 3. if any other duration specify ……..
17) How long you spend expose your baby on sunlight	1. Less than 30 minutes ( ) 2. More than 30 minutes ( ) 3. None ( )
18) How are you dressing your baby during sun exposure	1. Undressing the whole body ( ) 2. Undressing Arms, hand, legs and/or Face( ) 3. Dressing whole body( ) 4. If any other specify …….
19) Seasonal of birth	1. Rainy Season ( ) 2. Sunny/Dry Season ( )

MATERNAL FACTORS

20) Age of the mother	**…….in years**
21) Religion of the mother	1. Christian ( ) 2. Muslim ( ) 3. If any other specify ……
22) Are you exposed in Sunlight **If answer is No skip question number 23,24 and 25**	1. Yes ( ) 2. No ( ) tick appropriate response
23) If the answer for question 22 is yes, How many times a week?	1. less than 4 days in a week ( ) 2. four days or more than for days in a week ( ) 3. if any other duration specify ……..
24) How long you spend on sun exposure?	1. Less than 30 minutes ( ) 2. More than 30 minutes ( ) 3. None ( )
25) How are you dressing during sun exposure	1. Undressing Arms, hand, legs and/or Face 2. Covering Arms, hand, legs and/or Face 3. IF any other means specify……
26) Are you Vegetarian?	1. Yes ( ) 2. No ( )
27) Any history of maternal Vitamin D supplementation during pregnancy or after delivery?	1. Yes ( ) 2.No ( )
28) HIV status of the mother?	1. Negative ( ) 2. Positive ( )

29. Anthropometry

Weight	……. kilograms
Height	……centimeters
MUAC	……centimeters
WHZ	……

30. Features of Rickets (Clinician Section)

Bone deformities	Yes ( ) No ( )
Rachitic rosary	Yes ( ) No ( )
Harrison's groove	Yes ( ) No ( )
Widened wrist	Yes ( ) No ( )
Cranial tapes	Yes ( ) No ( )

31. Systematic Examination

CARDIOVASCULAR
RESPIRATORY
GI
NEUROLOGICAL EXAMINATION

32. Laboratory Findings

Vitamin D levels	Level; ……..
Calcium levels	Level ……..
Phosphate	Level …….
Alkaline Phosphate (ALP)	Level ……..
Alanine Transaminase (ALT)	Level ……..
Albumin	Level ……
Bilirubin Direct	Level ……
Bilirubin Total	Level …….
Creatinine	Level …….