Vitamin D Status in Botswana Children under 2 Years Old with and without Active Tuberculosis

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Abstract. Additional strategies are needed to prevent and treat tuberculosis (TB). Although vitamin D may have antimycobacterial effects, it is unknown whether low vitamin D status confers a risk for active TB in African children. This case-control study assessed serum 25-hydroxyvitamin D (25(OH)D) concentration in children with and without active TB in Gaborone, Botswana. A total of 80 children under 2 years old with and without active TB, seen at hospitals and clinics in the greater Gaborone area between September 2010 and November 2012, were enrolled. Of these, 39 cases did not differ from the 41 controls in median 25(OH)D levels ($P = 0.84$). The 25(OH)D was < 20 ng/mL in 8/39 (21%) cases and 7/41 (17%) controls ($P = 0.69$, χ^2). Univariate analyses of subject clinical characteristics (other than 25(OH)D levels) showed that any degree of weight loss was associated with a diagnosis of TB ($P = 0.047$). Other clinical characteristics, including age ($P = 0.08$) or weight below third percentile ($P = 0.58$), showed no association with TB. There was no significant difference in vitamin D status between children under 2 years old with and without active TB. Lower vitamin D status did not appear to be a risk factor for TB in this small Gaborone cohort.

INTRODUCTION

The burden of tuberculosis (TB) in Botswana is among the highest in the world. In 2012, the incidence of TB was 408/100,000, and the TB and human immunodeficiency virus (HIV) coinfection rate was 62%.¹ In an immunocompetent adult population, after TB exposure, there is a lifetime conversion risk of 10% from latent TB to active TB. However, in HIV-infected adult patients, this risk increases to 10% per year.¹ In children, this risk is even higher and increases to as much as 50% in infants exposed to an adult with a history of smear-positive TB.²

The precise burden of childhood TB in Botswana is unknown. In a tertiary hospital-based autopsy study before the widespread rollout of antiretrovirals, 12.5% of HIV-seropositive children were found to have pulmonary TB.³ In 2012, the Botswana National TB Program reported that childhood TB cases, being those under 15 years old, accounted for 10% of the national TB caseload.⁴

Vitamin D may have unique antimicrobial effects on TB.⁵ In adult TB patients receiving TB treatment, supplementation with vitamin D is associated with increased rates of smear conversion from positive to negative for acid-fast bacilli.⁶ A proposed mechanism is that vitamin D enhances macrophage function thereby augmenting human immunity to mycobacteria.⁷ In addition, vitamin D deficiency is suggested to be associated with mycobacterial diseases.⁸ Although vitamin D supplementation may decrease susceptibility to active TB, there are minimal data exploring vitamin D status in pediatric patients living in Africa, a TB-endemic setting.⁶

Our objective was to assess serum 25-hydroxyvitamin D (25(OH)D) status in children with and without active TB in

Gaborone, Botswana, a high-burden TB setting in southern Africa.

MATERIALS AND METHODS

Setting. This prospective case-control study evaluated serum 25(OH)D concentrations of infants and toddlers with and without TB in hospitals and clinics in the greater Gaborone area between September 2010 and November 2012.

Study population. Children in Botswana aged 0 to 2 years old, with and without active TB, admitted to the pediatric ward at Princess Marina Hospital in Gaborone, Bamalete Lutheran Hospital in Ramotswa, Scottish Livingstone Hospital in Molepolole, and Deborah Retief Memorial Hospital in Mochudi, as well as outpatients seen at the Gaborone city clinics were eligible for enrollment.

Eligibility criteria and study definitions. Cases were defined as patients diagnosed by a pediatrician as having active TB at the time of enrollment. Controls were defined as patients without active TB. Cases and controls were selected from patients admitted to one of the selected hospitals or visiting one of the Gaborone city clinics. Neither cases nor controls had a history of a previous episode of active TB before enrollment. Although study enrollment commenced before these criteria had been published, we retrospectively applied the consensus TB diagnostic definitions as described by Graham and others⁹ to classify enrolled TB cases. The diagnostic level for each TB case was at least at the level of “probable” for cases. This included cases diagnosed by chest X-ray and symptoms as well as those diagnosed by microbiological confirmation.¹⁰ Controls had to meet criteria for “not TB,” lacking clinical, radiographic, and laboratory evidence of TB. Microbiological confirmation included smear or culture of gastric aspirate or sputum induction samples for intrathoracic cases, and biopsy for extrathoracic diagnoses. The cases recruited were naive to a complete course of antituberculous therapy (ATT). This was defined as use of ATT for 21 days or less

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before the day of enrollment and drawing of the study 25(OH)D blood sample. Where ATT had been commenced before enrollment, the number of days of ATT was recorded. Vitamin D sufficiency was defined as a serum 25(OH)D of 20 ng/mL or greater. Vitamin D deficiency was defined as a serum 25(OH)D less than 20 ng/mL.

Outcomes. Information regarding demographics, HIV status, and TB status as well as treatment history were determined from medical records. 25(OH)D concentrations were obtained by a peripheral venous blood sample. The serum was separated, frozen for later testing, and then shipped to the South African Medical Research Council Developmental Pathways for Health Research Unit of the University of the Witwatersrand, South Africa. Serum samples were analyzed for 25(OH)D by chemiluminescent assay (DiaSorin Liaison, Stillwater, MN) in a laboratory, which participates in an international quality assurance program (International Vitamin D External Quality Assessment Scheme [DEQAS], London, United Kingdom). The coefficients for interassay variation for low and high controls were 8% and 6%, respectively, and for intraassay variation for low and high controls were 5% and 3%, respectively.

Analysis. The overall 25(OH)D concentrations were compared between groups using the Wilcoxon rank-sum test. The outcome was being a TB case or not (control). The exposure was explored in two ways: as a continuous variable (serum 25(OH)D) and as a dichotomous one (25(OH)D deficient or not). Statistical significance was defined as $P < 0.05$. A sample size of 80 participants was chosen with a goal of enrolling 40 cases and 40 controls. This sample size was not based on a power calculation as there were no prior studies on this topic in this or similar populations to inform such a calculation. This sample size was chosen to assess the variability of 25(OH)D in individuals with and without TB and to potentially inform the design of a larger study.

Ethics. This study was reviewed and approved by the institutional review boards of the Botswana Ministry of Health, Princess Marina Hospital, and the University of Pennsylvania. Written informed consent was obtained from a legal guardian of each subject before study enrollment.

RESULTS

A total of 39 cases and 41 controls were enrolled, and groups did not differ when comparing demographic characteristics (Table 1). The groups were not evenly matched in number as one control's TB workup was positive, changing this subject's status from control to case. Among cases with active TB, the mean age was 10.8 months, 41% were male, and six (15%) were HIV infected. Among the control group, the mean age was 8.5 months, 54% were male, and seven (18%) were HIV infected. Although a higher proportion (49% versus 27%) of TB cases as compared with controls were older than 12 months, there was no significant difference between the mean age of the two groups.

The median 25(OH)D level did not differ between cases (32.2 ng/mL) and controls (30.9 ng/mL; Table 2). A total of 15 children were vitamin D deficient with no difference in the proportion that was deficient in cases as compared with controls.

Univariate analyses of patient clinical characteristics in addition to vitamin D levels revealed that as age increased, the risk of TB increased as well ($P = 0.08$). However, of the factors explored (weight loss, HIV status, parental education, formula feeding, and sunlight exposure) only a history of any weight loss experienced by a patient was a significant predictor of TB ($P = 0.047$).

DISCUSSION

We evaluated vitamin D status in infants and toddlers with and without TB in an African setting. In this hospital and

TABLE 1
Characteristics of the study cohort ($n = 80$)

	No active TB ($N = 41$)	Active TB ($N = 39$)	Comparison
Age in months (mean, IQR)	8.5 (5, 13)	10.8 (5, 16)	$P = 0.15^*$
Age ≥ 12 months	11 (27%)	19 (49%)	$P = 0.04^\dagger$
Weight in kg (mean, IQR)	6.6 (5.2, 7.4)	7.0 (5.2, 8.5)	$P = 0.45^*$
Length in cm (mean, IQR)	65.8 (60.3, 72.3)	69.3 (61, 75)	$P = 0.35^*$
Male	22 (54%)	16 (41%)	$P = 0.26^\dagger$
HIV infected			
Yes	7 (18%)	6 (15%)	$P = 0.926^\dagger$
No	26 (65%)	25 (64%)	
Unknown	7 (18%)	6 (15%)	
Child has chronic diarrhea	8 (21%)	6 (17%)	$P = 0.71^\dagger$
Total sun protection score (high = more protection)	7.6 (4%)	8.1 (3%)	$P = 0.5^\dagger$
Child received only formula	23 (58%)	15 (41%)	$P = 0.14^\dagger$
Parental education level			$P = 0.96^\dagger$
Primary	6 (15%)	7 (18%)	
Secondary	29 (73%)	30 (77%)	
Tertiary	3 (8%)	1 (3%)	
Maternal exposure to HAART during pregnancy	19 (48%)	11 (31%)	$P = 0.16^\dagger$
Days on tuberculosis treatment (mean, median) ‡	NA	3.7, 3.0	
None	—	11 (29%)	
1–5	—	17 (45%)	
6–20	—	10 (26%)	

HAART = highly active antiretroviral therapy; HIV = human immunodeficiency virus; IQR = interquartile range; NA = not applicable; TB = tuberculosis.

*By rank-sum test.

† By χ^2 test.

‡ Data are for 38 subjects as "days on TB treatment" was not captured in one subject.

TABLE 2
25-Hydroxyvitamin D levels by study group

	No Active TB	Active TB	Comparison
	(N = 41)	(N = 39)	
Total 25(OH)D ng/mL, median (IQR)	30.9 (22.6, 41.9)	32.2 (21.5, 40)	$P = 0.84^*$
25(OH)D < 20 ng/mL	7 (17%)	8 (21%)	$P = 0.69^\dagger$
25(OH)D < 30 ng/mL	19 (46%)	17 (44%)	$P = 0.81^\dagger$

IQR = interquartile range; TB = tuberculosis.

*By rank-sum test.

†By χ^2 test.

clinic-based cohort, we found no difference in serum 25(OH)D between those with TB and those without. Similarly, although 18.8% of the overall cohort were vitamin D deficient (< 20 ng/mL), the proportion of those who were deficient did not differ between groups.

The vitamin D status of Botswana's children has not been described previously. However, a recent study conducted in Botswana evaluated maternal vitamin D status among HIV-infected, antiretroviral-naïve women, with CD4 counts ≥ 200 cells/mm³ and no acquired immunodeficiency syndrome (AIDS)-defining illnesses.¹¹ Although vitamin D insufficiency (defined as < 32 ng/mL) was common (32% of women), there was no association with morbidity, mortality, or growth delay in the study subjects' children.¹¹ By comparison, a 2011 South African study conducted among healthy 10-year olds that were part of a longitudinal Birth to Twenty cohort found a vitamin D deficiency (defined as < 20 ng/mL by this study) prevalence of 7%,¹² whereas a Tanzanian study in 6-month-old HIV-exposed uninfected infants found a prevalence of 34.6%.¹³ The proportion of 25(OH)D-deficient children in our study falls between these two studies.

There was no difference in serum 25(OH)D between children with TB and those without. This finding is consistent with recently conducted pediatric studies in Pune, India, and Westmead, Australia. In Pune, Jubulis and others explored modifiable risk factors for TB in children of age five or younger. They found a high prevalence of vitamin D deficiency (52%) among the 178 children in the study; however, there was no association between low 25(OH)D concentrations and TB infection.¹⁴ Similarly, a study conducted at a refugee clinic in Westmead, Australia, found a high incidence of vitamin D deficiency (56%) among children but without a correlation with TB infection.¹⁵ In addition, our findings are in keeping with a similar study exploring 25(OH)D concentrations in HIV-infected adults with and without TB in Botswana.¹⁶

There were several limitations to this study. First, our study population is modest and may not be adequately powered to detect small differences between groups. However, a comparison of the median 25(OH)D between groups shows that the values are very similar suggesting that no difference would have been found even with a larger sample size. Moreover, given that the study was conducted in and around Gaborone, which is an urban setting, our results may not be generalizable to a national or regional level. Third, our cross-sectional design assessed 25(OH)D concentrations at one point in time, that is, at diagnosis in cases and at admission or clinic visit in controls. Thus, we were not able to assess whether cases had a low 25(OH)D in the weeks or months before they were diagnosed with TB. Furthermore, diagnostic criteria for TB were applied retrospectively making it vulnerable to all limitations inherent to retrospective design.

CONCLUSIONS

We found no significant difference in vitamin D status between infants and toddlers with and without active TB. Our data suggest that low vitamin D status may not be a risk factor for TB in this small Gaborone cohort. Further studies are necessary to evaluate if there is a role for vitamin D in pediatric TB.

Received November 30, 2015. Accepted for publication February 5, 2016.

Published online March 14, 2016.

Acknowledgments: We thank colleagues in the Princess Marina Hospital Paediatric Department and to M. A. Poopedi of the Laboratory of the SA Medical Research Council Developmental Pathways for Health Research Unit of the University of the Witwatersrand, South Africa.

Financial support: This work was supported by funding from the Stokes Research Institute at the Children's Hospital of Philadelphia (to Andrew P. Steenhoff) and the Doris Duke Charitable Foundation to the University of Pennsylvania School of Medicine to fund medical student clinical research fellows (Jonathan Ludmir, Abiona Redwood). This study was made possible through core services and support from the Penn Center for AIDS Research, an National Institutes of Health-funded program (P30 AI 045008).

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REFERENCES

1. World Health Organization, 2013. *Global Tuberculosis Report 2013*. Geneva, Switzerland: World Health Organization.
2. Marais BJ, Gie RP, Schaaf HS, Hesselink AC, Obihara CC, Starke JJ, Enarson DA, Donald PR, Beyers N, 2004. The natural history of childhood intra-thoracic tuberculosis: a critical review of literature from the pre-chemotherapy era. *Int J Tuberc Lung Dis* 8: 392–402.
3. Nelson LJ, Wells CD, 2004. Global epidemiology of childhood tuberculosis. *Int J Tuberc Lung Dis* 8: 636–647.
4. Botswana National TB Program, 2012. *National TB Report 2012*. Available at: <http://finance-ebooks.rhcloud.com/read/bntpnational-tb-report-2012/>. Accessed December 27, 2014.
5. Coussens AK, Martineau AR, Wilkinson RJ, 2014. Anti-inflammatory and antimicrobial actions of vitamin D in combating TB/HIV. *Scientifica (Cairo)* 2014: 903680.

6. Nursyam EW, Amin Z, Rumende CM, 2006. The effect of vitamin D as supplementary treatment in patients with moderately advanced pulmonary tuberculosis lesion. *Acta Med Indones* 38: 3–5.
7. Martineau AR, Honecker FU, Wilkinson RJ, Griffiths CJ, 2007. Vitamin D in the treatment of pulmonary tuberculosis. *J Steroid Biochem Mol Biol* 103: 793–798.
8. Strachan DP, Powell KJ, Thaker A, Millard FJ, Maxwell JD, 1995. Vegetarian diet as a risk factor for tuberculosis in immigrant south London Asians. *Thorax* 50: 175–180.
9. Graham SM, Ahmed T, Amanullah F, Graham SM1, Ahmed T, Amanullah F, Browning R, Cardenas V, Casenghi M, Cuevas LE, Gale M, Gie RP, Grzemska M, Handelsman E, Hatherill M, Hesseling AC, Jean-Philippe P, Kampmann B, Kabra SK, Lienhardt C, Lighter-Fisher J, Madhi S, Makhene M, Marais BJ, McNeeley DF, Menzies H, Mitchell C, Modi S, Mofenson L, Musoke P, Nachman S, Powell C, Rigaud M, Rouzier V, Starke JR, Swaminathan S, Wingfield C, 2012. Evaluation of tuberculosis diagnostics in children: 1. Proposed clinical case definitions for classifications of intrathoracic tuberculosis disease. Consensus from an expert panel. *J Infect Dis* 205: 199–208.
10. Marais BJ, Gie RP, Hesseling AC, Schaaf HS, Lombard C, Enarson DA, Beyers N, 2006. A refined symptom-based approach to diagnose pulmonary tuberculosis in children. *Pediatrics* 118: e1350–e1359.
11. Powis K, Lockman S, Smeaton L, Hughes MD, Fawzi W, Ogwu A, Moyo S, van Widenfelt E, von Oettingen J, Makhema J, Essex M, Shapiro RL, 2014. Vitamin D insufficiency in HIV-infected pregnant women receiving antiretroviral therapy is not associated with morbidity, mortality or growth impairment in their uninfected infants in Botswana. *Pediatr Infect Dis J* 33: 1141–1147.
12. Poopedi MA, Norris SA, Pettifor JM, 2011. Factors influencing the vitamin D status of 10-year-old urban South African children. *Public Health Nutr* 14: 334–339.
13. Rwebembera A, Sudfeld CR, Manji KP, Duggan C, Aboud S, Fawzi WW, 2013. Prevalence and risk factors for vitamin D deficiency among Tanzanian HIV-exposed uninfected infants. *J Trop Pediatr* 59: 426–429.
14. Jubulis J, Kinikar A, Ithape M, Khandave M, Dixit S, Hotalkar S, Kulkarni V, Mave V, Gupte N, Kagal A, Jain S, Bharadwaj R, Gupta A, 2014. Modifiable risk factors associated with tuberculosis disease in children in Pune, India. *Int J Tuberc Lung Dis* 18: 198–204.
15. Gray K, Wood N, Gunasekera H, Sheikh M, Hazelton B, Barzi F, Isaacs D, 2012. Vitamin D and tuberculosis status in refugee children. *Pediatr Infect Dis J* 31: 521–523.
16. Steenhoff AP, Redwood A, Pettifor JM, Hove J, Bisson GP, Mosepele M, Pusoesele P, Thakur R, Kovarik C, Gross R, 2012. Vitamin D Status in HIV-infected patients with and without tuberculosis: a pilot study. *J Acquir Immune Defic Syndr* 61: e21–e23.