Vitamin D Deficiency Is Associated with Tuberculosis and Latent Tuberculosis Infection in Immigrants from Sub-Saharan Africa

Katherine B. Gibney,[1] Lachlan MacGregor,[2] Karin Leder,[1,6] Joseph Torresi,[1,3] Caroline Marshall,[1,4] Peter R. Ebeling,[3,5] and Beverley-Ann Biggs[1,3,4]

[1]Victorian Infectious Diseases Service and [2]Clinical Epidemiology and Health Service Evaluation Unit, Royal Melbourne Hospital, and [3]Department of Medicine, [4]Centre for Clinical Research Excellence in Infectious Diseases, and [5]Department of Medicine, Western Hospital, University of Melbourne, and [6]Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Victoria, Australia

Among African immigrants in Melbourne, Victoria, Australia, we demonstrated lower geometric mean vitamin D levels in immigrants with latent tuberculosis infection than in those with no *Mycobacterium tuberculosis* infection ($P = .007$); such levels were also lower in immigrants with tuberculosis or past tuberculosis than in those with latent tuberculosis infection ($P < .001$). Higher vitamin D levels were associated with lower probability of any *M. tuberculosis* infection ($P < .001$) and lower probability of tuberculosis or past tuberculosis (compared with latent tuberculosis infection; $P = .001$).

High rates of *Mycobacterium tuberculosis* (MTB) infection and vitamin D deficiency have been reported in African immigrants in Australia [1–3], the United States [4, 5], and Europe [6]. Vitamin D is known to have an important role in macrophage activation and the subsequent restriction of MTB growth [7], and it has been implicated as a risk factor for tuberculosis (TB) [8]. An association between 25(OH) vitamin D (25[OH]D) levels and TB has been described in several case-control studies [9–15]. An association between 25(OH)D levels and latent tuberculosis infection (LTBI) has not been well described.

The Royal Melbourne Hospital (Melbourne, Victoria, Australia) is a major tertiary referral center for refugee health and for patients with MTB infection. In this study, we examined the association between 25(OH)D levels and MTB infection status in adult immigrants from sub-Saharan Africa who attended the outpatient infectious diseases and refugee health clinics at the hospital.

Methods. A retrospective audit of patients attending the Victorian Infectious Diseases Service outpatient clinics at the Royal Melbourne Hospital from 1 January 2003 through 30 June 2006 was performed. All patients born in a sub-Saharan African country were included, except for those identified as white or Asian. Information was extracted from the patients' medical records and from hospital pathology and radiology records.

Investigation for exposure to MTB included Mantoux and QuantiFERON-TB Gold (QFT-G; Cellestis) testing [16]. The decision regarding which tests to perform was made by the referring doctor and depended on access to rebatable QFT-G tests. Mantoux tests were performed by specifically trained nurses using 0.1 mL (10 U) of PPD (CSL); the results were interpreted by the nurses as well. QFT-G tests were performed by personnel at accredited pathology laboratories, according to the manufacturer's instructions.

TB status was classified as follows: TB was defined as microbiological or histological evidence of MTB infection and/or receipt of treatment for TB during the study period, past TB was defined as documentation of having completed treatment for TB, LTBI was defined as a positive Mantoux test result (diameter, $\geqslant$10 mm) and/or a positive QFT-G test result and no evidence of TB or history of TB, any MTB infection was defined as evidence of MTB infection (i.e., past TB, TB, or LTBI), and no MTB infection was defined as a negative Mantoux and/or QFT-G test result and neither current TB nor past TB. Patients in the TB and past TB groups were combined (TB/past TB) for the analysis, because we were interested in those whose infection had progressed to TB.

Statistical analysis. Patients who had both MTB infection status and 25(OH)D levels documented were included in the analysis of associations between 25(OH)D level and MTB infection. Because the distribution of 25(OH)D levels was right-skewed, the data were log transformed for subsequent analysis. We compared geometric mean 25(OH)D levels in each of the 3 groups (no MTB infection, LTBI, and TB/past TB) using 2-sample Student's *t* tests. We then created 2 multivariable binary logistic regression models with the following outcome variables: (1) any MTB infection versus no MTB infection and (2) TB/past TB versus LTBI. These models incorporated age, sex, du-

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Reprints or correspondence: Dr. Katherine Gibney, Royal Darwin Hospital, PO Box 41326, Casuarina, Northern Territory, Australia, 0810 (katherinegibney@hotmail.com).

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ration of residence in Australia >1 year, and country of birth (East African countries vs. other African countries) a priori as covariates. Finally, we used 2 separate univariate binary logistic models to derive fitted values to graphically represent the relationship between 25(OH)D levels and probability of (1) any MTB infection and (2) TB/past TB (compared with LTBI). Statistical analysis was performed using Stata, version 9.0 (Stata).

Ethical considerations. To maintain confidentiality, patient names were not recorded. This work was performed as part of a clinic audit and, therefore, did not require approval from the hospital human research ethics committee.

Results. A total of 375 patients born in sub-Saharan Africa attended the Royal Melbourne Hospital infectious diseases outpatient clinics from 1 January 2003 through 30 June 2006. One-half of the patients were female (183 [49%] of 375 patients), and one-half were born in East Africa (196 [52%] of 375 patients). The median age was 32.3 years (range, 16.2–76.5 years), and the median duration of residence in Australia was 1.2 years (range, 6 days to 19.6 years). Overall, 276 (49%) of 375 patients had their MTB infection status documented, and 155 (41%) of 375 had both their MTB infection status and 25(OH)D level documented. Over one-half of the patients tested (152 [55%] of 276) had LTBI, and 73 (26%) of 276 patients had TB/past TB.

Moderate to severe 25(OH)D deficiency (25[OH]D level, ≤25 nmol/L) was reported in 31 (78%) of 40 patients with TB/past TB, 27 (33%) of 81 with LTBI, and 2 (6%) of 34 with no MTB infection. There was a difference in the geometric mean 25(OH)D levels when patients with LTBI were compared with patients with no MTB infection (37.3 vs. 54.6 nmol/L; $P = .007$), patients with TB/past TB were compared with patients with LTBI (16.1 vs. 37.3 nmol/L; $P < .001$), and patients with TB/past TB were compared with patients with no MTB infection (16.1 vs. 54.6 nmol/L; $P < .001$). There was no evidence of a difference in the geometric mean 25(OH)D levels between those with TB and those with past TB (17.1 vs. 13.3 nmol/L; $P = .275$).

Low vitamin D levels were predictive of any MTB infection and TB/past TB (compared with LTBI), and this relationship was seen across the range of 25(OH)D levels (figure 1). In the multivariate models (table 1), higher 25(OH)D levels were associated with lower probability of MTB infection (OR, 0.31 per doubling of 25[OH]D levels; 95% CI, 0.18–0.53; $P < .001$) and lower risk of TB/past TB, compared with LTBI (OR, 0.41 per doubling of 25[OH]D levels; 95% CI, 0.19–0.91; $P = .001$).

Fifty patients had results of both Mantoux and QFT-G tests documented. These results were discordant for 11 (22%) of 50 patients, with 9 patients having positive Mantoux test results and negative QFT-G test results and 2 patients having negative Mantoux test results and positive QFT-G test results. When these 11 patients were excluded from the analysis, the geometric mean 25(OH)D level in the LTBI group was 37.2 nmol/L, which differed from the geometric mean 25(OH)D levels in patients with no MTB infection ($P = .007$) and in those with TB/past TB ($P < .001$). When the LTBI group was restricted to those with a positive QFT-G test result (excluding those with only Mantoux test results), the geometric mean 25(OH)D level in the LTBI group was again 37.2 nmol/L, which differed from the geometric mean 25(OH)D levels in those with no MTB infection ($P = .014$) and in those with TB/past TB ($P < .001$). Vitamin D supplementation was documented in 93 (41%) of 225 patients with MTB infection and in 11 (22%) of 51 patients with no MTB infection.

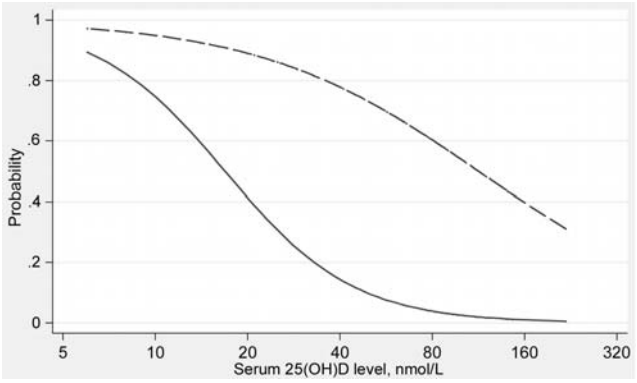

Figure 1. Logistic regression with fitted values showing probability of any *Mycobacterium tuberculosis* (MTB) infection and tuberculosis (TB) or past TB versus latent TB infection (LTBI) according to serum 25(OH) vitamin D (25[OH]D) levels. Curves represent fitted values derived from 2 separate univariate binary logistic regression models, in which the outcome was either (1) any MTB infection (TB, past TB, or LTBI) versus no MTB infection *(dashed line)* or (2) TB/past TB versus LTBI *(continuous line).* Thus, the probability shown on the Y-axis represents the expected probability or proportion of patients with the outcome as defined for any given level of serum 25(OH)D.

Discussion. Our study shows a strong association between vitamin D deficiency and MTB infection in African immigrants attending the infectious diseases clinics of a Melbourne tertiary care hospital. To our knowledge, this is the first study to clearly demonstrate an association between vitamin D deficiency and LTBI.

Our finding of moderate to severe 25(OH)D deficiency in 78% of patients with TB/past TB is similar to that reported for African patients receiving treatment for TB in London, United Kingdom [14, 17]. Reduced 25(OH)D levels have been reported in patients with TB, compared with healthy control subjects, in 7 case-control studies [9–15], although the difference achieved a P value of <.05 in only 5 of these studies [10, 11, 13–15]. None of these studies reported excluding patients with LTBI from the control group, and in 2 studies, the control group consisted entirely of patients with LTBI [14, 15]. Our finding of a relationship between low 25(OH)D levels and the

Table 1. Risk factors for *Mycobacterium tuberculosis* (MTB) infection.

	Any MTB infection versus no MTB infection				TB/past TB versus LTBI			
	Univariate analysis		Multivariate analysis[a]		Univariate analysis		Multivariate analysis[a]	
Variable	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Serum 25(OH) vitamin D level, nmol/L[b]	0.43 (0.29–0.65)	<.001	0.31 (0.18–0.53)	<.001	0.24 (0.13–0.43)	<.001	0.41 (0.19–0.91)	<.001
Female sex	1.19 (0.62–2.29)	.642	1.10 (0.46–2.62)	.832	1.18 (0.68–2.08)	.555	1.09 (0.30–4.00)	.891
Age, years	1.01 (0.98–1.04)	.535	1.00 (0.96–1.04)	.993	1.03 (1.01–1.06)	.018	1.08 (1.01–1.16)	.020
Residence in Australia >1 year	2.01 (1.02–4.00)	.030	1.64 (0.59–4.61)	.346	4.39 (2.21–8.97)	<.001	2.58 (0.69–9.59)	.157
East African country of birth[c]	0.95 (0.50–1.83)	1.000	0.18 (0.06–0.54)	.002	21.73 (9.26–50.96)	<.001	28.31 (6.37–125.86)	<.001

NOTE. LTBI, latent tuberculosis infection; TB, tuberculosis. Any MTB infection includes TB, past TB, and LTBI.

[a] Multivariate analysis includes all variables listed in this table.

[b] OR per doubling of serum 25(OH) vitamin D levels.

[c] Somalia, Ethiopia, Eritrea, Kenya, Uganda, Burundi, Malawi, Mozambique, Djibouti, and Tanzania.

risk of TB/past TB, compared with LTBI, raises the possibility that 25(OH)D deficiency increases the risk of progression from LTBI to TB, although our study does not show causality.

The clinical association between probability of any infection due to MTB and 25(OH)D deficiency has not been well documented previously, although in the control group in the study by Grange et al. [12] in Indonesia, those who had a lower 25(OH)D level (<81 nmol/L) were more likely to have a positive tuberculin skin test result than were those who had a 25(OH)D level ⩾81 nmol/L (86% vs. 40%; $P < .02$). Our findings raise the possibility that adequate 25(OH)D levels may protect against primary infection due to MTB, and this is supported by the work of Martineau et al. [18], in which a single dose of vitamin D improved immunity to mycobacteria in vitro in contacts of patients with TB.

The strengths of the study were the large number of patients included and the careful documentation of all forms of MTB infection. This allowed a more detailed analysis of the role of vitamin D deficiency in LTBI than has occurred in previous studies. The main limitation was the retrospective clinical audit design, which meant that not all patients had all variables of interest documented, including serum 25(OH)D level and MTB infection status. This may have affected the observed associations and would be overcome in a prospective study. A potential confounding factor is the effect of bacille Calmette-Guérin vaccination status (which was not recorded) on the results of Mantoux test results; however, the difference in geometric mean 25(OH)D levels remained statistically significant between the groups when QFT-G test results alone were used to determine LTBI status. Another limitation is that some patients may have been receiving vitamin D supplements at the time of having their serum 25(OH)D level measured. This may affect the associations shown, although overall vitamin D supplementation was more common in those with MTB infection.

There is increasing epidemiological and molecular evidence that vitamin D and MTB infection are intricately linked. Our study contributes to this evidence by documenting the inverse relationship between serum 25(OH)D levels and both the likelihood of having any MTB infection and the likelihood of having TB/past TB rather than LTBI. Now, the challenge is to perform appropriate studies to elucidate the role of vitamin D supplementation in (1) preventing MTB infection, (2) preventing progression from LTBI to TB, and (3) treating TB.

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Potential conflicts of interest. All authors: no conflicts.

References

1. Martin JA, Mak DB. Changing faces: a review of infectious disease screening of refugees by the Migrant Health Unit, Western Australia in 2003 and 2004. Med J Aust **2006**; 185:607–10.
2. Skull S, Ngeow J, Biggs B, Street A, Ebeling P. Vitamin D deficiency is common and unrecognized among recently arrived adult immigrants from The Horn of Africa. Intern Med J **2003**; 33:47–51.
3. Tiong AC, Patel MS, Gardiner J, et al. Health issues in newly arrived African refugees attending general practice clinics in Melbourne. Med J Aust **2006**; 185:602–6.
4. Lifson A, Thai D, O'Fallon A, Mills W, Hang K. Prevalence of tuberculosis, hepatitis B virus and intestinal parasitic infections among refugees to Minnesota. Public Health Rep **2002**; 117:69–77.
5. Centers for Disease Control and Prevention. Increase in African immigrants and refugees with tuberculosis—Seattle-King County, Washington, 1998–2001. MMWR Morb Mortal Wkly Rep **2002**; 51:882–3.
6. Lopez-Velex R, Huerga H, Turrientes M. Infectious diseases in immigrants from the perspective of a tropical medicine referral unit. Am J Trop Med Hyg **2003**; 69:115–21.
7. Chandra G, Selvaraj P, Jawahar M, Banurekha V, Narayanan P. Effect of vitamin D3 on phagocytic potential of macrophages with live *Mycobacterium tuberculosis* and lymphoproliferative response in pulmonary tuberculosis. J Clin Immunol **2004**; 24:249–57.
8. Chan T. Vitamin D deficiency and susceptibility to tuberculosis. Calcif Tissue Int **2000**; 66:476–8.
9. Chan T, Poon P, Pang J, et al. A study of the calcium and vitamin D metabolism in Chinese patients with pulmonary tuberculosis. J Trop Med Hyg **1994**; 97:26–30.
10. Davis P. A possible link between vitamin D deficiency and impaired host defence to *Mycobacterium tuberculosis*. Tubercle **1985**; 66:301–6.
11. Davies PDO, Church HA, Bovornkitti S, Charumilind A, Byrachandra

S. Altered vitamin D homeostasis in tuberculosis. Intern Med (Thailand) **1988**; 4:45–7.
12. Grange J, Davies P, Brown R, Woodhead J, Kardjito T. A study of vitamin D levels in Indonesian patients with untreated pulmonary tuberculosis. Tubercle **1985**; 66:187–91.
13. Sasidharan P, Rajeev E, Vijayakumari V. Tuberculosis and vitamin D deficiency. J Assoc Phycians India **2002**; 50:554–8.
14. Sita-Lumsden A, Lapthorn G, Swaminathan R, Milburn H. Reactivation of tuberculosis and vitamin D deficiency: the contribution of diet and exposure to sunlight. Thorax **2007**; 62:1003–7.
15. Wilkinson R, Llewelyn M, Toossi Z, et al. Influence of vitamin D deficiency and vitamin D receptor polymorphisms on tuberculosis among Gajarati Asians in west London: a case-control study. Lancet **2000**; 355:618–21.
16. Mazurek GH, Jereb J, Lobue P, Iademarco MF, Metchock B, Vernon A. Guidelines for using the QuantiFERON-TB Gold test for detecting *Mycobacterium tuberculosis* infection, United States. MMWR Recomm Rep **2005**; 54:49–55.
17. Ustianowski A, Shaffer R, Collin S, Wilkinson R, Davidson R. Prevalence and associations of vitamin D deficiency in foreign-born persons with tuberculosis in London. J Infect **2005**; 50:432–7.
18. Martineau A, Wilkinson R, Wilkinson K, et al. A single dose of vitamin D enhances immunity to mycobacteria. Am J Respir Crit Care Med **2007**; 176:208–13.