Tuberculosis 94 (2014) 51–54

Contents lists available at ScienceDirect

Tuberculosis

journal homepage: http://intl.elsevierhealth.com/journals/tube

MECHANISMS OF PATHOGENESIS

25-Hydroxyvitamin D levels after recovery from tuberculosis: Insights into pathogenesis

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ARTICLE INFO

Article history:
Received 10 July 2013
Received in revised form
22 October 2013
Accepted 25 October 2013

Keywords:
25-Hydroxyvitamin D
Vitamin D
Tuberculosis
Mycobacterium tuberculosis

SUMMARY

Objective: 25-Hydroxyvitamin D [25(OH)D] levels after recovery from tuberculosis (TB) may reflect pre-morbid levels and therefore provide insight into pathogenesis. We assessed 25(OH)D levels after recovery from TB disease, and compared to levels in persons without TB disease.
Methods: Case-control study. Cases were persons who had recovered from culture-confirmed *Mycobacterium tuberculosis* disease. Controls were persons without TB disease. Total 25(OH)D was measured from stored plasma specimens using liquid chromatography-mass spectrometry.
Results: 29 persons with prior TB disease and 36 controls were included. Median 25(OH)D levels were 24.7 ng/mL (IQR, 18.3–34.1) in prior TB disease, and 33.6 ng/mL (IQR, 26.2–42.4) in controls (Mann–Whitney; $P = 0.01$). Multivariable linear regression analysis showed that black race (adjusted mean difference [β] = −8.3 ng/mL; 95% CI −14.5, −2.2; $P < 0.01$), enrollment in winter ($\beta = -10.4$ ng/mL; 95% CI −17.0, −3.8; $P < 0.01$) and prior TB disease ($\beta = -5.8$ ng/mL; 95% CI −11.4, −0.3; $P = 0.05$) were associated with lower 25(OH)D levels.
Conclusions: Persons who had recovered from TB disease had lower 25(OH)D levels compared to controls without TB disease, after adjusting for important confounders. Larger, longitudinal studies are needed to further characterize the possible role of low 25(OH)D in the pathogenesis of TB disease and TB recurrence after recovery.

1. Introduction

Previous clinical, epidemiologic and in vitro data have suggested that low levels of vitamin D may predispose to tuberculosis (TB) [1,2]. Clinical observational studies have found low circulating 25-hydroxyvitamin D [25(OH)D] levels at the time of TB diagnosis and during treatment [3–7]. Epidemiologic studies have shown a higher incidence of TB disease in populations with diminished 25(OH)D levels such as persons of black race, and a peak of TB notifications in spring and early summer attributed to low 25(OH)D levels during winter [2,8,9]. In vitro, the biologically active metabolite of vitamin D, 1,25-dihydroxyvitamin D, has been shown to modulate cytokine responses to *Mycobacterium tuberculosis* antigens and to enhance the mycobactericidal activity of macrophages infected with *M. tuberculosis* [10–12].

Given that 25(OH)D levels could be affected by active TB disease itself or ongoing anti-TB treatment [2], we assessed 25(OH)D levels after recovery from TB disease as a possible marker of pre-morbid 25(OH)D levels, and compared them to levels in controls without TB disease.

2. Patients and methods

The population for this project was drawn from a case-control study conducted in Tennessee between 2008 and 2009 which recruited persons with prior extrapulmonary tuberculosis (EPTB), prior pulmonary tuberculosis (PTB), latent tuberculosis infection (LTBI), and uninfected contacts [13]. Inclusion criteria for the study

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http://dx.doi.org/10.1016/j.tube.2013.10.009

were as follows: ≥18 years at the time of TB diagnosis or enrollment; HIV seronegative; for EPTB and PTB, culture-confirmed *M. tuberculosis* disease and either completion or near completion (within one month) of anti-TB therapy. EPTB was defined as *M. tuberculosis* disease of any site other than the pulmonary parenchyma. Persons with both pulmonary and extrapulmonary involvement were classified as EPTB. PTB was defined as pulmonary disease with no extrapulmonary involvement. LTBI was defined as having a tuberculin skin test (TST) induration of ≥10 mm. Persons with LTBI could have received treatment, or not for LTBI. Uninfected contacts had a negative TST and had been exposed to culture-positive PTB patients. Exclusion criteria were as follows: serum creatinine >2 mg/dl; use of corticosteroids or other immunosuppressants at the time of diagnosis or enrollment; malignancy; diabetes mellitus; and pleural TB.

For our analysis, subjects were classified into two main study groups: (1) prior TB disease which included all persons with prior EPTB or PTB, and (2) non-TB disease which included all persons with LTBI and uninfected contacts, who served as controls. Persons who were still receiving anti-TB therapy at time of enrollment were excluded from our analysis because of the potential effect of anti-TB therapy on 25(OH)D levels [14].

For each person, 250 μL of stored plasma obtained from blood that had been drawn at the time of study enrollment was sent frozen in a microcentrifuge tube to Heartland Assays, Inc. (Ames, Iowa) for 25(OH)D analysis. Measurement of total 25(OH)D [25(OH)D_2 and 25(OH)D_3] was conducted using liquid chromatography-mass spectrometry. An amendment to the study protocol was approved by the Vanderbilt University Institutional Review Board to utilize the stored plasma specimens for this project.

Study data were collected and managed using a secure electronic data capture tool (REDCap) [15]. Data were analyzed using Stata software (version 12.0; StataCorp, Texas). 25(OH)D levels were compared between study groups by using the Mann–Whitney test. Multivariable linear regression was used to estimate the association between 25(OH)D levels and TB disease after adjusting for potential confounding factors. A secondary multivariable linear regression model log-transformed 25(OH)D levels, yielding similar results (data not shown). Because of the limited sample size, for multivariable analyses, only factors known to be potential predictors of the outcome that had a $P \leq 0.2$ in the univariable analyses were included in multivariable regression models. Significance was established with a $P \leq 0.05$.

3. Results

Twenty-nine persons with prior TB disease and 36 controls without TB disease were included in this analysis. The demographic and clinical characteristics of the study groups are shown in Table 1. TB disease was associated with lower 25(OH)D levels compared to controls without TB disease [median 25(OH)D, 24.7 ng/mL vs. 33.6 ng/mL; Mann–Whitney test, $P = 0.01$] (Figure 1). Other factors associated with significantly lower 25(OH)D included black race [median 25(OH)D, 16.8 ng/mL vs. 33.4 ng/mL; $P < 0.01$] and enrollment in winter [median 25(OH)D, 18.7 vs. 33.4; $P < 0.01$] (Table 2).

In multivariable linear regression modeling, black race (adjusted mean difference [β] = −8.3 ng/mL; 95% CI −14.5, −2.2; $P < 0.01$), enrollment in winter ($\beta = -10.4$ ng/mL; 95% CI −17.0, −3.8; $P < 0.01$) and prior TB disease ($\beta = -5.8$ ng/mL; 95% CI −11.4, −0.3; $P = 0.05$) were independently associated with lower 25(OH)D levels (Table 3).

The median number of months after completion of therapy in the TB disease group was 7 (IQR, 1–21). There was no association in the univariable or multivariable analyses between 25(OH)D levels and the number of months from completion of anti-TB therapy to when 25(OH)D levels were drawn (Pearson's $r = 0.26$; $P = 0.17$; and $\beta = 0.17$, −0.6 to 0.4; $P = 0.15$; respectively).

To assess if 25(OH)D levels differed by site of TB disease among cases, we compared 25(OH)D levels between patients with prior PTB ($n = 18$) and patients with prior EPTB ($n = 11$) and found no significant difference [median 25(OH)D, 26.1 ng/mL (IQR, 19.2–32.1) in PTB and 22.1 ng/mL (IQR, 15.6–40.6) in EPTB; $P = 0.81$]. We also compared 25(OH)D levels between persons with LTBI ($n = 16$) and uninfected contacts ($n = 20$) in the control group and found no significant difference [median 25(OH)D, 36.1 ng/mL (IQR, 29.5–43.9) in LTBI, 32.5 ng/mL (IQR, 23.1–40.5) in uninfected contacts; $P = 0.31$].

4. Discussion

We found lower 25(OH)D levels among persons who recovered from TB disease compared to controls without TB disease, after adjusting for important confounding factors. To our knowledge, this is the first report of 25(OH)D levels in persons who have recovered from TB disease.

Prior cross-sectional studies have reported low levels of 25(OH)D at the time of TB diagnosis and during treatment [2]. 25(OH)D levels after recovery from TB disease may reflect pre-morbid levels. If true, it would suggest that low 25(OH)D is a risk factor for TB rather than a consequence of the disease process or anti-TB treatment. Furthermore, low levels of 25(OH)D after recovery from TB disease may be a predisposing factor for developing recurrent TB, as 25(OH)D insufficiency affects cellular immune responses and therefore may facilitate relapse or reinfection [16,17]. If 25(OH)D levels remain low, they may contribute to the higher risk of TB disease seen among persons who have recovered from prior TB compared to the general population, even among those who completed fully active anti-TB regimens [18]. We were unable to

Table 1
Clinical and demographic characteristics of study groups.

Characteristics*	Prior TB disease ($n = 29$)	No TB disease ($n = 36$)	P†
Age in years§	48 (35–59)	44 (37–54)	0.58
Male sex	16 (55)	13 (36)	0.12
Black race	11 (38)	7 (19)	0.10
Hispanic	4 (14)	1 (3)	0.10
Non US-born	13 (45)	2 (6)	<0.01
Enrollment in winter	8 (28)	6 (17)	0.29
25(OH)D ng/mL§	24.7 (18.3–34.1)	33.6 (26.2–42.4)	0.01

* Data are presented as numbers (%) of individuals except indicated.
† Obtained by using Mann–Whitney or Pearson's chi-square test.
§ Data are presented as median numbers (interquartile range).

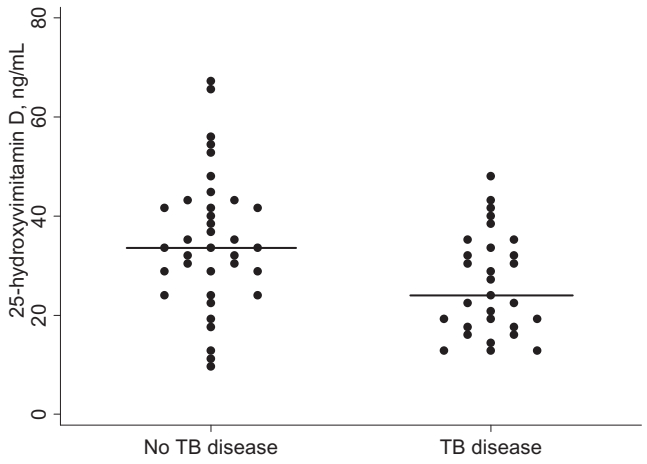

Figure 1. Median levels of total 25-hydroxyvitamin D [25(OH)D] by study group. Each dot represents the level of 25(OH)D for an individual patient. Bars represent medians.