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Vitamin D Status in HIV-Infected Patients with and without Tuberculosis: A Pilot Study

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INTRODUCTION

Tuberculosis (TB) is a leading cause of death in human immunodeficiency virus (HIV)-infected individuals. Efforts to reduce the burden of TB include isoniazid prophylactic therapy (IPT) for latent TB infection (LTBI).[1] However, IPT confers a risk of hepatotoxicity and requires at least six months of therapy.[2] Therefore, additional strategies to reduce the burden of active TB are needed.

Vitamin D supplementation may decrease the progression of LTBI to active TB. Primarily, in vitro studies demonstrate that 1,25-dihydroxyvitamin D (1,25$[OH]_2$D) enhances macrophage function, thereby augmenting immunologic control of mycobacteria.[3] The benefit of vitamin D supplementation is suggested by the observation of increased rates of clearance of TB from sputum in HIV-uninfected individuals.[4]

Vitamin D, however, may have detrimental effects in HIV-infected patients. In vitro, vitamin D depresses cell mediated immune function, [5] which could hasten progression of LTBI to active disease. This may limit vitamin D supplementation as an adjunct for TB control. Given vitamin D's conflicting mechanisms of action on the immune system, we conducted a study in Botswana, an area of high TB and HIV prevalence,[6] to determine if there was evidence for differences in 25-hydroxyvitamin D (25-OHD) levels in HIV-infected individuals with and without active TB. We hypothesized that 25-OHD levels would be significantly lower in HIV-infected individuals with active TB as compared to those without active TB.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
Andrew Steenhoff, Abiona Redwood, John Pettifor and Robert Gross were involved in the study conception, design, implementation, data acquisition and analysis, drafting and final approval of version to be published. Gregory Bisson and Carrie Kovarik were involved in aiding the study design, article revision and final approval of version to be published. Phillip Pusoesele, Rameshwari Thakur, Mosepele Mosepele and Joseph Hove were involved in data acquisition, article revision and final approval of the version to be published.

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STUDY POPULATION AND METHODS

Study Population

Between December 2008 and January 2010, we conducted a case-control pilot study in Gaborone, Botswana with HIV-infected Batswana subjects recruited from HIV and TB clinics. Participants were at least 21 years old with Bacillus Calmette-Guerin (BCG) vaccination (documented by medical record or scar) and HIV-infection (diagnosed by ELISA).

Cases

Cases of TB had either active pulmonary or extrapulmonary disease defined as having at least one positive sputum smear for acid fast bacilli (AFB), positive culture, or a biopsy consistent with *Mycobacterium tuberculosis* as documented in the medical records. Cases were also anti-retroviral therapy (ARV) naïve, and had been receiving anti-tuberculosis therapy (ATT) for less than 21 consecutive days prior to enrollment, a time period chosen to limit the effect of TB treatment on 25-OHD levels.[7] ATT consisted of rifampicin, isoniazid, pyrazinamide and ethambutol.

Controls

Controls were ARV naive without active TB and were assumed to be latently infected. Controls were matched to cases by sex and CD4 counts in a 1:1 ratio to achieve the smallest difference possible in CD4 count between cases and controls, given the strong relationship between CD4 count and active TB in HIV infected individuals.[8]

Data collection

This included CD4 count and treatment history abstracted from clinical records. Serum 25-OHD was measured in two separate batches using the Diasorin Liaison assay at the Medical Research Council Mineral Metabolism Research Unit of the University of the Witwatersrand, South Africa, a member of the International Vitamin D External Quality Assessment Program (http://wwww.deqas.org/).

Analysis

Comparison of the 25-OHD levels was performed by calculating the difference in 25-OHD levels for each case-control pair, then the median difference and interquartile range (IQR) among case-control pairs was determined. The overall median (and IQR) 25-OHD level for subjects with and without active TB was also calculated.

Ethics

The study was approved by the Ethics Boards of the Botswana Ministry of Health, Princess Marina Hospital and the University of Pennsylvania.

RESULTS

A total of 43 HIV infected patients were recruited, and the 25-OHD levels of 38 patients were compared. Nineteen patients with active TB (cases) were matched by CD4 count to 19 patients without TB (controls). A total of five patients were not included in the analysis: two withdrew from the study, CD4 information could not be located on one, and two patients' data were excluded because the matched control had a CD4 count that was too high (174 cells/mm^3) compared to the potential case (10 cells/mm^3). The table displays the characteristics of study subjects and shows the 25-OHD levels and CD4 counts and their

respective differences. Of the patients enrolled, 78% (15/19) of both cases and controls were vitamin D deficient (<50nmol/L).

DISCUSSION

The absolute difference in 25-OHD levels of 1.3 nmol/L between HIV patients with and without TB was not consistent with previous studies of HIV-uninfected individuals. These studies had found significantly lower 25-OHD levels in those with active TB versus those without. For example, 375 HIV-uninfected sub-Saharan African immigrants to Australia with TB had a median 25-OHD level of 21.2 nmol/L lower than those without TB.[9] A Tanzanian study with 655 subjects, found that 25-OHD levels were 7.4 nmol/L lower in patients with sputum culture positive pulmonary TB than in those who were sputum culture negative.[10]

The absence of such differences in our study may be the lack of a vitamin D immune-enhancing effect in immunocompromised individuals. Conversely, the immunosuppressive effect of vitamin D on cell-mediated immunity may dampen the enhancing effect on innate immunity in the setting of pre-existing T cell suppression, as is the case with HIV.

Interestingly, there was a global vitamin D deficiency in both cases and controls and perhaps the controls had not yet developed TB because of other differences from the cases. However, current definitions of vitamin D deficiency are based on bone health, not immunological health. Future steps should include ascertaining 25-OHD levels that enhance TB immunity in HIV infected patients and then evaluating how vitamin D supplementation may optimize immunity against TB.

This study had several limitations. First, the sample size was small and thus the power to detect small differences was likely low. Yet the very small observed differences suggest that a larger study may not have detected either clinically or statistically significant differences either. Second, 25-OHD levels in cases may have been affected by ATT since rifampicin and isoniazid induce hepatic enzymes to metabolize 25-hydroxyvitamin D, although we mitigated this to some extent by only including individuals with <3 weeks of therapy.[7] Third, sun exposure, diet and nutritional status were not assessed.

CONCLUSIONS

The insignificant absolute difference in 25-OHD levels between HIV-infected patients with and without TB was not consistent with similar previous studies of HIV-uninfected individuals. This suggests that hypovitaminosis D is not a major risk factor for TB in HIV infected as compared to HIV-uninfected individuals. Further studies are needed to explore the role of vitamin D as a factor in the development of TB in HIV- infected individuals.

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List of abbreviations

25-OHD 25-hydroxyvitamin D

1	25[OH]$_2$D, 1,25-dihydroxyvitamin D
AFB	Acid fast bacilli
ARV	Antiretroviral therapy
ATT	Anti-tuberculosis therapy
BCG	Bacillus Calmette-Guerin
ELISA	Enzyme linked immunosorbent assay
HIV	Human immunodeficiency virus
IPT	Isoniazid prophylactic therapy
IQR	Interquartile range
LTBI	Latent tuberculosis infection
TB	Tuberculosis

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Table 1

Characteristics, vitamin D levels and CD4 counts of subjects.

	Active TB* (n=19)	No Active TB† (n=19)	Difference between Cases & Controls
Site of care:			
Princess Marina Hospital	1 (5%)	11 (58%)	
Bontleng Clinic	5 (26%)	8 (42%)	
Old Naledi Clinic	13 (68%)	0	
Age‡ (years, IQR)	36 (32, 37)	32 (31, 38)	
Male	7 (37%)	7 (37%)	
Female	12 (63%)	12 (63%)	
25-OHD level			
Median (IQR) nmol/L	35.1 (27.7, 42.8)	38.3 (32.9, 46.6)	
Median difference among case- control pairs (IQR) nmol/L‡	-	-	1.3 (−9.2, 16)
CD4 count			
Median (IQR) nmol/L	169 (87, 294)	176 (77, 236)	
Median difference among case control pairs (IQR) nmol/L^{a}	-	-	3 (−78, 11)

*Subjects with active tuberculosis.

†Subjects without active tuberculosis.

‡Median age

^{a}Median of the differences in vitamin D levels/ CD4 count among case-control pairs, as matched by CD4 count. Cases had active TB and controls did not have active TB.

TB = tuberculosis; IQR = interquartile range