

Vitamin D In Gambian Children With Discordant Tuberculosis Infection Status Despite Matched TB Exposure: A Case Control Study

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Short Report

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Abstract

Using a matched case-control design we measured vitamin D levels in pairs of Gambian children with discordant infection status despite the same sleeping proximity to the same adult TB index case. Vitamin D levels were 2.05ng/mL higher in 24 highly TB-exposed uninfected children compared with 24 matched highly TB-Exposed infected children (p=0.08). The findings warrant further investigation in larger studies to understand the implications and significance. Conclusion: Vitamin D levels were higher in TB-uninfected children compared with TB-infected despite equal high exposure to a TB case.

Introduction

Vitamin D deficiency has been associated with increased risk of tuberculosis (TB) disease ¹, and infection ². Host vitamin D Receptor (VDR) genetic variants are also associated with risk of TB disease ³. Although vitamin D supplementation has recently been shown not to prevent latent TB infection in Mongolian children ⁴, vitamin D levels are influenced by genetics, cultural factors, diet and latitude, making it unclear how these findings may relate to tropical African settings ⁵. Vitamin D is an immunomodulator with IFN- γ -dependent and IFN- γ and TNF- α -independent host mechanisms to control mycobacteria ⁶. Vitamin D-dependent antimicrobial peptides are hypothesised to down-regulate host pro-inflammatory responses responsible for lung pathology seen in TB disease ⁷. A negative Tuberculin Skin Test (TST) or interferon-gamma release assay (IGRA) in an individual with a high risk of *Mycobacteria tuberculosis (M.tb)* exposure is likely evidence of elimination of the infection via innate immune responses or acquired immune response without T cell priming or memory ⁸. We hypothesized that highly TB-exposed infected Gambian children would have lower levels of vitamin D compared to matched highly TB-exposed uninfected children. We therefore measured vitamin D levels in samples from pairs of children with discordant latent tuberculosis infection status despite matched exposure to the same household adult tuberculosis index case ⁹.

Materials And Methods

Participant characteristics: As previously reported ⁹, pairs of asymptomatic children (5–15 years old) with discordant TST status, despite matched exposure to the same adult index case with smear-positive pulmonary tuberculosis were recruited in The Gambia (highly TB-exposed uninfected and highly TB-exposed infected children). Highly TB-exposed uninfected children were defined as TST negative three months after enrolment, despite exposure. Samples from highly TB-exposed children at their initial clinic visit were also tested using an in-house IGRA. All highly TB-exposed infected children were HIV negative, as were the adult TB index cases. No participants developed active TB disease during 12 months of follow-up.

Sample procedures: Venous blood was collected and serum separated from lithium heparin vacutainer tubes (BD, Oxford, UK). Samples were stored at -80°C and shipped on dry ice prior to thawing for

experiments.

Vitamin D quantitation: Vitamin D (25(OH)D) in serum was measured using a commercial FDA-approved, validated enzyme immunoassay according to manufacturer instructions (Immunodiagnostic Systems, UK). Vitamin D levels in ng/mL were read from a standard curve.

Statistical analyses:

Shapiro-Wilk test was used to assess data distribution. Paired students' t test was conducted on the difference in ng/mL 25(OH)D within matched pairs. Wilcoxon's matched pairs signed rank test was used for age comparisons. Analyses were conducted using Graphpad Prism.

Ethical approval: The study was approved by The Gambia Government/MRC Joint Ethics Committee (SCC1405 and SCC1273) and the Imperial College Healthcare Tissue Bank (R13071).

Results

Of the 58 children (29 pairs) recruited to the study, five pairs did not have sufficient stored serum volume for vitamin D measurement for both children in a pair. The remaining 24 pairs (48 children) were included in analyses. Median ages did not significantly differ between infected (10.0 years) and uninfected (8.8 years; $p=0.13$). There was no significant difference in the proportion of males to females in either group (1M:1F in highly TB-exposed infected children, 1M:0.85F in matched uninfected children). Four highly TB-exposed infected children had negative or indeterminate IGRA.

Mean levels of vitamin D for 24 highly TB-exposed infected children was 18.72 ng/mL; range 10.9-28.6 ng/mL (46.8 nmol/L; range 27-71.5 nmol/L) and 24 highly TB-exposed uninfected children was 20.76ng/mL; range 12.6-29.7 ng/mL (51.9 nmol/L; range 31.5-74.3 nmol/L), difference 2.05ng/mL, 95% CI -0.288 to 4.38, $p=0.083$; Fig1A).

As was conducted previously⁹, a subgroup analysis was performed excluding the four pairs where the highly TB-exposed infected children had discordant IGRA/TST results. This subgroup analysis included 40 children (median ages 9.8 years and 9.1 years for infected and uninfected respectively; $p=0.44$). Highly TB-exposed infected children had a mean vitamin D level of 18.08 ng/mL; range 10.9-28.6 ng/mL (45.2 nmol/L; range 27.3-71.5 nmol/L), whereas the matched highly TB-exposed uninfected children had a mean level of 20.5 ng/mL; range 12.6-29.7 ng/mL (51.3 nmol/L; range 31.5- 74.3 nmol/L), difference 2.46 ng/mL, 95% CI -0.17 to 5.09, $p=0.065$, Fig 1B). All children (infected and uninfected) included in this study were above the threshold of 10 ng/mL (25 nmol/mL) for vitamin D deficiency (Fig 1).

Discussion

Understanding paediatric protective immunity to acquisition of TB infection is vital in order to guide targeting of preventative and adjunctive therapy, vaccine design and evaluation. Here, we compared

vitamin D levels in latently infected children in a TB endemic country in children with a persistently negative TST despite matched household *M.tb* exposure. We identified a trend towards lower vitamin D levels in children with TB infection than those without evidence of infection, however this did not reach significance. The trend was consistent in the subgroup analysis restricted to pairs of children where the highly TB-exposed infected children had consistent IGRA/TST results at baseline.

A 2020 household contact study in UK children found a stepwise decline in vitamin D levels from non-infected children to those with TB infection and then children with TB disease, with a significant difference between those with TB infection and TB disease ¹⁰. In a large study of 9810 Mongolian school children, Ganmaa *et al*/report an adjusted risk ratio of 1.23 [95% CI, 1.08–1.40], p=0.002 for vitamin D deficiency, defined as <10 ng/mL and TB infection as determined by the QuantiFERON-TB (QFT) Gold assay ². Despite this finding, a subsequent Phase 3 randomised controlled study in over 8,800 Mongolian children found that vitamin D oral supplementation over three years was not associated with any difference in QFT positivity, despite a mean increase of over 20ng/mL vitamin D in the supplemented group ⁴.

A meta-analysis of 3,544 participants included in prospective trials investigating vitamin D and TB risk found a median vitamin D level of 26 ng/mL (65.0 nmol/L; IQR 19.5–33.4 ng/mL) and a dose-dependent relationship between deficiency of vitamin D (<10ng/mL) and increased risk of incident TB, a finding which was significantly exacerbated by HIV ¹. The applicability of these findings to the Gambian population being studied here is not clear. While no Gambian children studied here would be classed as deficient using the 10ng/mL (25 nmol/L) threshold for vitamin D deficiency, the Mongolian Phase 3 study found 31.8% of children had vitamin D levels below 10ng/mL, and between 24% (of uninfected children) and 63% (of children with TB disease) included in the UK study were deemed vitamin D deficient using the same threshold.

While the trend between elevated vitamin D and absence of TB infection despite high TB-exposure reported here is consistent with results from existing literature ^{1,10}, a study in Gambian adults reporting higher serum vitamin D levels in adults with TB disease compared to household contacts ⁵ points towards variation in associations even within the same country.

Strengths of this study include the careful exposure-matched study design, comparing experimental data on samples from school-age children with TB infection to those who remain persistently uninfected despite defined household contact with an adult with smear-positive pulmonary tuberculosis. The original study was not powered to detect differences in vitamin D levels. Therefore, the small sample size of pairs of children from whom sufficient sample was available for this exploratory analysis is a limitation. IGRA status was available at baseline for the highly TB-exposed infected children but was not available for the highly TB-exposed uninfected children. Potentially confounding factors that may affect vitamin D levels (such as diet) are likely to be equally distributed within pairs living in the same household compound, therefore the magnitude of vitamin D differences may be small.

Our data from this largely vitamin D-sufficient group of children with household tuberculosis exposure in a tropical African climate contribute to the body of evidence that higher vitamin D levels may be linked to lower risk of acquisition of TB infection. Larger studies utilising similar epidemiological designs in high TB-prevalence countries with distinct climates are required to further elucidate the connection between vitamin D levels and immunity against tuberculosis infection.

Declarations

FUNDING

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Figures

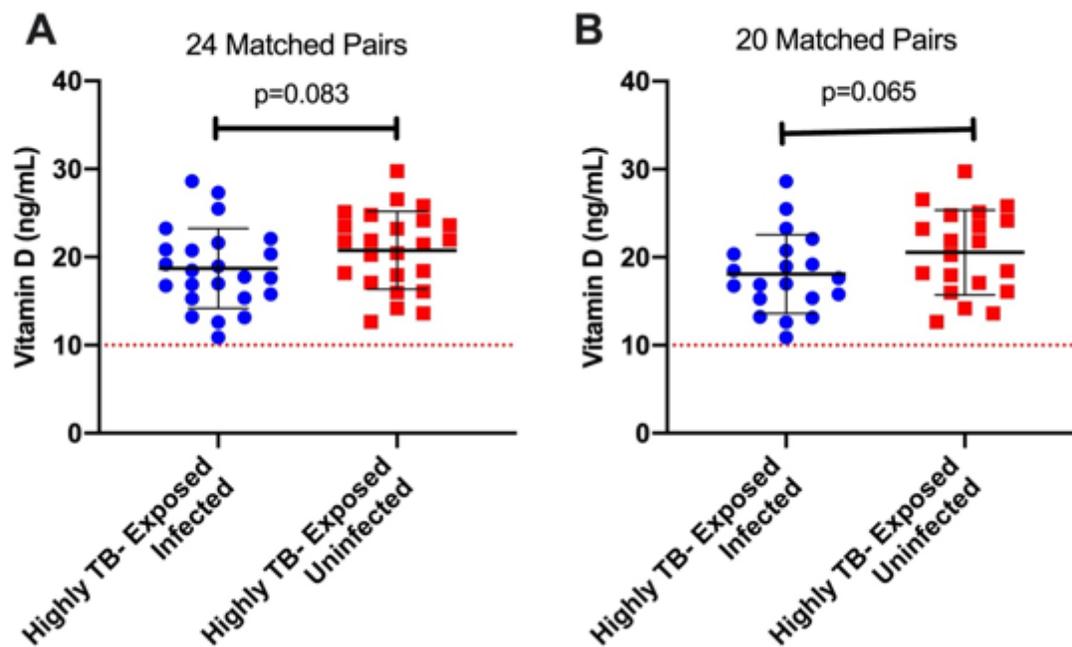


Figure 1

Vitamin D levels among 24 matched pairs (A) and 20 matched pairs (B) of highly TB-exposed uninfected and infected children. Red dotted line represents vitamin D deficiency threshold of 10ng/mL