Tuberculosis 98 (2016) 86–91

Contents lists available at ScienceDirect

Tuberculosis

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

EPIDEMIOLOGY

Elevated serum 25-hydroxy (OH) vitamin D levels are associated with risk of TB progression in Gambian adults

Olumuyiwa Owolabi [a, *], Schadrac Agbla [a], Patrick Owiafe [a, b], Simon Donkor [a], Toyin Togun [a], Abdou K. Sillah [a], Martin O.C. Ota [a, c], Jayne S. Sutherland [a]

[a] Vaccines & Immunity Theme, Medical Research Council Unit, Atlantic Road, Fajara, Gambia
[b] Department of Medical Laboratory Science, University of Health and Allied Sciences, PMB 31 Ho, Volta Region, Ghana
[c] World Health Organisation Regional Office, Brazzaville, Congo

ARTICLE INFO

Article history:
Received 8 September 2015
Received in revised form
22 February 2016
Accepted 29 February 2016

Keywords:
Tuberculosis
Incident cases
25(OH) D
Vitamin D binding protein
Latent TB infection

SUMMARY

Background: Vitamin D is essential in the host defence against tuberculosis (TB) as an immune modulator. The aim of this study was to determine the level of 25-hydroxyvitamin D (25 (OH) D) from adult TB index cases before and after treatment and their exposed household contacts (HHC) in The Gambia.
Methods: Serum from adult index TB cases and their TB-exposed household contacts (HHC) was analysed for 25(OH) D and Vitamin D binding protein (VDBP) concentrations. Tuberculin skin test (TST) status was used as a measure of *Mycobacterium tuberculosis* (Mtb) infectivity in the HHC. In addition, HHC who later progressed to active TB (incident cases) were assessed alongside non-progressors to determine the influence of 25 (OH) D levels on TB risk.
Results: Eighty-three TB cases, 46 TST+ and 52 TST− HHC were analysed. Generally levels of 25(OH) D were considered insufficient in all subjects. However, median levels of 25(OH) D and VDBP were significantly higher in TB cases compared to both TST+ and TST− HHC at recruitment and were significantly reduced after TB therapy ($p < 0.0001$ for all). In addition, levels of serum 25(OH) D at recruitment were significantly higher in TB progressors compared to non-progressors (median (IQR): 25.0(20.8–29.2) in progressors and 20.3 (16.3–24.6) ng/ml in non-progressors; $p = 0.007$).
Conclusion: In The Gambia, an equatorial country, 25(OH) D levels are higher in serum of TB progressors and those with active disease compared to latently infected and uninfected subjects. These results contrast to findings in non-equatorial countries.

1. Introduction

The global burden of Tuberculosis (TB) is huge, with an estimated one-third of the world population latently infected [1,2], 9 million new cases and 1.5 million deaths per year [3]. In a life-time, 10% of infected individuals progress to active TB disease in immune competent individuals increasing to 10% per year in HIV co-infected individuals [4]. One of the major contributing factors to disease progression in HIV uninfected individuals is malnutrition [5] with micronutrient deficiencies such as vitamins A, B6, E, thiamine, folate, and zinc reported in patients with active TB disease [6]. 25-hydroxyvitamin D (25(OH) D) has been implicated in the host defence against TB as an immune modulator [1,7,8]. Indeed, treatment of TB decades ago included exposure to the sun for enhanced 25(OH) D synthesis, but the mechanism was not clear [9]. 25(OH) D has been shown to down-regulate the pro-inflammatory response and therefore may help to protect the host against increased lung pathology induced by exacerbated inflammation [10]. It also has anti-microbial effects by inducing activation of macrophages [11]. A seminal paper in Science demonstrated the link between 25(OH) D and IFN-γ induced macrophage antimicrobial responses [12].

The majority of studies analysing the role of 25(OH) D in TB have shown an association between low 25(OH) D and susceptibility to active TB disease [13–16]. However, there are still discrepancies in terms of genetic predisposition [17,18] and serum levels in TB and non-TB subjects [19,20]. This is likely due to geographic location, exposure to sunlight, genetic differences, cultural practices, religion and the presence of other disease conditions [21]. A recent study in Greenland, for example, showed large variation of Serum 25(OH) D

* Corresponding author. MRC Unit, PO Box 273, Banjul, Gambia. Tel.: +220 4495442x3032; fax: +220 4495919.
E-mail address: oowolabi@mrc.gm (O. Owolabi).

http://dx.doi.org/10.1016/j.tube.2016.02.007

concentrations in TB patients, with supplementation likely to increase the risk of TB amongst those with normal or high concentrations [22]. Indeed, clinical trials in Guinea-Bissau and Georgia and a meta-analysis of all trials that used 25(OH) D supplementation as adjunctive therapy have shown no clinical benefits [23–25]. In HIV-infected patients in Uganda, no difference in 25(OH) D levels was seen in subjects with and without TB [23], while a Malawi study among TB patients with over 50% HIV co-infection rates showed that deficiency in the levels of 25(OH) D was common and had no influence on response to treatment [27].

The genetic predisposition to TB associated with Vitamin D receptor (VDR) gene polymorphisms was considered a prime candidate for TB susceptibility but results from a meta-analysis have proved inconclusive [17] although a link between VDR polymorphisms and response to therapy in terms of time to fast sputum conversion rate with anti TB treatment in FF; Taql Tt and non-FF; TT genotypes respectively for pulmonary TB has been suggested [24]. These discrepancies are further highlighted by a recent study showing no benefit of 25(OH) D supplementation on response to therapy in an Indian population [25] although it may limit lung pathology and thus reduce disability-associated life-years (DALYs) in vulnerable populations [26].

In West Africa, there appears to be a role for VDR haplotypes rather than genotypes in susceptibility to TB [18], although another study in Guinea Bissau showed VDR polymorphisms when analysed together with ethnicity were associated with increased risk of TB disease [27]. Interestingly, there also appears to be an influence of variation within the gene encoding Vitamin D binding protein (VDBP) and Serum 25(OH) D levels in Gambian children [28], which has implications for interpretation of 25(OH) D status across different groups. However, no study has been performed in The Gambia to determine the serum levels of 25(OH) D in TB patients, TST+ and TST− contacts.

Due to our powerful TB case-contact platform in The Gambia [29], we are able to analyse individuals across the spectrum of TB infection and disease. Thus, the aim of this study was to compare the level of Serum 25(OH) D and VDBP in adult TB cases before and after treatment and their exposed household contacts (both TST+ and TST−). Importantly, we also analysed exposed subjects who progressed to active TB disease between 3 and 24 months from recruitment. This is the first study in Gambia, an equatorial country, to determine 25(OH) D levels and association with TB. In complete contrast to the majority of studies in non-equatorial countries, we found higher 25(OH) D levels in TB cases compared to TST+ and TST− HHC. Importantly, levels decreased with treatment and were higher in subjects who progressed to active TB, indicating that in our setting higher 25(OH) D is associated with TB risk.

2. Materials and methods

2.1. Study participants and design

Adult TB cases were recruited from the Medical Research Council (MRC) TB clinic in Fajara, The Gambia. The diagnosis of active TB was established on the basis of smear positivity for acid-fast bacilli of *Mycobacterium tuberculosis* (Mtb). All mycobacterial cultures were identified and confirmed using standard procedures. Household contacts (HHC) of confirmed index cases were visited to assess their TB infection status and were followed for 2 years from recruitment. Tuberculin skin test (TST) was used as a measure of mycobacterial infection and was performed using 2 tuberculin units of purified peptide derivative RT23 (SSI, Denmark) injected intra dermally into the volar aspect of the forearm and read at 48–72 h. TST was performed prior to any blood sampling of study participants. A reading of ≥10 mm was considered positive, and indicative of mycobacterial infection. The diagnosis of latent TB infection was established on the basis of absence of TB symptoms, absence of radiologic abnormalities of active TB (all contacts had chest X-ray performed) and positivity for TST.

25(OH) D was measured at recruitment and 6 months after recruitment for adult TB cases but measured only at recruitment for TST+ and TST− HHC. The VDBP was measured for a random subsample of TB cases, TST+ and TST− HHC (n = 24 cases at 0 and 6 months; 27 TST+ HHC and 28 TST− HHC). These had similar age and sex distributions to the larger cohort (not shown). A matched case–control study was also conducted to analyse TB progressors (defined as asymptomatic at recruitment but progressing to active disease between 3 and 24 months [29] and matched (by age and sex)) to non-progressors in a 1:3 ratio. Serum samples from all subjects were collected and stored at −20 °C until analysis. All participants in this study gave written informed consent.

2.2. Measurement of 25(OH) D levels

Serum 25(OH) D concentrations were determined using a Vitamin D total ELISA (DIAsource Immunoassays, Belgium) according to the manufacturer's instructions. A 4-parameter logistic function curve was used to determine the 25(OH) D concentrations of the samples from the calibration curve. 25(OH) D levels were defined as: Deficiency: 0–10 ng/ml; Insufficiency: 10–30 ng/ml; Sufficiency: 30–150 ng/ml and toxicity: >150 ng/ml.

2.3. Measurement of vitamin D binding protein levels

In order to determine the free, bioactive levels of 25(OH) D, we analysed Serum Vitamin D binding protein (VDBP) using an ELISA-based method (Immundiagnostik, Germany) according to manufacturer's instructions. A 4-parameter standard curve was constructed and levels of VDBP determined in each sample. Results were multiplied by 40,000 and converted to mg/dl.

2.4. Statistical analysis

The 25(OH) D level at recruitment was compared between TB groups using Wald adjusted test accounting for clustering within households. Unadjusted and adjusted mixed effects models were performed with age, gender and season at recruitment as potential confounders. We investigated the relationship between 25(OH) D and BMI using a multilevel structural equation modelling accounting for clustering within households. Wilcoxon signed rank test and Wald adjusted test were used to compare the 25(OH) D and VDBP levels between TB groups at 0 and 6 months. Pearson chi-squared test with second-order correction of Rao and Scott accounting for clustering within households was used to assess association between TB groups and categorical variables such as gender and 25(OH) D category (i.e. insufficient versus sufficient) at recruitment. Wald test with adjustment for confounders using linear regression was used to analyse progressors and non-progressors.

3. Results

3.1. Participant demographics

We analysed a total of 181 HIV sero-negative participants consisting of 83 confirmed TB cases, 46 TST+ HHC and 52 TST− HHC (Table 1). A total of 12 incident cases (progressing to active TB between 3 and 24 months from recruitment) were detected and matched by age and sex with 32 HHC who did not progress to active TB. There was no difference in age between TB cases, TST+ HHC and TST− HHC but a significant difference in the proportion of males in the TB index case group (71.1%), TST+ (52.2%) and TST− (36.5%)