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Case-control study of vitamin D status and adult multidrug-resistant pulmonary TB

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SUMMARY

BACKGROUND: India has the highest prevalence of multidrug-resistant TB (MDR-TB) globally. Vitamin D deficiency is potentially an important risk factor for MDR-TB.

METHODS: We conducted a case-control study of 90 newly diagnosed adult MDR-TB cases, 180 household controls and 82 non-household controls in Mumbai, India. Serum 25-hydroxyvitamin D (25(OH)D), anthropometry, clinical status and history, dietary data and sociodemographic data were collected from each participant. Interferon-gamma release assay (IGRA) was also performed in controls to assess latent TB. Multivariable regression was performed to estimate associations between 25(OH)D vs. case status and IGRA positivity.

RESULTS: Mean participant age was 33.8 ± 12.0 years; 72.8% had 25(OH)D <20 ng/ml. Mean 25(OH)D was significantly ($P < 0.05$) lower in cases (12.5 ± 7.9) than both household (17.5 ± 11.2) and non-household controls (16.4 ± 9.1). In multivariable models, 25(OH)D concentration was inversely associated with MDR-TB case status among cases and household controls (OR 0.95 per 1 ng/ml, 95% CI 0.92–0.99; $P = 0.015$), and among cases and non-household controls (OR 0.94 per 1 ng/ml, 95% CI 0.89–1.00; $P = 0.033$); 53.6% of controls were IGRA-positive. 25(OH)D status was not associated with IGRA positivity.

CONCLUSION: Vitamin D status was independently associated with MDR-TB case status. Research should evaluate the effectiveness of vitamin D supplementation in prevention and adjunctive treatment of MDR-TB.

KEY WORDS: host-directed therapy; nutrition support; clinical management; nutritional epidemiology; slum-dwelling populations

Drug-resistant TB (DR-TB) has emerged as a major global health threat in recent years. India is the worst affected country, with more than 124,000 DR-TB cases (27% of the global total), most of whom are multidrug-resistant (MDR).[1] India also has the highest national prevalence of latent TB infection (LTBI), estimated at 48% using the QuantiFERON-TB Gold (QFT-G; Qiagen, Hilden, Germany) interferon-gamma release assay (IGRA) and 42% based on tuberculin skin test (TST).[2]

Despite plentiful sun exposure, the Indian population also has an extremely high prevalence of vitamin D deficiency (70–100% have 25-hydroxyvitamin D (25(OH)D) concentrations <50 ng/ml).[3] This is attributable to a lack of dietary vitamin D and supplementation, limited skin exposure given cultural practices and increasingly indoor lifestyles, air pollution, skin pigmentation, and unspaced and unplanned pregnancies.[4]

The long duration and high cost of multidrug-resistant TB (MDR-TB) treatment[5] motivate efforts to develop cost-effective preventive and adjunctive therapies,[6] including vitamin D supplementation. Vitamin D influences the expression of genes mediating immune response, improving innate immunity by boosting production of anti-microbial peptides (including cathelicidins and β defensin 2) and cytokine response.[7] Vitamin D enhances adaptive immunity by suppressing the production of inflammatory cytokines by Type 1 T-helper cells.[8] Vitamin D receptor polymorphisms have been found to increase risk of TB[9] and time to sputum conversion,[10] further supporting a clinical role for vitamin D in TB.

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Despite plausible mechanisms, epidemiologic studies of vitamin D and DR-TB are limited. The objective of the present case-control study was to examine associations between vitamin D status and active MDR-TB infection among cases and controls, and between vitamin D status and LTBI among controls in a largely slum-dwelling population in municipal wards of Mumbai, India, where rates of DR-TB are historically high.[11,12]

METHODS

Study population

Pulmonary MDR-TB, including extensively drug-resistant (XDR-) and pre-XDR-TB cases residing in the M-East, M-West and H-East wards of Mumbai, and receiving treatment at two DR-TB treatment centres were identified by study staff using the national Programmatic Management of Drug Resistant TB register. Inclusion criteria for cases were 18–60 years of age, residents of the study wards for ≥6 months, had initiated MDR-TB treatment <1 month ago, had no history of MDR-TB in the past 2 years and had ≥2 eligible household controls to whom they had disclosed their TB status and who agreed to join the study. Sample size analysis was based on prior data comparing vitamin D status in MDR-TB patients and household contacts in urban India, as described in our protocol paper.[13]

Two household controls and one non-household control aged 18–60 years were recruited for each case. Household controls resided with the index case for at ≥1 year prior to the case's MDR-TB diagnosis, and had no symptoms of active TB at screening. Non-household controls were recruited from dermatology departments at the hospitals where the cases were identified and were required to have no active TB symptoms and no family member with a history of TB in the past 2 years. Non-household controls were matched with cases by age (±10 years), sex and ward of residence (as the pool of eligible household controls was limited, these were not matched to cases).

Once identified as potentially eligible based on age and time since MDR-TB treatment initiation, cases and household controls were contacted by trained field workers from a local community-based NGO who explained the study in detail, obtained informed consent and scheduled a convenient time for their interview. Non-household controls were recruited when they presented for dermatological consultation. Participants were recruited between January and December 2020.

The study was approved by Institutional Review Board of Harvard T H Chan School of Public Health, Boston, MA, USA; Institutional Research Ethics Committee of the Foundation for Medical Research, Mumbai, and the Indian Health Ministry's Screening Committee, New Delhi, India.

Assessments

Participants' sociodemographic characteristics, clinical status and history, and food consumption frequency[14] were assessed using SurveyCTO software (Dobility Inc, Cambridge, MA, USA). Height, weight, mid-upper arm circumference and waist circumference were measured. Haemoglobin and random blood glucose measurements were taken using respectively Mission® ULTRA haemoglobinometer (Acon Labs, San Diego, CA, USA) and Contour® TS glucometer (Ascensia Diabetes Care UK Limited, Newbury, UK). A certified phlebotomist collected blood to measure serum 25(OH)D (all participants) and QFT-G IGRA (controls only). 25(OH)D was measured at Metropolis Healthcare (Mumbai), a reputable laboratory using a liquid chromatography-mass spectrometry platform subject to stringent quality assurance measures set by the India National Accreditation Board for Testing and Calibration Laboratories (Gurgaon, India). Controls underwent chest X-ray and voluntary HIV testing at local imaging centres and integrated counselling and testing centres, respectively.

Statistical analysis

Descriptive statistics on sociodemographic, anthropometric, clinical and biochemical variables were tabulated by study group (cases, household controls and non-household controls). We computed an asset index using principal component analysis.[15] Within categories of population characteristics, we computed median and interquartile range values for 25(OH)D, and estimated bivariate associations between 25(OH)D and selected characteristics using generalised estimating equations (clustering by index case and employing an exchangeable correlation structure). Given the potential heterogeneity between cases and controls, bivariate analysis was conducted among all participants and after excluding cases. We tested differences in mean 25(OH)D between study groups using Mann-Whitney *U*-tests and differences in distributions between 25(OH)D categories (0–10, 10–20, 20–30 <30 ng/mL[16]) using Fisher's exact tests. In analyses involving 25(OH)D, 7 cases, 3 household controls and 2 non-household controls (3% of participants) with 25(OH)D <3.55 ng/ml (assay detection limit) were imputed as 3.55 ng/ml. Four cases, 6 household controls and 1 non-household control (3% of the study population) with 25(OH)D >50 ng/ml were excluded as outliers (these were retained in sensitivity analyses; Supplementary Tables S1–S4).

We conducted logistic regression analyses, conditioned on matching factors, to evaluate associations between 25(OH)D and either MDR-TB case status or

IGRA positivity. Details of Analysis (1) were as follows: outcome: MDR-TB case status; population: cases and household controls matched by household. Details of Analysis (2) were as follows: outcome: MDR-TB case status; population: cases and non-household controls matched by ward, age, and sex. Details of Analysis (3) were as follows: outcome: IGRA positivity; population: household and non-household controls matched by index case (and, therefore, effectively matched by ward). For each analysis, we ran an unadjusted model, age- and sex-adjusted model (except in analysis (2), in which age and sex were conditioned on as matching factors), and multivariable models (further adjusted for religion, marital status, education, occupation, asset quartile, history of smoking and number of household members sleeping per room). In each model, we compared results treating 25(OH)D as a continuous variable and as a categorical variable using the following cut-offs: 0–10, 10–20, and <20 ng/ml (20–30 and <30 ng/ml were condensed to prevent low cell counts in some models; results did not differ when categories were disaggregated). In continuous models, we evaluated non-linearity in associations using cubic polynomials, and interaction between 25(OH)D and sex, and between 25(OH)D and age.

RESULTS

Data were collected from 352 participants (90 cases, 180 household controls and 82 non-household controls). Mean age of participants was 33.8 ± 12.0 years and 53.7% were female (Table 1). Household controls were significantly older ($P < 0.05$) (mean age: 36.8 years) than cases (29.8 years) and non-household controls (31.6 years). Cases had significantly lower body mass index than control groups (66% of cases were underweight compared, with 23% of household and 15% non-household controls, and 9% of cases being overweight or obese compared with 48% of household and 51% of non-household controls). Anaemia was significantly more prevalent in cases (22%) than household (9%) and non-household (10%) controls. Two household controls had findings suggestive of cavitary lesions on chest X-ray, but were not considered cases as they did not have signs or symptoms of TB.

The overall prevalence of vitamin D deficiency (25(OH)D <10 ng/ml), inadequacy (10–<20 ng/ml) and adequacy (≥20 ng/ml) was respectively 35.2%, 36.7% and 28.2% (Table 2, Figures 1 and 2). Vitamin D deficiency was prevalent in 47% of cases (median 25(OH)D: 10.5 ng/ml), 33% of household controls (median: 14.2 ng/ml) and 28% of non-household controls (median: 15.7 ng/ml). Mean 25(OH)D was significantly ($P < 0.05$) lower in cases than in household and non-household controls, while control groups did not significantly differ from one another. In bivariate analysis among controls, 25(OH)D was inversely associated with blood draw in summer months, <40 years of age, female sex, unmarried status and living in a household with ≥5 members sleeping per room (Table 3). In multivariable analysis among controls (not shown), adjusting for all predictors listed in Table 3 and study group (household vs. non-household controls), each 5-year difference in age was significantly associated with 1.2 ng/ml (95% confidence interval [CI] 0.5–1.9) higher mean 25(OH)D; men had 4.2 ng/ml (95% CI 1.3–7.1) higher mean 25(OH)D than women; and blood draw in monsoon or winter seasons was associated with a 4.5 ng/ml (95% CI 1.3–7.7) or 5.1 ng/ml (95% CI 2.4–8.2) higher mean in 25(OH)D than summer, respectively.

Among the population of cases and household controls, each 1-unit increase in 25(OH)D was significantly ($P < 0.05$) associated with MDR-TB case status in the multivariable-adjusted model (odds ratio [OR] 0.94, 95% CI 0.91–0.97; Table 4). Compared to individuals with 25(OH)D <10 ng/ml, the 20–50 ng/ml range was marginally associated ($P = 0.085$) with lower odds of case status (OR 0.46, 95% CI 0.19–1.11). Similarly, restricting to cases and non-household controls, each 1-unit increase in 25(OH)D was significantly associated with an OR of 0.94 (95% CI 0.89–1.00) of MDR-TB case status in the multivariable model, while 25(OH)D 20–50 ng/ml was marginally associated ($P = 0.069$) with an OR of 0.33 (95% CI 0.10–1.1) upon multivariable adjustment. Significant and marginally significant associations between 25(OH)D and MDR-TB case status remained when including 11 participants with 25(OH)D values >50 ng/ml (Supplementary Table S3). We found no evidence of non-linearity in associations between continuous 25(OH)D and MDR-TB in either contrast examined (cases vs. household controls or vs. non-household controls), nor interaction by age or sex.

The prevalence of IGRA positivity did not significantly differ between household and non-household controls (36% vs. 32%, respectively; $P = 0.186$) (Table 1). Mean 25(OH)D did not differ between IGRA-negative and IGRA-positive controls (15.3 ng/ml vs. 13.4 ng/ml, respectively) (Table 3). Neither continuous nor categorical 25(OH)D were significantly associated with IGRA positivity in multivariable analysis (Table 5, Supplementary Table S4).

DISCUSSION

This case-control study in Mumbai, India, identified lower vitamin D levels as an independent risk factor for active MDR-TB among the population of cases and household controls, and among cases and non-household controls. We found no association between vitamin D status and IGRA positivity.

Table 1 Background characteristics among multidrug-resistant TB cases and controls in Mumbai, India

Variable	Category	Cases ($n = 90$)		Household controls ($n = 180$)		Non-household controls ($n = 82$)	
		n	%	*n*	%	*n*	%
Season of blood draw	Summer (March–May)	15	17	30	17	6	7
	Monsoon (June–September)	29	32	58	32	2	2
	Winter (October–February)	46	51	92	51	74	90
Ward	M: East	56	62	112	62	52	63
	M: West	24	27	48	27	21	26
	H: East	10	11	20	11	9	11
Age, years	<25	44	49	43	24	24	29
	25–40	27	30	50	28	39	48
	≥40	19	21	87	48	19	23
Female sex		48	53	96	53	45	55
Religion	Hindu	42	47	85	47	45	55
	Muslim	29	32	58	32	16	20
	Buddhist	16	18	35	19	21	26
	Other	3	3	2	1	0	0
Marital status	Single	46	51	53	29	28	34
	Married	41	46	122	68	51	62
	Separated	0	0	2	1	0	0
	Divorced	1	1	1	1	0	0
	Widowed	2	2	2	1	3	4
Education level	No formal education (illiterate)	9	10	25	14	7	9
	No formal education (literate)	4	4	11	6	2	2
	Primary (<4th standard)	3	3	9	5	1	1
	Secondary (<9th standard)	26	29	56	31	24	29
	Senior secondary (SSC/HSC)	41	46	64	36	42	51
	Graduate	7	8	14	8	5	6
	Post-graduate	0	0	1	1	1	1
Occupation	Unemployed	13	14	7	4	4	5
	Homemaker	19	21	60	33	23	28
	Student	21	23	15	8	10	12
	Daily wage worker/casual labour	5	6	24	13	5	6
	Salaried	15	17	49	27	33	40
	Self-employed	14	16	24	13	7	9
	Retired	2	2	0	0	0	0
	Other	1	1	1	1	0	0
BMI, kg/m^2	Underweight (<18.5)	59	66	41	23	12	15
	Normal (18.5–22.9)	23	26	52	29	28	34
	Overweight (23–24.9)	5	6	24	13	11	13
	Overweight (≥25)	3	3	63	35	31	38
IGRA-positive (controls only)				65	36	26	32
Ever smoked		12	13	17	9	12	15
Currently taking vitamin D supplement		2	2	0	0	0	0
Fish intake in last month, g/day	0	39	43	90	50	40	49
	0–32.4	29	32	42	23	20	24
	≥32.4	22	24	48	27	22	27
Household members sleeping per room	<3	24	27	49	27	27	33
	3–5	45	50	82	46	36	44
	≥5	21	23	49	27	19	23
Asset quartile	1	20	22	49	27	19	23
	2	29	32	44	24	15	18
	3	18	20	50	28	20	24
	4	23	26	37	21	28	34

SSC/HSC = Secondary School Certificate/Higher Secondary Certificate; BMI = body mass index; IGRA = interferon-gamma release assay.

Table 2 Serum 25(OH)D among MDR-TB cases and controls*

25(OH)D ng/ml	Cases ($n = 86$) *n* (%)	Household controls ($n = 174$) *n* (%)	Non-household controls ($n = 81$) *n* (%)
<10	40 (47)	57 (33)	23 (28)
10–20	32 (37)	59 (34)	34 (42)
20–30	9 (10)	32 (18)	17 (21)
≥30	5 (6)	26 (15)	7 (9)
Mean ± SD	12.5 ± 7.9	17.5 ± 11.2	16.4 ± 9.1
Median [IQR]	10.5 [6.4–15.7]	14.2 [8.4–24.2]	15.7 [9.1–21.9]

* 4 cases, 6 household controls, and 1 non-household control with 25(OH)D ≥50 ng/ml were excluded from the analyses.
MDR-TB = multidrug-resistant TB; 25(OH)D = serum 25-hydroxyvitamin D SD = standard deviation; IQR = interquartile range.

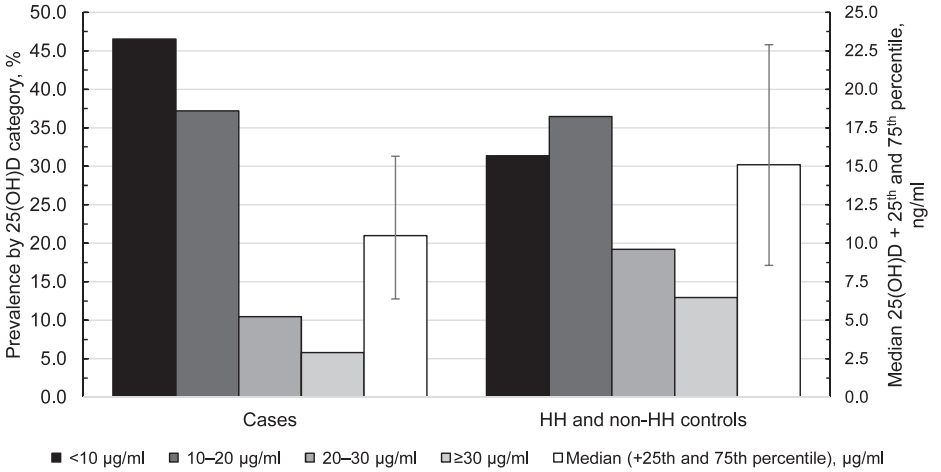

Figure 1 Prevalence of serum 25(OH)D ranges among MDR-TB cases and controls. Mann-Whitney *U*-test for difference in 25(OH)D between cases and controls: $P < 0.001$. Fisher's exact tests for difference in distribution of 25(OH)D categories between cases and controls: $P = 0.038$; 4 cases, 6 household controls and 1 non-household control with 25(OH)D ≥50 ng/ml were excluded from analyses. 25(OH)D = serum 25-hydroxyvitamin D; HH = household; MDR-TB = multidrug-resistant TB.

An inverse relation between vitamin D status and MDR-TB infection agrees with prior systematic reviews of drug-susceptible TB (DS-TB).[17–20] However, meta-analyses of clinical trials have shown little or no positive effects of vitamin D supplementation for DS-TB management.[21–23] The benefits of vitamin D supplements may be greater among severely ill patients, but this warrants further investigation. In a recent trial of vitamin D supplementation among HIV patients in Tanzania, those with TB at baseline had lower mortality when provided vitamin D supplements vs. placebo.[24]

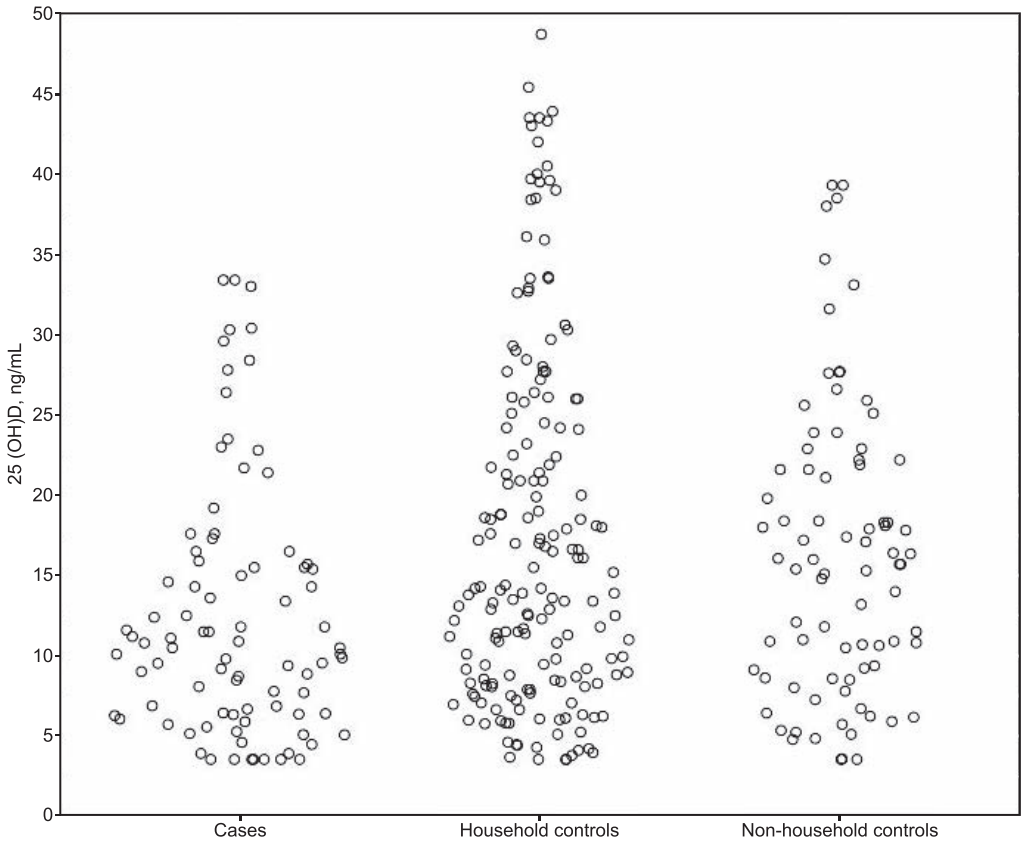

Figure 2 Serum 25(OH)D values among MDR-TB cases and controls; 4 cases, 6 household controls and 1 non-household control with 25(OH)D ≥50 ng/ml were excluded from analyses. 25(OH)D = serum 25-hydroxyvitamin D; MDR-TB = multidrug-resistant TB.

Table 3 Serum 25(OH)D by population characteristics among household and non-household controls ($n = 255$)

Variable	Category	*n*	Median	IQR	*P* value*
Season of blood draw	Summer (March–May)	34	11.8	6.3–17.2	Reference
	Monsoon (June–September)	60	16.8	11.5–25.9	0.001†
	Winter (October–February)	161	15.7	8.5–23.9	0.009†
Ward	M: East	160	15.0	8.4–22.9	Reference
	M: West	66	14.1	9.4–19.9	0.764
	H: East	29	16.1	9.2–27.7	0.368
Age, years	<25	66	9.3	6.4–18.3	Reference
	25–40	88	14.4	8.2–21.9	0.070
	≥40	101	17.8	12.5–26	0.000†
Sex	Female	137	12.9	6.7–22.2	Reference
	Male	118	16.7	11.4–23.7	0.025†
BMI, kg/m^2	Underweight (<18.5)	53	10.8	6.2–20.9	0.272
	Normal (18.5–22.9)	78	13.2	8.7–23.7	Reference
	Overweight (23–24.9)	33	16.5	12.5–22.9	0.371
	Overweight (≥25)	91	16.6	10.6–26	0.224
Religion	Hindu	126	16.1	8.9–21.9	Reference
	Muslim	72	10.9	6.2–27.8	0.846
	Buddhist/Other	57	15.2	10.9–22.2	0.952
Marital status	Not married	88	12.9	8–21.4	Reference
	Married	167	15.7	9.6–24	0.023†
Education	Primary school or lower	54	15.9	8.9–23.8	Reference
	Secondary school	78	15.4	8.4–22.2	0.873
	Senior secondary school or higher	123	14.2	8.6–22.7	0.778
Occupation	Homemaker	80	14.5	7.2–26.2	Reference
	Salaried	80	15.9	11–19.2	0.815
	Other occupation	95	14.2	8.7–24.2	0.791
Asset quartile	1	66	15.2	8.4–21.1	Reference
	2	58	16.1	9–22	0.546
	3	68	14.0	8–24.7	0.701
	4	63	15.5	9.9–22.8	0.659
Fish intake in previous month, g/day	0	126	13.3	8.0–23.7	Reference
	0–32.4	62	15.0	10.5–21.5	0.590
	≥32.4	67	18.1	10.9–24.2	0.100
Household members sleeping per room	<3	75	17.0	11.2–26.1	Reference
	3–5	114	15.3	8.5–23.8	0.214
	≥5	66	12.4	7.9–20.4	0.008†
IGRA result (controls only)	Negative	166	15.3	8.9–23.1	Reference
	Positive	89	13.4	8.1–21.9	0.356

* *P* values were estimated using generalized estimating equations clustering by index case; 4 cases, 6 household controls, and 1 non-household control with 25(OH)D ≥50 ng/ml were excluded from analyses.
† Statistically significant.
25(OH)D = serum 25-hydroxyvitamin D; IQR = interquartile range; BMI = body mass index; IGRA = interferon-gamma release assay.

Some prior observational studies evaluating the relationship between vitamin D status and MDR-TB found that vitamin D levels were lower among MDR-TB patients than DS-TB cases and healthy controls,[25] and that vitamin D receptor polymorphisms were associated with slower culture conversion among active MDR-TB patients.[26] Unlike in DS-TB, some meta-analyses showed vitamin D to be effective in accelerating sputum conversion in MDR-TB patients,[27,28] although existing trials have been small, and not all enrolled patients received second-line treatment.[27]

It has been hypothesised that effects of second-line treatment for DR-TB on immune response (and treatment outcomes) may be modified differently by vitamin D as compared with first-line treatment.[26,29] In addition to novel cellular targets, reduced anti-TB activity and increased immune suppression of current MDR-TB regimens may generally offer a greater window of opportunity for a modestly active adjunctive therapy to demonstrate clinical benefit.[21] Furthermore, behavioural changes (reduced dietary intake and sun exposure), resulting from comparatively long durations of MDR-TB and MDR-TB treatment are plausibly associated with greater reductions in vitamin D status than in DS-TB, and thus a greater benefit of supplementation.

The observed null association between 25(OH)D and LTBI in the current study contrasts with some prior studies showing vitamin D status to be associated with lower risk of LTBI.[30,31] This may be due to our smaller sample size, as our study was primarily powered to understand the relationship between vitamin D status and MDR-TB case status. However, a large randomised controlled trial recently conducted among Mongolian schoolchildren also found no association between vitamin D supplementation and TB infection.[32]

The high prevalence of inadequate 25(OH)D levels in our study agrees with findings from other Indian populations.[3] In line with studies from low- and middle-income countries,[33] we observed lower vitamin D levels among women and participants with low socio-economic status (represented by residence

Table 4 Conditional logistic regression of the association between serum 25(OH)D and MDR-TB case status*

Population	Covariates conditioned on or adjusted for	25(OH)D category (referent: 0–10 ng/ml) ng/ml	OR for a 1 ng/ml increase in 25(OH)D (95% CI)	*P* value
Analyses in which 25(OH)D is modelled as a continuous predictor				
Cases and household controls matched by household (n = 260; 86 cases)	Conditioned on matching factor (household)		0.941 (0.910–0.974)	<0.001†
	...further adjusted for age and sex		0.954 (0.919–0.989)	0.011†
	...further adjusted for religion, marital status, education, occupation, asset quartile, ever smoker, and number of household members sleeping per room		0.954 (0.918–0.991)	0.015†
Cases and non-household controls matched by ward, age, and sex (n = 167; 86 cases)	Conditioned on matching factors (ward, age, and sex)		0.936 (0.895–0.980)	0.005†
	...further adjusted for religion, marital status, education, occupation, asset quartile, ever smoker, and number of household members sleeping per room		0.940 (0.889–0.995)	0.033†
Analyses in which 25(OH)D is modelled as a categorical predictor (0–10 ng/ml, 10–20 ng/ml, 20–50 ng/ml)				
Cases and household controls matched by household (n = 260; 86 cases)	Conditioned on matching factor (household)	10–20	0.771 (0.428–1.390)	0.387
		20–50	0.335 (0.157–0.715)	0.005†
	...further adjusted for age and sex	10–20	1.015 (0.533–1.932)	0.965
		20–50	0.467 (0.206–1.060)	0.069
	...further adjusted for religion, marital status, education, occupation, asset quartile, ever smoker, and number of household members sleeping per room	10–20	1.102 (0.529–2.299)	0.795
		20–50	0.459 (0.189–1.114)	0.085
Cases and non-household controls matched by ward, age, and sex (n = 167; 86 cases)	Conditioned on matching factors (ward, age, and sex)	10–20	0.627 (0.282–1.395)	0.253
		20–50	0.281 (0.108–0.728)	0.009†
	...further adjusted for religion, marital status, education, occupation, asset quartile, ever smoker, and number of household members sleeping per room	10–20	0.608 (0.212–1.740)	0.353
		20–50	0.332 (0.101–1.089)	0.069

* 4 cases, 6 household controls, and 1 non-household control with 25(OH)D ≥50 ng/ml were excluded from analyses.
† Statistically significant.
25(OH)D = serum 25-hydroxyvitamin D; MDR-TB = multidrug-resistant TB.

in households with ≥5 sleepers per room); this may be attributable to malnutrition and protein deficiency which affect poorer households and which suppress vitamin D binding protein.[34] In contrast with other studies,[33] we found a positive association between vitamin D levels and age in this population. Higher vitamin D levels in winter may be attributable to reduced outdoor exposure during extremely hot and humid months.

Our study has several strengths. Inclusion of both household and non-household controls (matched to cases on age, sex and ward) allowed us to quantify the influence of vitamin D on MDR-TB disease in the general population and a highly susceptible subgroup. Assessment of LTBI using QFT-G allowed us to further explore vitamin D's potential role in preventing TB infection.

This study had some limitations. The cross-sectional design limited our ability to ascertain the temporal relationship between vitamin D status and infection (it is possible that lower vitamin D status among cases rendered them more susceptible to infection or that case status itself suppressed vitamin D status).[35] Second, age- and sex-matching was only possible for non-household controls given the limited pool of eligible household controls; however, we adjusted for age and sex in regression models. Third, the study population was restricted to residents of M and H wards of Mumbai; while results of this study are likely representative of slum populations in Mumbai and other cities in India (populations most affected by TB), care is warranted when generalising further. Furthermore, due to COVID-19 related restrictions, a high proportion (90%) of matched non-household controls were recruited in just 2 months (November and December, when restrictions were eased). Fourth, serum 25(OH)D measurements ≥50 ng/ml may have been affected by laboratory error, given that none of the 11 outlying participants consumed vitamin D supplements (except three who consumed multivitamins with small amounts of vitamin D) and their

Table 5 Conditional logistic regression of the association between serum 25(OH)D and IGRA positivity*

Population	Covariates conditioned on or adjusted for	25(OH)D category (referent: 0–10 ng/ml) ng/ml	OR for a 1 ng/ml increase in 25(OH)D (95% CI)	*P* value
Analyses in which 25(OH)D is modelled as a continuous predictor				
Household and non-household controls matched by index case (living in the same ward) ($n = 255$; 89 IGRA+)	Conditioned on matching factor (index case)		0.976 (0.941–1.012)	0.186
	...further adjusted for age and sex		0.974 (0.938–1.012)	0.179
	...further adjusted for religion, marital status, education, occupation, asset quartile, ever smoker, and the number of household members sleeping per room		0.977 (0.934–1.021)	0.302
Analyses in which 25(OH)D is modelled as a categorical predictor (0–10 ng/ml, 10–20 ng/ml, 20–50 ng/ml)				
Household and non-household controls matched by index case (living in the same ward) ($n = 255$; 89 IGRA+)	Conditioned on matching factor (index case)	10–20	0.834 (0.371–1.872)	0.659
		20–50	0.448 (0.172–1.187)	0.100
	...further adjusted for age and sex	10–20	0.902 (0.374–2.176)	0.819
		20–50	0.433 (0.161–1.162)	0.096
	...further adjusted for religion, marital status, education, occupation, asset quartile, ever smoker, and the number of household members sleeping per room	10–20	0.870 (0.273–2.773)	0.814
		20–50	0.459 (0.142–1.487)	0.194

* 6 household controls, and 1 non-household control with 25(OH)D ≥50 ng/ml were excluded from analyses.
25(OH)D = serum 25-hydroxyvitamin D; IGRA = interferon-gamma release assay; CI = confidence interval; + = positive.

dietary vitamin D intake was extremely low (averaging 15 IU/day).

Results of this well-designed observational study are promising. Given plausible mechanisms and limited supporting evidence from prior trials, clinical benefits of vitamin D supplementation in treating active MDR-TB and preventing progression from latent to active disease would be worth evaluating in a large randomised controlled trial.

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RÉSUMÉ

CONTEXTE : L'Inde présente la plus forte prévalence de TB multirésistante (MDR-TB) au monde. La carence en vitamine D est potentiellement un important facteur de risque de MDR-TB.

MÉTHODES : Nous avons mené une étude cas-témoins auprès de 90 adultes chez qui une MDR-TB a été récemment diagnostiquée, de 180 témoins vivant à domicile et de 82 témoins ne vivant pas à domicile à Mumbai, en Inde. La 25-hydroxyvitamine D sérique (25(OH)D), l'anthropométrie, l'état et les antécédents cliniques, les données diététiques et les données sociodémographiques ont été recueillies auprès de chaque participant. Un test de libération d'interféron-gamma (IGRA) a également été réalisé chez les témoins pour évaluer la TB latente. Une régression multivariable a été effectuée pour estimer les associations entre la 25(OH)D et le statut du cas et la positivité de l'IGRA.

RÉSULTATS : L'âge moyen des participants était de 33,8 ± 12,0 ans ; 72,8% avaient une 25(OH)D <20 ng/ml. La moyenne de la 25(OH)D était significativement plus basse ($P < 0,05$) chez les cas (12,5 ± 7,9) que chez les témoins à domicile (17,5 ± 11,2) et les témoins hors domicile (16,4 ± 9,1). Dans les modèles multivariables, la concentration de 25(OH)D était inversement associée au statut de cas de MDR-TB chez les cas et les témoins vivant à domicile (OR 0,95 pour 1 ng/ml, IC 95% 0,92–0,99 ; $P = 0,015$), et chez les cas et les témoins ne vivant pas à domicile (OR 0,94 pour 1 ng/ml, IC 95% 0,89–1,00 ; $P = 0,033$) ; 53,6% des témoins étaient IGRA-positifs. Le statut 25(OH)D n'était pas associé à la positivité IGRA.

CONCLUSION : Le statut en vitamine D était indépendamment associé au statut de cas de MDR-TB. La recherche devrait évaluer l'efficacité de la supplémentation en vitamine D dans la prévention et le traitement d'appoint de la MDR-TB.