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Dose-response association between salivary cotinine levels and *Mycobacterium tuberculosis* infection

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Summary

SETTING—Tijuana, Mexico.

OBJECTIVE—To describe the association between salivary cotinine levels and interferon-release assay results.

DESIGN—We conducted a cross-sectional study among persons who inject drugs. Salivary cotinine levels were measured using NicAlert, a semi-quantitative dipstick assay. QuantiFERON TB Gold In-tube (QFT) was used to determine *Mycobacterium tuberculosis* (*Mtb*) infection.

RESULTS—Among 234 participants, the prevalence of QFT positivity for NicAlert cotinine categories 0 (nonsmoking), 1 (secondhand smoke exposure or low-level smoking), and 2–6 (regular smoking) were 42.1%, 46.4%, and 65.2%, respectively ($P_{\text{trend}} = 0.012$). We found increasing trends in QFT positivity ($P_{\text{trend}} = 0.003$) and interferon- concentrations (Spearman's $r = 0.200$; $P = 0.002$) across cotinine levels 0 to 6. In multivariable log-binomial regression models adjusted for education, cotinine levels were not associated with QFT positivity when included as smoking categories (1 and 2–6 vs. 0), but were independently associated with QFT positivity when included as an ordinal variable (prevalence ratio = 1.09 per +1 cotinine level; 95% confidence interval = 1.02 – 1.16).

CONCLUSION—Our findings suggest that a dose-response relationship exists between tobacco smoke exposure and *Mtb* infection. Longitudinal studies that use biochemical measures for smoking status are needed to confirm our findings.

Keywords

tuberculosis; tobacco; substance abuse; mycobacterium infections; smoking; interferon gamma release assay

INTRODUCTION

Cigarette smoking is a major driver of the global tuberculosis (TB) epidemic and has been shown to increase the risk for active TB disease.^{1–4} Smoking also impairs immune functions

in the pulmonary system, which might increase the risk for *Mycobacterium tuberculosis* (*Mtb*) infection among exposed TB contacts.^{5–7} However, a major limitation of previous epidemiological research on this topic is that few studies employed biochemical measures of smoking status, such as cotinine assays, to evaluate the relationship between cigarette smoking and *Mtb* infection.^{8–10} Cotinine is a metabolite of nicotine and a commonly used biomarker for cigarette smoke exposure.¹¹ For epidemiological studies, cotinine assays might be preferable to self-reported measures of smoking status, which are susceptible to misclassification due to social desirability bias and recall bias.^{12,13} Furthermore, variations in smoking behavior that could affect the level of exposure to cigarette smoke by the respiratory system, such as the depth of cigarette smoke inhalation, are difficult to capture via self-report.¹²

The World Health Organization and the International Union Against Tuberculosis and Lung Disease have jointly released recommendations for integrated efforts to combat the dual threat of smoking and TB.¹⁴ However, improved understanding of the relationship between smoking and TB is needed to guide the development of specific interventions. The objective of the present study is to describe the association between salivary cotinine levels and *Mtb* infection determined by an interferon-gamma (IFN- γ) release assay (IGRA). We hypothesized that salivary cotinine levels consistent with smoking would be associated with increased prevalence of *Mtb* infection compared to salivary cotinine levels indicating non-smoking.

STUDY POPULATION AND METHODS

Study population

We conducted a cross-sectional study among persons enrolled in *Proyecto El Cuete*, a longitudinal cohort study of HIV risk behavior among persons who inject drugs in Tijuana, Baja California, Mexico.¹⁵ Previous studies have found high prevalence of smoking (97%) and *Mtb* infection (67%) in this population.^{16,17} Given that substance use has been shown to be an important driver of TB outbreaks, improved understanding of *Mtb* infection among persons who use illicit drugs is critical for global TB prevention efforts.^{18,19} Study participants were recruited through word-of-mouth and community-based outreach. Eligible participants were 18 year-old Tijuana residents who had injected drugs during the month prior to study enrollment. Study interviewers administered a detailed questionnaire that included questions on demographics and cigarette smoking at baseline and every six months for 30 months. Study participants were also administered a rapid HIV antibody test (Advanced Quality Rapid Anti-HIV 1&2 Test; In Tec Products, Inc; Xiamen, China) at each visit. A subset of 279 participants presenting for their 12 month visit was consecutively enrolled to participate in the present study of smoking and *Mtb* infection. We obtained written informed consent from all participants and offered an additional US\$5 for participation in this sub-study. The study protocol and instruments were approved by the University of California San Diego Human Research Protection Program and the Tijuana General Hospital Ethics Committee.

Measures

Self-reported smoking status was obtained by asking, “Have you smoked a cigarette during the previous six months?” Participants were also asked about the number of cigarettes smoked per day and the age at smoking initiation.

We used NicAlert (Nymox Pharmaceutical Corporation; Hasbrouck Heights, NJ), a rapid semi-quantitative dipstick assay, to measure salivary cotinine levels. Salivary NicAlert has been shown to have a sensitivity of 95%-99% and specificity of 93%-96% for determining smoking status.^{20,21} NicAlert involves collecting participant’s saliva in a collection tube

and depositing eight drops on an immunochromatographic strip containing gold particles coated with monoclonal antibodies to measure cotinine concentrations.^{20,21} Results range from cotinine levels 0 to 6, with higher values corresponding to higher cotinine concentrations (Figure 1). Given that the standard salivary cotinine concentration cutoff for determining smoking status is 15 ng/ml, a NicAlert cotinine level of 0 (cotinine concentration <10 ng/ml), 1 (cotinine concentration 10–30 ng/ml), and 2–6 (cotinine concentration >30 ng/ml) indicate nonsmoking, secondhand smoke exposure or low-level smoking, and regular smoking, respectively.¹¹

We used QuantiFERON TB Gold In-tube ([QFT] Cellestis; Victoria, Australia) IGRA to determine *Mtb* infection.²² IFN- γ concentrations >10 IU/ml were set as 10 IU/ml to account for the high level of imprecision in the >10 IU/ml range. IFN- γ concentrations above the cutoff of 0.35 IU/mL were considered positive for QFT.

Statistical analysis

We used the Pearson's χ^2 test or Fisher's exact test to compare the distribution of categorical variables and the Kruskal-Wallis test for continuous variables between cotinine categories and to determine their associations with QFT status. We assessed agreement between self-reported smoking and cotinine levels dichotomized at cutoffs of 1 and 2 by calculating Cohen's κ . The Cochran-Armitage test was used to evaluate trends in the prevalence of QFT positivity across ordinal variables and to determine whether a dose-response association exists between cotinine levels (0 to 6) and QFT positivity. Cotinine levels 5 and 6 were combined in dose-response analysis due to the small number of participants with cotinine level 6 ($n = 5$).

Log-binomial regression models were constructed to determine correlates of QFT positivity and to derive prevalence ratios (PRs).^{23,24} Cotinine levels were included in separate models as a categorical variable (cotinine levels 2–6 and 1 vs. cotinine levels 0) and an ordinal variable (cotinine levels 0 to 6). We also constructed bivariate log-binomial models to evaluate the association between QFT positivity and the following possible confounders: sex, age, education, unstable housing, average monthly income, and HIV infection. Factors associated with QFT positivity with $P < 0.10$ in bivariate models were considered for inclusion with cotinine level variables in the final multivariable log-binomial models. Only statistically significant covariates with $P < 0.05$ were retained with cotinine variables in the final models. SAS 9.3 (Cary, North Carolina) was used for all analyses.

RESULTS

Of the 279 enrolled participants, we excluded participants with invalid NicAlert results ($n = 20$), QFT specimen collection or handling error ($n = 24$), and indeterminate QFT result ($n = 1$) from the analysis. Of the 234 (83.9%) participants included in this analysis, 19 (8.1%), 28 (12.0%), and 187 (79.9%) had NicAlert cotinine levels 0, 1, and 2–6, respectively (Figure 1). Participants were more likely to be male (57.7%); median age was 38 years (interquartile range [IQR], 31 – 45 years); 79 (33.8%) attained primary school or lower level of education; 34 (14.5%) reported having unstable housing; and 121 (51.7%) reported an average monthly income of less than 2500 pesos (USD \$200) (Table 1). Five (2.7%) participants tested positive for HIV. No statistically significant differences were observed in demographic characteristics between participants with cotinine levels 0, 1, and 2–6 (Table 1).

Overall, 213 (91.0%) participants reported smoking cigarettes during the previous six months (Table 1). Participants smoked a median of 10 cigarettes per day (IQR, 5 – 20 cigarettes), and had smoked for a median of 24 years (IQR, 16 – 30 years). The prevalence of self-reported cigarette smoking during the previous six months for participants with

cotinine levels 0, 1, and 2–6 were 63.2%, 57.1%, and 98.9%, respectively ($P < 0.001$). Of the 21 participants who reported no cigarette-smoking during the previous six months, 19 (90.5%) had cotinine levels 0 or 1. Among participants who reported smoking cigarettes during the past six months, 185 (86.9%) had cotinine levels between 2 and 6. A higher level of agreement between NicAlert scores and self-reported smoking was observed when using a cotinine level cutoff of 2 ($\kappa = 0.496$) compared to a cutoff of 1 ($\kappa = 0.289$). Participants with cotinine levels 0 and 1 reported smoking fewer cigarettes per day compared to participants with cotinine levels 2–6 (medians of 3 and 1 vs. 10, respectively; $P < 0.001$) (Table 1).

The prevalence of QFT positivity was 61.1% and higher among male participants compared to female participants (67.4% vs. 52.5%; $P = 0.021$) (Table 2). No other demographic or self-reported smoking-related factors were found to be associated with QFT positivity. The prevalence of QFT positivity for cotinine level categories 0, 1, and 2–6 were 42.1%, 46.4%, and 65.2%, respectively ($P_{\text{trend}} = 0.012$). We also found an increasing trend in QFT positivity across cotinine levels 0 to 5/6 (from 42.1% to 65.8%; $P_{\text{trend}} = 0.003$) (Figure 2). The median IFN- γ concentration was 1.40 IU/ml (IQR, 0.04 – 10.0 IU/ml) and increased across cotinine levels 0 to 5/6 (from 0.28 IU/ml to 3.99 IU/ml; Spearman's $r = 0.200$; $P = 0.002$) (Figure 3).

In bivariate log-binomial analysis, sex and education level were found to be associated with QFT positivity with $P < 0.10$. In the bivariate model with cotinine levels included as a categorical variable, the prevalence of QFT positivity was higher for participants with cotinine levels 2–6 (PR = 1.55; 95% confidence interval [CI] = 0.91 – 2.65) or 1 (PR = 1.10; 95% CI = 0.57 – 2.13) compared to that among participants with cotinine level 0, but these differences did not reach statistical significance (Table 3). In the final multivariable model containing education and cotinine categories, only education was found to be independently associated with QFT positivity (Table 3).

In bivariate log-binomial analysis with cotinine levels included as an ordinal variable, cotinine levels were associated with increased prevalence of QFT positivity (PR = 1.09 per increase of 1 cotinine level; 95% CI = 1.02 – 1.16) (Table 3). In the final model including education and the ordinal cotinine variable, lower levels of educational attainment and increased cotinine levels were independently associated with QFT positivity (Table 3).

DISCUSSION

We found evidence of a dose-response relationship between salivary cotinine levels (0 to 6) and QFT positivity and a positive correlation between salivary cotinine and IFN- γ concentrations. However, we found insufficient evidence of an association between salivary cotinine levels indicative of cigarette smoking status (NicAlert cotinine levels 1 and 2–6 vs. 0) and QFT positivity. The lack of association observed between QFT positivity and cotinine-based smoking categories might be due to the relatively small sample size of participants with cotinine levels 0 ($n = 19$) and 1 ($n = 28$). Furthermore, we analyzed cotinine levels 0 and 1 as separate categories in the primary analysis because NicAlert cotinine level 1 is indicative of cotinine concentration 10–30 ng/ml and, therefore, could include smokers and non-smokers based on the standard smoking cutoff of 15 ng/ml.¹¹ When cotinine levels 0–1 and 2–6 were compared in post-hoc analysis, we found a statistically significant difference in the prevalence of QFT positivity between the two categories (data not shown).

We are aware of only one other study exploring the association between biochemical measures of smoking status and IGRA results. That study found no evidence of an

association between serum cotinine results and IGRA positivity, and the paper did not report whether a dose-response analysis was performed.⁹ The study population consisted of patients seeking care for respiratory symptoms consistent with TB, and the prevalence of IGRA positivity for the 108 patients without TB disease was uniformly high for smokers and nonsmokers (~80%).⁹ Therefore, that study might have been insufficiently powered to detect a difference in IGRA positivity between smokers and nonsmokers in such a high TB prevalent setting.

Previous studies examining the association between self-reported smoking and IFN- γ release assay results have reported mixed results.^{16,25–28} Notably, two recent studies reported a negative association between cigarette smoking and IGRA positivity.^{27,28} The authors of these studies speculated that impaired T cell response due to smoking might have compromised the performance of the IFN- γ release assay. However, since these studies were conducted among persons with HIV-infection or active TB disease, their findings might only apply to persons who are already immune-compromised. Our findings among a community sample of mostly HIV-uninfected participants suggest that cigarette smoking does not impair T cell function to the extent that IFN- γ release assay results are negatively affected among immune-competent persons. Additional research is needed to determine the reasons for these apparently contradictory findings.

We found relatively low agreement between salivary cotinine levels and self-reported smoking. This might be due in part to the differences in reference periods; our cigarette smoking questions asked about smoking behavior during the previous six months while salivary cotinine corresponds to smoking that occurred within the past seven days. Only two participants who reported no cigarette smoking had high levels of cotinine, suggesting that our findings were not significantly affected by social desirability bias. Contrary to previous studies, a NicAlert cutoff of 2 resulted in higher level of agreement with self-reported smoking compared to a cutoff of 1.^{20,21} This could be due to the higher level of secondhand smoke exposure among our study population, many of whom live and congregate in poorly-ventilated housing. Furthermore, given the high prevalence of smoking in this population, secondhand exposure in social settings is likely to be common for nonsmokers. Thus, nonsmokers in our study might have had elevated salivary cotinine levels, resulting in a higher cutoff needed for optimal discrimination of smokers and non-smokers. However, we did not ask participants regarding secondhand smoke exposure and, therefore, were unable to further explore this possible explanation. Salivary cotinine levels were a stronger predictor for QFT positivity compared to self-reported smoking and smoking intensity (number of cigarettes smoked daily). The relatively low precision of self-reported measures for smoking might have contributed to the mixed findings among previous studies of dose-response relationship between self-reported smoking intensity and the risk of *Mtb* infection.^{16,29–31}

Our findings should be interpreted with consideration of the following limitations. First, given that our study population was composed entirely of persons who inject drugs, our findings might not be generalizable. However, the prevalence ratio of 1.6 that we found for *Mtb* infection between smoking and non-smoking cotinine categories was similar to previously-reported effect sizes, which suggests that our findings are generalizable to other populations.⁴ For example, Slama et al. reported a pooled odds ratio of 1.8 for smoking and *Mtb* infection in their systematic review.⁴ As with previous studies on this topic, we employed a cross-sectional study design and, therefore, were unable to determine the temporality between cigarette smoke exposure and *Mtb* infection. Given that salivary cotinine results are indicative of current cigarette smoke exposure whereas QFT results correspond to lifetime history of infection, participants infected with *Mtb* as a child and who later started smoking cigarettes would contribute to a positive association between smoking

and *Mtb* infection in this study. Longitudinal studies should be conducted among persons at high risk for *Mtb* infection to provide more robust evidence for the relationship between cigarette smoking and *Mtb* infection.

High quality longitudinal studies provide strong evidence that smoking increases the risk for active TB disease.^{4,32,33} Findings from our study suggest that cigarette smoke exposure might also increase the risk for *Mtb* infection. However, given that no longitudinal studies have yet been conducted to determine whether cigarette smoking is a risk factor for *Mtb* infection, demonstrated this association,, the evidence for a causal relationship between cigarette smoking and *Mtb* infection remains weak. Despite this gap in knowledge, sufficient evidence exists to support the integration of smoking and TB prevention activities, and such integrated efforts are urgently needed to accelerate the progress towards achieving global elimination of TB.^{1,34}

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Figure 1.
Distribution of NicAlert salivary cotinine levels among persons who inject drugs; Tijuana, Mexico, 2012–2013.

Figure 2. QFT positivity by NicAlert salivary cotinine levels among persons who inject drugs; Tijuana, Mexico, 2012–2013

The grey bar represents the prevalence of QFT positivity at each NicAlert level. The dotted line represents predicted prevalence of QFT positivity at each NicAlert level based on the bivariate log-binomial model. Note: QFT = QuantiFERON TB Gold In-tube

Figure 3. Median interferon-gamma concentrations by NicAlert salivary cotinine levels among persons who inject drugs; Tijuana, Mexico, 2012–2013

The circle represents the median IFN- concentration and the vertical line represents the interquartile range. An increasing trend in IFN- concentrations was observed across cotinine levels (Spearman's $r=0.002$; $P=0.002$).

Table 1
Demographic and behavioral characteristics among persons who inject drugs; Tijuana, Mexico, 2012–2013

Characteristic	All Participants N=234 n (%)	Cotinine Categories (Concentration)			P value
		0 (0–10 ng/mL) N=19 n (%)	1 (10–30 ng/mL) N=28 n (%)	2–6 (>30 ng/mL) N=187 n (%)	
Sex					0.208 ^a
Male	135 (57.7)	8 (42.1)	14 (50.0)	113 (60.4)	
Female	99 (42.3)	11 (57.9)	14 (50.0)	74 (39.6)	
Age					0.790 ^c
Median (IQR)	38 (31–45)	35 (31–45)	40 (33–46)	38 (31–45)	
Education level					0.028 ^a
Up to Primary School	79 (33.8)	7 (36.8)	7 (25.0)	65 (34.8)	
Secondary School	96 (41.0)	9 (47.4)	7 (25.0)	80 (42.8)	
Prep School or higher	59 (25.2)	3 (15.8)	14 (50.0)	42 (22.5)	
Unstable housing ^d					0.547 ^b
No	200 (85.5)	17 (89.5)	22 (78.6)	161 (86.1)	
Yes	34 (14.5)	2 (10.5)	6 (21.4)	26 (13.9)	
Average monthly income (pesos)					0.436 ^b
<1500	90 (38.5)	7 (36.8)	9 (32.1)	74 (39.6)	
1500–2499	31 (13.2)	3 (15.8)	6 (21.4)	22 (11.8)	
2500–3499	45 (19.2)	1 (5.3)	6 (21.4)	38 (20.3)	
3500	68 (29.1)	8 (42.1)	7 (25.0)	53 (28.3)	
HIV infection					1.000 ^b
No	229 (97.9)	19 (100)	28 (100)	182 (97.3)	
Yes	5 (2.1)	0 (0)	0 (0)	5 (2.7)	
Self-reported cigarette smoking, past 6 months					<0.001 ^b
No	21 (9.0)	7 (36.8)	12 (42.9)	2 (1.1)	
Yes	213 (91.0)	12 (63.2)	16 (57.1)	185 (98.9)	
Number of cigarettes ^e smoked per day					<0.001 ^c
Median (IQR)	10.0 (4.0–15.0)	3.0 (0.0–10.0)	1.0 (0.0–10.0)	10.0 (5.0–20.0)	
Years of cigarette ^f smoking					0.009 ^c

Characteristic	Cotinine Categories (Concentration)				P value
	All Participants N=234 n (%)	0 (0–10 ng/mL) N=19 n (%)	1 (10–30 ng/mL) N=28 n (%)	2–6 (>30 ng/mL) N=187 n (%)	
Median (IQR)	24.0 (14.0 – 30.0)	15.0 (0.0 – 25.0)	18.5 (0.0 – 30.0)	24.0 (16.0 – 30.0)	

^aPearson's χ^2 test

^bFisher's exact test

^cKruskal-Wallis test

^dParticipants were categorized as having unstable housing if they had slept in a car, abandoned building, shelter, or on the street during the past six months.

^eParticipants who did not smoke during the past six months were included as having smoked 0 cigarettes per day.

^fParticipants who had never smoked were included as having smoked for 0 years.

Table 2

Bivariate analysis of factors associated with QFT positivity among persons who inject drugs; Tijuana, Mexico, 2012–2013.

Characteristic	N	QFT positive	
		n (%)	P value
All Participants	234	143 (61.1)	
Sex			0.021 ^a
Male	135	91 (67.4)	
Female	99	52 (52.5)	
Age			0.722 ^c
<30	41	25 (61.0)	
30–39	92	55 (59.8)	
40–49	75	46 (61.3)	
50	26	17 (65.4)	
Education level			0.060 ^c
Up to Primary School	79	52 (65.8)	
Secondary School	96	63 (65.6)	
Prep School or higher	59	28 (47.5)	
Unstable housing ^d			0.933 ^a
No	200	122 (61.0)	
Yes	34	21 (61.8)	
Average monthly income (pesos)			0.758 ^c
<1500	90	55 (61.1)	
1500–2499	31	15 (48.4)	
2500–3499	45	32 (71.1)	
3500	68	41 (60.3)	
Self-reported cigarette smoking, past 6 months			0.815 ^b
No	21	12 (57.1)	
Yes	213	131 (61.5)	
Number of cigarettes smoked per day			0.361 ^c
<6	84	53 (63.1)	
6–9	29	19 (65.5)	
10–19	67	41 (61.2)	
20	54	30 (55.6)	
Years of cigarette smoking			0.162 ^c
1) 0 – 9	40	18 (45.0)	
2) 10 – 19	52	36 (69.2)	
3) 20 – 29	77	47 (61.0)	
4) 30	65	42 (64.6)	
Cotinine categories (equivalent concentration)			0.012 ^c
0 (0–10 ng/mL)	19	8 (42.1)	

Characteristic	QFT positive		
	N	n (%)	P value
1 (10–30 ng/mL)	28	13 (46.4)	
2–6 (>30 ng/mL)	187	122 (65.2)	

Note: QFT = QuantiFERON TB Gold In-tube

^aPearson's χ^2 test

^bFisher's exact test

^cCochran-Armitage test for trend

^dParticipants were categorized as having unstable housing if they had slept in a car, abandoned building, shelter, or on the street during the past six months.

Table 3

Factors associated with QFT positivity in log-binomial regression models among persons who inject drugs; Tijuana, Mexico, 2012–2013.

Variable		Bivariate PR (95% CI)	Final PR (95% CI)
Categorical Models			
Cotinine Level (Categorical)	0	1.00	1.00
	1	1.10 (0.57, 2.13)	1.23 (0.64, 2.38)
	2–6	1.55 (0.91, 2.65)	1.58 (0.92, 2.69)
Education	Prep school or higher		1.00
	Secondary school or lower		1.35 (1.01, 1.80)
Dose-Response Models			
Cotinine Level (Ordinal)	+1	1.09 (1.02, 1.16)	1.09 (1.02, 1.16)
Education	Prep school or higher		1.00
	Secondary school or lower		1.38 (1.04, 1.83)

Note: PR = Prevalence ratio; QFT = QuantiFERON TB Gold In-tube