Correlation of ambient pollution levels and heavily-trafficked roadway proximity on the prevalence of smear-positive tuberculosis

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SUMMARY

Objectives: Varying levels of evidence exist for the contribution of indoor air pollution and environmental tobacco smoke as a risk factor for tuberculosis (TB). Despite a similar mechanism of action, the influence of outdoor air pollution exposure as an independent contributor to TB disease has yet to be explored. This area of inquiry is of increasing importance given the level of pollution in the rising economies of many TB-endemic nations. Los Angeles' unique physical environs and traffic patterns mirror other global megacities with a greater burden of TB therefore allowing for preliminary correlative studies. This preliminary study hypothesizes that individuals who reside proximal to elevated pollutant exposures are likely to have a greater burden of disease – as evidenced by sputum smear-positive TB.

Study design: Retrospective medical records review.

Methods: Medical records of non-homeless individuals (n = 196) diagnosed with culture positive TB at Los Angeles County and University of Southern California Medical Center Hospital were analyzed from January 2007 to December 2008. The study population was grouped according to acid-fast bacilli (AFB) smear-positive (n = 111) and smear-negative (n = 85) status. Air pollutant exposure was captured using measurements of ozone (O₃) and particulate matter with an aerodynamic diameter of less than 2.5 (PM₂.₅). Individual assignment to O₃ and PM₂.₅ exposures were based on residential proximity to the nearest US Environmental Protection Agency's monitoring station. Proximity of home residences to traffic-related pollutants occurred by measurement of distance to the nearest freeway and major non-freeway road.

Results: Single factorial models yielded a significant correlation of smear-positive status and residential exposure to PM₂.₅. Residential distance to freeways and major arterial roads did not yield an association.

Conclusions: This is the first report linking ambient pollution exposure as a risk factor for TB. PM₂.₅ may have the potential to impact TB lung pathology as evidenced by the linkage of fine particulate matter levels and smear-positive TB.
**Introduction**

Although relatively efficacious treatment for tuberculosis (TB) has existed for decades, the disease continues to remain as one of the leading causes of mortality attributed to an infectious disease. Globally, approximately 1 million deaths among HIV-negative and 0.35 million deaths among HIV-positive persons were related to TB in 2010. The literature is profuse with articles exploring the socio-clinical determinants of TB disease outcomes but comparatively few exist that explore environmental influences. Increasing epidemiologic evidence exists that environmental tobacco smoke (ETS) is an independent risk factor for TB infection, progression of primary TB and development of cavitary disease.5 A select number of studies have also shown a significant link between TB prevalence and indoor air pollution from combustion of solid fuels. However, ambient air pollution from outdoor sources has yet to be studied in TB epidemiologic research despite a similar mechanism of disease to ETS and biofuel combustion. This subject matter is of great import given the rise in outdoor pollution in increasingly industrializing, TB-endemic nations such as India and China.

A major source of outdoor air pollution is from roadway traffic; whose global prevalence is increasing due to the increased availability of affordable automobile options. The combustion of fuel for automobiles has been linked as a key contributor to particulate matter and gaseous pollutant exposures in areas located proximal to highly trafficked roads. Moreover, an increasing amount of literature has noted the link between residential proximity to roadways with acute respiratory infections (e.g. Influenza, Streptococcus pneumoniae). These works, much like the current study, have several patient-specific factors that may influence the results of environmental linkage studies. TB is a disease whose prevalence has been significantly associated with socio-economic risk factors, that are more likely to be seen in urban settings where highly concentrated levels of air pollution exist. These risk factors may include residence in crowded home environments, contact with recent immigrants from TB-endemic nations, exposure to cigarette smoke, and engagement in high risk behaviour patterns. Moreover, the host genotype may influence TB disease based on gene activation patterns from ambient pollutant generated oxidative stressors.

Southern California is a unique environment to study the effects of ambient pollutants on TB outcomes given the physical landscape, higher traffic-based patterns and prevalence of disease relative to other US urban settings. The Los Angeles (LA) metropolitan area and its surrounding locales represent a diverse geographic patient population with varying exposure levels. The interface between rural agricultural and urban landscapes also generates a unique study environment; due to such varying sources as farming modalities or proximity to heavily-trafficked roads. Moreover, the increasing LA urban sprawl, along with its secondary population-derived byproducts of technologies (e.g. air conditioning), produces even greater combustion products via photochemical gases and airborne particles.

This 2 year retrospective study explores the association of smear-positive TB to select ambient pollutants among a cohort of patients at a major metropolitan hospital in Los Angeles. Particulate matter less than 2.5 μm in diameter (PM2.5) and ozone (O3) levels were chosen as correlative variables due to their well-documented influence on lung immunology via the production of oxidative and nitrosative stressors. Both pollutants have been shown in multiple models to impair alveolar macrophage activity and mucociliary clearance mechanisms that are critical in the innate defense against Mycobacteria tuberculosis (M. tb). Individual pollutant exposure was spatially assigned based on residential proximity to the nearest US Environmental Protection Agency (EPA) pollutant monitoring station. Traffic-based exposures were measured in proxy by spatially correlating home residences to select roadways. Higher levels of residential pollutant exposures, an indirect marker of individual pollutant exposure, diminish host M. tb immune defenses and increase bacillary load – as evidenced by smear-positive TB, were hypothesized. The ultimate aim of this preliminary analysis is to contribute to the literature of environmental determinants on pulmonary TB disease and to serve as the basis for future work that will explore the epidemiologic, genotypic and immunologic mechanisms underlying this potential risk factor.

**Methods**

**Study population**

Patients with culture-proven tuberculosis evaluated at the Los Angeles County and University of Southern California Medical Center (LAC + USC Medical Center) from January 2007 through December 2008 were identified. The Mycobacteriology laboratory data and medical records of the patients were reviewed. Non-homeless individuals (n = 196) with culture positive M. tb were included in the analysis. Demographic variables, including ethnicity, were self-identified on hospital intake forms and listed in the hospital electronic record. Age was calculated from date of birth to the time of confirmation of culture positive TB status. Insurance status was captured but not displayed since all patients had a public insurance carrier provided by state or federal-based sources. Other payment modalities often implied no insurance carrier or hospital-based financial support mechanisms. Patients with no listed address or described as being ‘homeless’ in the address section of their hospital intake forms were excluded from the study. The study was approved the Health Sciences Institutional Review Board (HSIRB) of the University of Southern California. As per 45 CFR 46.116, HSIRB waived written consent based on fulfilment of criteria that the study minimized risk to subjects, lessened any impact on the welfare of the subjects, deidentified all participants and documented the impracticality of locating patients given the historical nature of the research.

**Microbiology**

Acid-fast bacilli (AFB) smears were performed on all samples sent for mycobacterial cultures. Specimens for culture derived from induced or expectorated sputum, pleural fluid, blood, lymph node and gastric fluid. A positive AFB stain involved detectable bacilli on both auramine-rhodamine and Ziehl–Neelsen stains (Becton, Dickinson and Company, New York, NY).
Jersey, USA). All specimens are cultured on both Lowenstein-Jensen Medium Slants and Mycobacteria Growth Indicator Tubes (Becton, Dickinson and Company, New Jersey, USA). HIV antibody screening was performed on the ADVIA Centaur® CP Immunoassay System (Siemens Healthcare Diagnostics, Illinois, USA). Viral load was determined by HIV-1 RNA Ampliprep-Taqman real time PCR assay (Roche Molecular Diagnostics, New Jersey, USA). CD4 counts were performed by flow cytometry and the absolute number CD4 cells were determined by multiplying the appropriate percentages by the absolute number of lymphocytes, as determined on a complete blood count.

Exposure data

Individual pollution exposure was based on the AirData EPA online database of Southern California air pollutant concentration levels. Maximal annual 1 h O₃ and annual mean PM₂.₅ values were averaged over the 2-year interval. Monitors that did not capture annual data from January 2007 to December 2008 were excluded. Values were spatially correlated to the EPA monitoring station's coordinates. Individual assignment to O₃ and PM₂.₅ exposures were based on residential proximity to the nearest EPA monitoring station. Residences were determined based on demographic intake forms obtained from LAC + USC Medical Center's medical records and geocoded using non-parsed postal addresses. Residential and monitoring station mappings were based on World Geodetic System (WDS) 1984 coordinates. Spatial mapping and geometric measurements were analyzed in ArcGIS version 10.0 software (Environmental Systems Research Institute Inc, Redlands, CA, USA).

Exposure of the study population to traffic-related pollutants occurred by two measurements – proximity to the nearest freeway and nearest major non-freeway road. A freeway was defined as a US state or interstate highway. A major road was defined as a major arterial route. Estimates of residence to each roadway category were based on the shortest distance from home residence to the proximal side of a major road and freeway.

Statistical analyses

Logistic regression was used to calculate the association of AFB smear-positive TB with residential air pollutants and traffic exposures. The derived odds ratios were corrected for age, ethnicity, year of TB diagnosis, type of insurance and HIV status. HIV status was corrected for based on the established correlation of the infection with TB pathology.¹

Adjustment for confounding was determined by change in significance level after introduction of each covariate into the single and polynomial pollutant model analyses. Fitted multivariate logistic models were used to predict the additive value of each covariate on smear-positive TB status and used a smoothing spline with 2 degrees of freedom. Significance was defined as two-sided P < 0.05 for all analyses. Statistical procedures were performed in JMP® version 9.0.0 (SAS Institute Inc, NC, USA).

Results

Geo-demographic and clinical characteristics of the study population

The study population was subdivided into AFB smear positive (n = 111) and smear negative (n = 85) (Table 1). Each grouping derived from LA County Hospital’s patient catchment area that predominantly serves individuals in the LA Metropolitan region. The largest portion of the population resided near downtown, Eastern and Southern LA areas with diverse ethnic enclaves. The majority of patients had identified themselves as Hispanic with Asians represented the second most common minority population. Greater than 75% of each grouping was over 21 years of age. All individuals had either public or subsidized hospital-based payment mechanisms. The majority presented with pulmonary symptoms that led to over 80% of specimens deriving from sputum or pleural fluid sources. Of those with AFB-positive results, the largest amount derived

<table>
<thead>
<tr>
<th>Variable</th>
<th>AFB negative (n = 85)</th>
<th>AFB positive (n = 111)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>5 (6)</td>
<td>7 (6)</td>
</tr>
<tr>
<td>21–40</td>
<td>30 (35)</td>
<td>45 (41)</td>
</tr>
<tr>
<td>41–60</td>
<td>33 (39)</td>
<td>44 (40)</td>
</tr>
<tr>
<td>&gt;60</td>
<td>17 (20)</td>
<td>15 (13)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>20 (25)</td>
<td>21 (19)</td>
</tr>
<tr>
<td>Black</td>
<td>7 (8)</td>
<td>10 (9)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>53 (62)</td>
<td>73 (66)</td>
</tr>
<tr>
<td>White</td>
<td>3 (3)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Other</td>
<td>2 (2)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Year of TB diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2007</td>
<td>43 (50)</td>
<td>56 (50)</td>
</tr>
<tr>
<td>2008</td>
<td>42 (50)</td>
<td>55 (50)</td>
</tr>
<tr>
<td>Microbiologic TB source</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sputum</td>
<td>65 (76)</td>
<td>100 (90)</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>3 (4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Blood</td>
<td>3 (4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Lymph node</td>
<td>7 (8)</td>
<td>7 (6)</td>
</tr>
<tr>
<td>Gastric fluid</td>
<td>7 (8)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>HIV&lt;sup&gt;+&lt;/sup&gt; positive</td>
<td>13 (15)</td>
<td>18 (16)</td>
</tr>
<tr>
<td>CD4 count (cells/mm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>10</td>
<td>16</td>
</tr>
<tr>
<td>&gt;200</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Viral load&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undetectable</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Detectable</td>
<td>11</td>
<td>17</td>
</tr>
<tr>
<td>Negative</td>
<td>72 (85)</td>
<td>93 (84)</td>
</tr>
</tbody>
</table>

¹ Age was determined at the time of confirmation of *M. tb.* from mycobacterial culture.

² Ethnicity was based on classification criteria listed in the electronic patient records at LAC + USC Medical Center.

³ Source of TB cultures and HIV data were ascertained from the medical records in the Mycobacterial Laboratory at LAC + USC Medical Center.

⁴ Viral load was considered undetectable with less than 48 copies of HIV/mL.
from sputum specimens. Approximately 15% of each subgroup was HIV positive with a low CD4 count (<200 cell/mm³) and a detectable viral load.

**Correlation of smear-positive status with residential proximity to highly trafficked roads**

Measures of central tendency indicate that both AFB status subgroups are located closer to a major road given the higher likelihood of residing to more prevalent major arterial roads (Table 2). The 25–75% interquartile ranges for both roadways indicate a high variability in both groups. The range is smaller among the major roadways given their greater frequency of distribution. Individual proximity to both freeways and major roads are not significantly correlated with AFB-positive status (Table 3). Odds ratios were corrected for age, ethnicity, insurance, year of TB diagnosis, insurance, HIV status and HIV biomarkers (CD4 and viral load). Odds were based on the assumption that residential proximity to roadways was associated with AFB-positive smears.

**Correlation of smear-positive status with residential proximity to elevated air pollutant levels**

EPA monitoring stations were more densely located in the LA metropolitan area – as opposed to other locations within LA County. Levels of PM2.5 and O3 exceeded stricter California EPA standards in both subgroups (Table 2). Federal standards were minimally surpassed among the AFB negative population. Measures of central tendency indicated higher PM2.5 and O3 levels among those with smear-positive TB. Significant correlation was noted in single pollutant models analyzing PM2.5 levels and smear-positive TB (OR = 25.3, 95% CI = 3.38–29.1, P = 0.0044) (Table 3). O3 levels could not be significantly correlated with AFB-positive smears (OR = 1.56, 95% CI = 0.49–1.56, P = 0.4486). Polynomial logistic regression analyses yielded solely one significant model that incorporated both O3 and PM2.5 values (OR = 4.72, 95% CI = 4.26–61.22, P = 0.0297). All single and multiple pollutant models were adjusted for demographic and clinical criteria listed in Table 1.

**Discussion**

This paper serves as the first investigation linking ambient pollution exposures with increased TB bacillary load — as evidenced by smear-positive TB. LA served as a unique environment to conduct this study given its varied sociodemographic population, higher traffic-based patterns and the proximal interface between rural agricultural and urban landscapes. Two criteria air pollutants that have been extensively associated with lower airway disease and lung inflammation (PM2.5 and O3) were compared. Proximity to highly-trafficked roadways was used as a proxy for gaseous and particulate matter that arises from vehicular exhaust. Culture positive TB disease was primarily from pulmonary sources and 90% of all AFB-positive specimens were derived from sputum samples. The study revealed that PM2.5 levels were significantly correlated with higher odds of smear-positive TB. The smaller sized aerodynamic particles could directly impact alveolar and distal airway generation epithelial surfaces serving as the primary defense against pulmonary TB. However, no single pollutant models could spatially predict a linkage among O3 levels and highly trafficked roadways to home residences. Polynomial analyses did detect a significant association of smear-positive TB using both PM2.5 and O3 levels.

**Table 2 — Roadway distance and air pollution values by AFB status.**

<table>
<thead>
<tr>
<th>Exposure Category</th>
<th>AFB positive status</th>
<th>AFB negative status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Median (IQR)</td>
</tr>
<tr>
<td>Freeway distance from home residence (m)</td>
<td>1118 ± 882</td>
<td>884 (417–1538)</td>
</tr>
<tr>
<td>Major road distance from home residence (m)</td>
<td>225 ± 284</td>
<td>164 (69–290)</td>
</tr>
<tr>
<td>PM2.5 (μg/m³)a,b</td>
<td>15.55 ± 1.46</td>
<td>15.53 (13.75–16.86)</td>
</tr>
<tr>
<td>Ozone (ppm)c</td>
<td>0.114 ± 0.017</td>
<td>0.111 (0.108–0.115)</td>
</tr>
</tbody>
</table>

a PM2.5 and ozone values were derived from averaging annual mean levels and maximum 1-hour values, respectively. The most proximal pollution monitoring station to a study participant’s home address is used to define individual levels of exposure.

b Federal and California EPA standards for maximum average annual PM2.5 levels are 15.0 μg/m³ and 12.0 μg/m³, respectively.

c Federal and California EPA standards for 1-hour maximum ozone annual levels are 0.12 ppm and 0.075 ppm, respectively.

**Table 3 — Single model association of smear-positive status with residential distance to roadways and air pollutant exposures.**

<table>
<thead>
<tr>
<th>Exposure category</th>
<th>AFB positive OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freeway distance from home residence (m)</td>
<td>0.77 (0.16–3.60)</td>
<td>0.7440</td>
</tr>
<tr>
<td>Major road distance from home residence (m)</td>
<td>1.99 (0.17–24.22)</td>
<td>0.5696</td>
</tr>
<tr>
<td>PM2.5 (μg/m³)</td>
<td>25.5 (3.38–29.1)</td>
<td>0.0044</td>
</tr>
<tr>
<td>Ozone (ppm)</td>
<td>1.56 (0.49–5.05)</td>
<td>0.4486</td>
</tr>
</tbody>
</table>

a All exposure category models were controlled for age, ethnicity, insurance, year of sampling, HIV status and source of AFB samples.
Limited research exists on the association of outdoor air pollution and TB. Tremblay had utilized historical TB data to show that coal combustion associated with industrialization in Canada and the USA could have aggravated the epidemic in Western nations. Using current coal consumption in China, he had also suggested that the endemicity of TB in such a rapidly industrializing economy may contribute to the nation’s current epidemiologic patterns. Cohen and Mehta of the Health Effects Institute, in a commentary to the Lin et al. meta-analysis linking ETS and indoor biofuel pollutants with TB, had speculated that combustion source outdoor urban air pollution may also be associated with TB.

The basis for this ecologic argument may be that TB, similar to other chronic inflammatory diseases, are influenced by air pollutants which generate oxygen and nitrogen free radicals. Palanisamy et al. revealed the byproducts of these radicals were associated with advanced lung lesions in the guinea pig model of TB. Patients infected with TB have also been shown to have decreased antioxidant levels and therefore further stressors may enhance inflammatory-based lung pathology. Moreover, particulate matter derived from industrial and vehicular sources may impact alveolar macrophage phagolysosomal activity and thus impair bacillary containment. Particulate matter-associated transition metals (e.g. iron) may also contribute to an increased bacillary load via pathogen-derived pathways.

Tremblay had suggested in his review article that coal combustion, via carbon monoxide toxicity, may down regulate TNF-α and therefore impair TB granulomata formation. TNF-α is a cytokine that controls mycobacterial infections by influencing chemokine expression and immune cell recruitment during sequestration of bacilli within the granuloma.

There are notable limitations within the study design. A major limitation had been the lack of inclusion of key socioeconomic and demographic variables that affect determinants of TB disease and transmission patterns. Patients with select social and economic factors may be more likely to reside in high areas of urban pollution that contain TB-risk factors (e.g. crowded home environments, recent immigrants from TB-endemic nations, high risk behaviour patterns). Future studies will require mapping socio-economic factors alongside ambient pollution levels to better determine the influence of these possible confounders. Another major issue is the lack of obtainment of active and passive exposure to cigarette smoke. ETS has been associated with advanced lung lesions in the guinea pig model of TB. Patients infected with TB have also been shown to have decreased antioxidant levels and therefore further stressors may enhance inflammatory-based lung pathology. Moreover, particulate matter derived from industrial and vehicular sources may impact alveolar macrophage phagolysosomal activity and thus impair bacillary containment. Particulate matter-associated transition metals (e.g. iron) may also contribute to an increased bacillary load via pathogen-derived pathways.

Increasing evidence exists for the linkage of TB prevalence and select environmental pollutants — specifically, ETS and indoor air pollution from combustion of biofuels. This preliminary study serves as the first inquiry into the correlation of outdoor air pollution on TB clinical outcomes in the modern age of rapid industrialization and automobile-centric cultures. Data indicate that exposure to higher PM2.5 levels is significantly associated with smear-positive TB. Dual pollutant models incorporating PM2.5 and O3 also yield a significant correlation. However, no significant association could be found among the population residing closer to roadways – a proxy for traffic-related pollutants. Future definitive studies will benefit from addressing key socio-economic, demographic, immunologic, and environmental covariates that may serve as potential covariates.

Conclusions

Increasing evidence exists for the linkage of TB prevalence and select environmental pollutants — specifically, ETS and indoor air pollution from combustion of biofuels. This preliminary study serves as the first inquiry into the correlation of outdoor air pollution on TB clinical outcomes in the modern age of rapid industrialization and automobile-centric cultures. Data indicate that exposure to higher PM2.5 levels is significantly associated with smear-positive TB. Dual pollutant models incorporating PM2.5 and O3 also yield a significant correlation. However, no significant association could be found among the population residing closer to roadways – a proxy for traffic-related pollutants. Future definitive studies will benefit from addressing key socio-economic, demographic, immunologic, and environmental covariates that may serve as potential covariates.

Author statements

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**Ethical approval**

The study was approved by the Health Sciences Institutional Review Board (HSIRB) of the University of Southern California. As per 45 CFR 46.116, HSIRB waived written consent based on fulfilment of criteria that the study minimized risk to subjects, lessened any impact on the welfare of the subjects, deidentified all participants and documented the impracticability of locating patients given the historical nature of the research.

**Funding**

None declared.

**Competing interests**

None declared.

**REFERENCES**