Air pollution and its effects on health – Case studies, India

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Abstract

To investigate the health impact of vehicular pollution of Kolkata (former Calcutta), a cross-sectional study was carried out during 2007-2010 among 932 male non-smoking residents of the city and 812-age- and gender-matched rural subjects as control. The urban group included 460 men who were occupationally exposed to vehicular pollution: 56 traffic policemen, 188 street hawkers, 82 auto rickshaw drivers, 78 bus drivers and 56 motor mechanics. Remaining 472 participants from the city were office employees. Compared with control, urban subjects had increased prevalence of respiratory symptoms, asthma, headache and reduced lung function, chronic obstructive pulmonary disease and hypertension. The changes, more prevalent among occupationally exposed subjects, were positively associated with PM₁₀ level in ambient air. The urban subjects also showed increased levels of pro-inflammatory cytokines and chemokines in plasma, cytological changes suggesting inflammation in the airways, altered immunity (depleted number of CD4+ T cells and CD19+ B cells but increased number of CD16+CD56+ NK cells), leukocyte activation in terms of expression of CD11b/CD18 and production of myeloperoxidase and elastase, along with upregulation of platelet P-selectin expression and raised soluble P-selectin in plasma, implying platelet hyperactivity. Urban participants illustrated excess generation of reactive oxygen species concomitant with depletion of superoxide dismutase and total antioxidant status, suggesting oxidative stress. Moreover, they showed increased chromosomal breakage and DNA damage and elevated level of expression of γ-H2AX protein suggests that the damage was at the level of DNA double strands. Despite this, the expressions of DNA damage repair proteins Mre-11 and Ku70 were only modestly up-regulated, implying insufficient DNA repair. Besides, pro-survival signaling by protein kinase B was activated and metaplasia and dysplasia of airway cells were increased suggesting triggering of the carcinogenesis process in the lung. In essence, chronic exposure to vehicular pollution of Kolkata reduces lung function, increases blood pressure, suppresses immunity, and enhances cancer risk in the lung.

Introduction

Air pollution has remained a major health concern in India. In the past decades, several studies highlighted the important contribution of ambient air pollution to excess morbidity and mortality (Schwartz, 2001, Le et al., 2010). In particular,
exposure to particulate air pollution has been found to be associated with increase in hospital admissions for cardiovascular and respiratory disease and mortality in many countries (Samet et al. 2000; Dockery, 2009) including India (Kumar et al., 2010; Balakrishnan et al., 2011; Rajarathnam et al., 2011). Epidemiologic studies also depicted a close link between air pollution and asthma and allergic diseases (Kelly and Fussell, 2011). Health impact of air pollution depends on the pollutant type, its concentration in the air, length of exposure, other pollutants in the air, and individual susceptibility. Poor people, undernourished people, very young and very old, and people with pre-existing respiratory disease and other ill health are more at risk (Vichit-Vadakan et al., 2010). The ambient air of most of the Indian cities contains respirable suspended particulate matter in levels that are above the national ambient air quality standards. The most important contributor to air pollution in the cities is exhausts from petrol- and diesel-fueled vehicles. Millions of people are exposed to this poor quality of air for years. The consequence could be adverse health effects that could be sub-clinical or overt. Despite these, little is known about the health impact of urban air pollution at the cellular and sub-cellular levels among people residing in the Indian mega cities. Against this background, this cross sectional study was undertaken to examine the health impact of air pollution in Kolkata (former Calcutta), a city with high level of air pollution from vehicular traffic.

**Material and methods**

Participants

A cross sectional study was carried out to investigate the health impact of vehicular pollution of Kolkata during 2007-2010. A total number of 932 non-smoking adult males, aged 25-58 years, median age 44 years, who were residents of Kolkata for the past 10 years or more were enrolled as subjects exposed to urban air pollution mostly from vehicular sources. Among the participants, 460 individuals were occupationally exposed to vehicular pollution: 56 were traffic policemen managing road traffic, 188 were road-side hawkers doing business on pavements of busy roads, 82 were drivers of petrol-fueled auto rickshaws, 78 were drivers of diesel-fueled buses, and 56 were workers of motor mechanics working in motor repairing/servicing agencies. Remaining 472 participants were engaged in office jobs within the city.

As control, 812 non-smoking adult males, aged 24-57 years (median age 43 years) were enrolled from 16 villages of Hooghly, Howrah, Burdwan, Nadia and North 24-Parganas district of West Bengal where traffic-related air pollution was comparatively less due to lesser number of motor vehicles (cycle and cycle rickshaws were principal mode of transport) and there was no air polluting industry including brick kilns within 5 km radius. The control subjects were small businessmen, teachers, and handloom workers.

Inclusion and exclusion criteria

Apparently healthy, non-smoker and non-chewer of tobacco, and user of LPG for domestic cooking were enrolled. Persons currently under medication or have a past history of malignancy were excluded. The study protocol was approved by the
Respiratory symptoms, pulmonary function test, COPD

The participants were interviewed with structured, validated questionnaire to get background information about socio-demographic characteristics, prevalence of respiratory symptoms in past three months following respiratory questionnaire of British Medical Research Council (Cotes, 1987) and history of other ailments. PFT was done by spirometry following ATS (1995) criteria. Chronic obstructive pulmonary disease (COPD) was diagnosed following the criteria of Global Initiative for Chronic Obstructive Lung Diseases (GOLD) [Pauwels et al., 2001].

Assessment of cellular lung reaction

Cellular lung response to air pollution was measured by sputum cytology using Papanicolaou staining (Hughes and Dodds, 1968), Perl's Prussian Blue and Non-specific esterase staining, and immunocytochemistry. Changes in blood profile were evaluated by hematology.

Measurement of Hematological and immunological parameters

Relative distribution of immune cells (CD4+ and CD8+ T-cells, , CD19+ B-cells, CD16+CD56+ NK cells, CD4+CD25+ Treg cells) P-selectin (P-sel, CD62P) expression on platelets were measured by flow cytometry (FACS Calibur with sorter, Becton Dickinson, USA) following the procedure of Hu et al. (2000). Platelet aggregation induced by agonists was measured by platelet aggregometer (Chrono-Log, USA), Soluble p-selectin (sP-sel) and by ELISA (soluble P-selectin).

Evaluation of inflammatory response

The levels of pro-inflammatory cytokines C-reactive protein (CRP), interleukin-6 (IL-6), , -12 (IL-12), tumor necrosis factor –alpha (TNF-alpha); chemokine interleukin-8 (IL-8); along with anti-inflammatory cytokines interleukin-10 (IL-10) were measured in plasma by enzyme-linked immunosorbent assay (ELISA). Neutrophil and monocyte activation was measure by assessing surface expression of beta-2 Mac-1 integrin (CD11b/CD18) by flow cytometry.

Hypertension and markers of cardiovascular health

Arterial blood pressure was measured by digital sphygmomanometer. Hypertension was diagnosed if systolic blood pressure rose to 130 mmHg or above and/or diastolic blood pressure rose to90mmHg or above (JNC-7). Molecules which are important for cardio-vascular diseases ( CVD) such as soluble platelet endothelial cell adhesion molecule-1 (sPECAM-1), anti-cardiolipin antibodies IgG and IgM, catecholamines, serotonin, oxidized low density lipoprotein (oxLDL) were measured in plasma by ELISA.
Genotoxicity and cancer risk

Micronuclei (MN) test was done to examine chromosomal breakage (Schmid, 1975), comet assay for DNA damage (Singh et al., 1988), AgNOR expression for ribosome biogenesis, gamma H2AX expression for DNA double strand break, MRN complex and Ku70 for DNA damage repair. Expression of tumor suppressor gene p53 was localized in airway cells by immunocytochemistry.

Measurement of oxidative stress

Air pollution is believed to affect different health parameters by generating reactive oxygen species (ROS) and by reducing antioxidant defense. We measured ROS generation by leukocytes in flow cytometry using DCFH-DA. Antioxidant defense was in erythrocytes and plasma (superoxide dismutase [SOD] and total antioxidant status [TAS] was measured by colorimetric methods. and particulate pollutant levels were measured by aerosol monitor.

Statistical analysis of data

All data are expressed as mean± standard deviation. The collected data were processed and analyzed in EPI info 6.0 and SPSS (Statistical Package for Social Sciences) software. Logistic regression analysis using generalized estimating equations (GEEs) was used to examine the relationship between measured outcome and possible confounders such as RSPM levels. Spearman’s rank test for continuous variables and Chi-square test for categorical variables were done. P<0.05 was considered as significant.

Results

Air quality of Kolkata and rural areas

Air quality data were obtained from West Bengal Pollution Control Board. The annual average PM<sub>10</sub> in ambient air in Kolkata during the study period was 174.8±98.7 µg/m<sup>3</sup> in contrast to 56.6 ± 16.4 µg/m<sup>3</sup> in control (rural) areas (p<0.001). The concentrations of NO<sub>2</sub> and SO<sub>2</sub> in Kolkata’s air during this period were 64.3 ± 22.7µg/m<sup>3</sup> and 7.2 ± 2.5µg/m<sup>3</sup> respectively.

Vehicular pollution increases respiratory symptoms and declines lung function

Residents of Kolkata had greater prevalence of upper respiratory symptoms such as sore throat, sneezing and sinusitis (63.3% vs. 38.0% in control) and lower respiratory symptoms like chronic cough and chest discomfort (62.2% vs. 35.8%). Dyspnea (44.6% vs. 19.5%), physician-diagnosed asthma (4.4% vs. 2.1%), headache (76.3% vs. 36.6%) and eye irritation (18.4% vs. 4.3%) were also more common among city dwellers.
All the measured lung function parameters - forced vital capacity (FVC), forced expiratory volume at one second (FEV$_1$), forced expiratory flow at 25-75% (FEF$_{25-75}$) and peak expiratory flow rate (PEFR) were significantly reduced among urban subjects. Overall, lung function was reduced in 384/932 (41.2%) residents of Kolkata compared with 146/812 (18.0%) of rural controls (Fig. 1; p<0.0001). The prevalence of reduced lung function was more (218/460, 47.4%) in persons occupationally exposed to vehicular pollution compared with those with office jobs (166/470, 35.3%, p<0.001), particularly among traffic policemen and bus drivers (Table 1).

![Figure 1. Prevalence of lung function deficits (A) and chronic obstructive pulmonary disease (COPD) among residents of Kolkata (urban) and rural West Bengal (control). Note significantly increased prevalence (p<0.0001 in Chi-square test) of lung function reduction and COPD among urban subjects chronically exposed to vehicular pollution of the city.](image)

Moreover, 32 (3.4%) urban subjects had chronic obstructive pulmonary disease (COPD) in contrast to 6 (0.7%) of control (p<0.0001, Fig. 1). Among urban subject, COPD was more prevalent among occupationaly exposed participants (4.3% vs. 2.6% among others, p<0.05)- traffic police (5.3%), road-side hawkers (3.7%), auto rickshaw drivers (3.6%), bus drivers (5.1%) and garage workers (7.1%). After controlling potential confounders like body mass index (BMI), education, environmental tobacco smoke and income, PM$_{10}$ level in ambient air were positively associated with decrement in FVC (Odds ratio [OR] = 2.02, 95% confidence interval [95%CI] 1.32-3.84) and COPD (OR = 3.82, 95%CI 2.12-6.39).

Inflammation and covert hemorrhage in the lung

The sputum samples of urban subjects were more cellular and contained increased number of inflammatory cells than the controls. The absolute number of neutrophils in sputum was increased by 1.2-fold, eosinophils by 1.7-fold, lymphocytes by 1.4-fold and alveolar macrophages (AM) by 2.3-fold in urban group. The number of AM per high power field was 3-times higher than the controls. Also, a large number of AM of air pollution-exposed subjects were multinucleated, highly keratinized, heavily loaded with phagocytosed particles and were larger in size. Iron-laden macrophages
(siderophages) were more frequent in sputum of air pollution-exposed individuals, indicating covert pulmonary hemorrhage following sustained exposures to air pollution.

Table 1. A comparison of the prevalence of lung function deficits, chronic obstructive pulmonary disease (COPD) and hypertension between urban and rural subjects

<table>
<thead>
<tr>
<th>Urban subjects</th>
<th>Reduced lung function (%)</th>
<th>COPD (%)</th>
<th>Hypertension (%)</th>
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<tbody>
<tr>
<td>Traffic police (n = 56)</td>
<td>51.8</td>
<td>5.3</td>
<td>42.8</td>
</tr>
<tr>
<td>Street hawkers (n= 188)</td>
<td>40.9</td>
<td>3.7</td>
<td>35.1</td>
</tr>
<tr>
<td>Auto rickshaw drivers (n= 82)</td>
<td>48.8</td>
<td>3.6</td>
<td>36.6</td>
</tr>
<tr>
<td>Bus drivers (n=78)</td>
<td>56.4</td>
<td>5.1</td>
<td>37.1</td>
</tr>
<tr>
<td>Motor mechanics (n= 56)</td>
<td>50.0</td>
<td>7.1</td>
<td>33.9</td>
</tr>
<tr>
<td>With office jobs (n=470)</td>
<td>35.3</td>
<td>2.6</td>
<td>21.9</td>
</tr>
<tr>
<td>Urban, total (n=932)</td>
<td>41.2*</td>
<td>3.4*</td>
<td>29.1*</td>
</tr>
<tr>
<td>Rural control (n= 812)</td>
<td>18.0</td>
<td>0.7</td>
<td>10.6</td>
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*, p<0.001 compared with rural control in Chi-square test

Hematological and immunological changes

Compared with control, urban subjects had lowered hemoglobin levels, reduced number of circulating erythrocytes, hypochromic red cells, red cell anisopoikilocytosis and abundance of ‘target’ cells, suggesting prevalence of anemia and altered liver function with regard to cholesterol metabolism. In addition, they had increased number of total leukocyte and immature neutrophils along with toxic granulation in neutrophils, suggesting greater prevalence of bacterial infection. Residents of Kolkata demonstrated a fall in CD4+ T cells and CD19+ B-cells but increase in the number of natural killer (NK) and T-regulatory cells, suggesting alteration in immunity.

Hypertension and tachycardia

Participants from Kolkata had elevated blood pressure (hypertension) and increased pulse rate (tachycardia) which are considered as risk factors for CVD. Hypertension was diagnosed in 29.1% of urban subjects (36.5% in occupationally exposed and 21.9% in others, p<0.05) against 10.6% in control (p<0.001). Tachycardia was present in 15.1% of air pollution-exposed group in contrast to 3.1% of control (p<0.001). Hypertension was more prevalent among occupationally exposed subjects, especially among traffic policemen (Table 1). In conformity with the present observation, a recent study has shown increase in systolic blood pressure following inhalation of diesel exhaust particles, and the change was found to be mediated by the nanoparticles (Mills et al., 2011).
Platelet and leukocyte hyperactivity

Subjects exposed to urban air pollution had 1.3-times more platelets in peripheral blood than the controls. In addition, platelet aggregation to collagen was increased by 32.9%, and 4.7% of platelets of citizens of Kolkata expressed P-selectin against 1.6% in controls. Thus, urban subjects had 4-times more activated platelets in circulation than the control. Besides, they had 2.6-times more soluble P-selectin (sP-sel) in blood plasma, 40-80% increase in CD11b/CD18 expressions on neutrophil and monocyte surface, 3-times more monocyte-platelet aggregates in blood, upregulation of myeloperoxidase and elastase activities in neutrophils, marked increase in oxLDL in plasma, and increased concentrations of serum anti-cardiolipin IgG, corroborating the risk of CVD among city dwellers.

Participants from Kolkata had 3.5-fold increase in circulating levels of IL-6 and CRP, 2.8-times more TNF-α and 2.4-times more IL-8 in plasma. Moreover, about 60% of urban subjects had more than 3.0 mg/l serum CRP levels, implying high risk of CVD. Serum cortisol level was also increased by 1.2-fold over control in urban subjects, indicating increased level of stress. IL-6, CRP, TNF-α and IL-8 levels correlated strongly with PM10. Serum IL-6 and cortisol levels displayed robust association with both systolic and diastolic blood pressure and hypertension. Similarly, positive correlation was found between tachycardia and serum CRP and cortisol levels.

Oxidative stress

Generation of ROS in airway cells and blood leukocytes was increased by 80-109% concomitant with 28-31% decline in SOD and 19-53% depletion of TAS. The findings indicate oxidative stress in subjects exposed to urban air pollution. After controlling possible confounders, a strong positive association was found between PM10 level and generation of oxidative stress. Collectively, the present findings suggest greater risk of CVD in people chronically exposed to vehicular pollution of the city, and the cardiovascular effects of air pollution were mediated by inflammation and oxidative stress.

Impact on chromosomes and the DNA

Vehicular exhausts contain a large number of organic chemicals, some of which like benzo(a) pyrene, 1,3-butadiene and benzene are human carcinogens. These carcinogens elicit their effects via mutagenesis. We have examined this possibility by evaluating MN formation and DNA damage in exposed cells. Compared with control, urban subjects had 2.2-times more MN-containing cells in buccal epithelium (p<0.001, Table 2). Similarly, DNA damage was more prevalent among urban subjects. Compared with 12.5% lymphocytes with damaged DNA in control, 67.8% of cells in urban subjects showed DNA damage. The tail length of Comet, an indicator of the extent of DNA damage, was also doubled in urban subjects (p<0.001, Table 2). Results from MN and Comet assays suggest rise in chromosomal and DNA damage among the residents of Kolkata.

Immunocytochemical (ICC) study showed 5.4-times increase in γ-H2AX protein expression in airway epithelial cells of urban subjects, suggesting marked rise in DNA
double strand breaks. Among the proteins involved in non-homologous end-joining type of DNA damage repair, expression of MRE-11 was increased insignificantly, while a 2.8-fold increase was observed in the expression of Ku70 (Table 2). The results suggest that cumulative exposure to vehicular pollution of Kolkata was associated with 5-times more DNA double strand breaks in cells that line the airways, but the repair machinery was insufficiently stimulated to repair this damage. As a consequence, some injury to the DNA may remain unhealed and that may lead to cell death or carcinogenesis.

Table 2: Comparison of chromosomal and DNA damage and efficiency of DNA repair mechanism between rural and urban subjects

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Urban</th>
</tr>
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<tbody>
<tr>
<td>Micronucleus per1000 buccal epithelial cells</td>
<td>1.36 ± 0.56</td>
<td>3.09 ± 1.46*</td>
</tr>
<tr>
<td>Comet tail length in µm</td>
<td>12.72 ± 4.32</td>
<td>28.85 ± 8.85*</td>
</tr>
<tr>
<td>Nuclear expression of γ-H2AX in airway cells (%)</td>
<td>2.65 ± 1.12</td>
<td>14.36 ± 4.14*</td>
</tr>
<tr>
<td>Expression of Mre-11 in airway cells (%)</td>
<td>2.15 ± 1.28</td>
<td>2.68 ± 1.46</td>
</tr>
<tr>
<td>Nuclear expression of Ku70 (%)</td>
<td>1.62 ± 1.17</td>
<td>4.53 ± 3.21*</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD; *, p < 0.001 compared with control in Student’s t-test

Cancer risk

Metaplasia (15.3% in urban vs. 3.8% in control) and dysplasia (3.6% in urban vs. 0.5% in control) of airway epithelial cells were more frequent in urban subjects. Moreover, ICC showed up-regulated expressions of p53 and activated (phosphorylated) form of protein kinase B (p-Akt<sub>thr308</sub> and p-Akt<sub>ser473</sub>) in these cells. Collectively, the findings indicate higher risk of lung cancer among urban subjects.

Figure 2. Prevalence of metaplasia (A) and dysplasia (B) of airway epithelial cells among urban and control subjects. Note significantly increased prevalence (p<0.0001 in Chi-square test) of both meta- and dysplasia among urban subjects, suggesting increased risk of lung cancer among city dwellers compared with rural subjects.
Diesel and petrol-powered internal combustion engines emit high numbers of ultrafine or nanoparticles (UFP, particles <100 nm) [Oberdorster and Utell, 2002] and may contribute to adverse respiratory and cardiovascular effects of particulate matter (Oberdörster et al., 2004). Depending on their particle size, inhaled UFPs are efficiently deposited in nasal, tracheobronchial, and alveolar regions due to diffusion. Pulmonary and systemic inflammation among urban subjects can be attributed in part to UFPs as they have increased potential to induce inflammation when compared on an equal mass basis with larger particles (Oberdorster, 2001).

UFPs diffuse through the lipid cell membrane and enter the cytoplasm. From there they enter the nucleus or mitochondria and interfere with cellular energy process. They can cross the epithelium, enter vascular endothelial cells and even red blood cells and possibly be transported via the blood to other organs including the brain (Oberdörster et al., 2004; Schmid et al., 2009). UFPs activate platelets (Rückerl et al., 2007) and they are more active in generating ROS than coarse and fine particles (Li et al., 2003). These nanoparticles are most potent in inducing heme oxygenase expression, depleting intracellular glutathione and inducing oxidative stress (Cho et al., 2005). Therefore, the depletion of SOD and TAS and excess generation of ROS observed among urban subjects of this study could be related to cumulative inhalation of UFPs. A recent study has shown that inhalation of diesel exhaust particles increased oxLDL, its primary receptor on endothelial cells, and vascular ROS generation (Lund et al., 2011).

In conclusion, this study has shown that chronic exposure to air pollution of Kolkata that arises mostly from vehicular exhausts of more than 1.2 million motor vehicles plying in the city adversely affect the health of its residents. It impairs lung function, increases the risk of life-threatening COPD, elicits pulmonary and systemic inflammation, causes covert pulmonary hemorrhage, alters immunity that may make the citizens susceptible to infection, increases the risk of hypertension and consequent cardio-vascular diseases, damages DNA and the chromosomes, interferes with DNA damage repair mechanism and enhances dysplasia of airway cells thereby increasing the risk of cancer in the lung and the airways. Carcinogenic changes were mediated by up-regulation of Akt signal transduction pathway, and PM$_{10}$ level in breathing air was positively associated with most these changes via generation of oxidative stress.

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