Original Article

Carbon Particles in Airway Macrophage as a Surrogate Marker in the Early Detection of Lung Diseases

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Abstract

Background: It has been shown that inhalation of carbonaceous particulate matter may impair lung function in children.

Objective: Using the carbon content of airway macrophages as a marker of individual exposure to particulate matter derived from fossil fuel, we sought direct evidence for this association.

Methods: 300 children from puffed rice industrial areas and 300 children from population living in green zone were selected randomly. Airway macrophages were obtained from healthy children through sputum induction, and the grading of ultrafine carbon particles in airway macrophages was measured. Pulmonary function was also measured by spirometry.

Results: Pulmonary function tests showed that in industrial area 42.6% and 20.3% of children had moderate obstructive airway disease and restrictive airway disease, respectively. In the green zone area, 7% of children had obstructive airway disease and 6% had restrictive airway disease. Evaluation of airway macrophages for ultrafine carbon particles revealed that in industrial area there were ultrafine carbon particles of grade 2 in 23% of subjects and grade 3 in 8.33% of individuals with obstructive airway disease. In the green zone area, the rates were 1.67% and 0.7%, respectively.

Conclusion: The study provides a first evidence of the strong association between air pollution and development of airway diseases. Carbon particles in the sputum can be used as a marker for air pollution.

Keywords: Macrophages, alveolar; Particulate matter; Air pollutants; Spirometry; Lung diseases

Introduction

The incidence of allergic respiratory diseases and bronchial asthma appears to be increasing worldwide.[1] People living in urban areas more frequently experience these conditions than those living in rural areas.[2] One of the several causes of the rise in morbidity associated with allergic respiratory diseases is the increased presence of outdoor air pollutants resulting from more intense energy consumption and exhaust emissions from cars, other vehicles and industries.[3,4] One of the important health problems is urban air pollution. It has

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been shown that air pollution can adversely affect lung function in patients with asthma.[5] Air pollution can injure the airway mucous membranes and impair mucociliary clearance which in turn may facilitate access of inhaled allergens to the cells of the immune system, thus promoting sensitization of the airway.[6] Consequently, a more severe immunoglobulin (IgE) mediated response to aeroallergens and airway inflammation could account for increasing prevalence of allergic respiratory diseases in polluted urban areas.[7] Although air pollutants include a heterogeneous mixture of gases and particles, recent research studies have concerned the adverse effects of particulate matter (PM) which consists of primary particles, such as diesel soot or other combustion-derived particles emitted directly into the atmosphere, and secondary particles which are produced in the atmosphere through complex physicochemical reactions of various gases.[8,9] Air-borne PM is generally defined based on the size distribution of the particles. Thus, PM_{10} and $PM_{2.5}$ stand for PM with median aerodynamic diameters of less than 10 μm and 2.5 μm, respectively. Ultrafine particles as defined those particles with a diameter of <100 nm, are likely to deposit in human alveoli at a high rate.[9] Inhaled air pollutants can cause lung inflammation, which can in turn alter the autonomic nervous control of heart rhythm and release of inflammatory mediators into the blood which affect extrapulmonary organs.[10] Both pulmonary and systemic inflammation may occur through oxidative stress responses to reactive oxygen species. Another possibility is that ultrafine particles are able to translocate into the systemic circulation, and affect cardiovascular endpoints more directly.[11]

Among various states in India, Karnataka has some of the largest number of clusters of puffed rice units. It is estimated that there are about 2000 such units in Karnataka state and some of the major clusters are in the towns of Davangere, Hubli, Dharwad and Belgaum. Fuels used in the ovens for making puffed rice are mainly rice husk, wood, wood shavings, used automobile tire, groundnut shell, and agricultural residues. The used automobile tire is used in view of high heat it generates at low cost. Burning these fuels in highly inefficient conventional ovens generates high levels of particulate matter, carbon monoxide and other pollutants. We therefore conducted the present study to determine the role of carbon particles in airway macrophage and the pulmonary functions in children living in an industrial area and a green zone and to evaluate the utility of carbon particle in airway macrophage for early detection of respiratory disorders.

Materials and Methods

This study was conducted between September and December 2010. Ethical clearance was obtained from the Institutional Review Board.

Subjects

Inclusion criteria: Three-hundred apparently healthy children were randomly selected from a school with 380 students located in an industrial area. Another 300 apparently healthy children who served as comparison group were randomly selected from a school with 400 students located in a green zone far away from city, vehicular traffic and industrial area. Children were included if they were 8 to 16 years of age, were living in Davangere, and were living in the same house they had lived in for at least one year before the study began and if their parents reported that they had normal levels of activity and spirometry reading peak expiratory flow rate (PEFR)>80%.

TAKE-HOME MESSAGE

- The incidence of allergic respiratory diseases and bronchial asthma are increasing worldwide.
- The predominant source of carbon particles in alveolar macrophages is from combustion of fossil fuels.
- Carbon particles in alveolar macrophages as well as its grade is higher in children residing in industrial area than green zone.

Exclusion criteria: We excluded children who had any chronic respiratory condition, symptoms consistent with a respiratory infection over the previous three months, and those who were smokers. To ensure that the carbon content of airway macrophages reflected exposures in Davangere, we also excluded children who had spent more than five days outside the city in the previous three months as the half-life of particles in airway macrophages after a single instilled dose is 3.9 months.[12,13]

On the study day, children and parents were asked to complete a questionnaire that requested the child's age, sex, number of siblings, race, and home address. The child's body mass index (BMI) was calculated from the height and weight on that day.

Lung Function

Lung function was recorded no more than 20 minutes before sputum induction with the use of a spirometer (RMS Helios 401) and software. Spirometry was conducted according to the recommendations of the American Thoracic Society b a single operator.[14] Forced vital capacity (FVC), forced expiratory flow between 25% and 75% of the FVC (FEF_{25-75}) and the FEV_1/FVC ratio were used as the primary measures to assess lung function. Each plot of the flow rate against volume during an FVC maneuver (flow volume curve) was visually examined for each child and if the final expiratory phase was stopped due to a Valsalva maneuver or hesitation, only the first portion of the curve was considered and only the FEV_1 was calculated. If FEV_1/FVC is >88%, then the patient has restricted lung disease; if it is ≤69% of the predicted value, then the patient has obstructive lung disease.[14]

Sputum Collection

Children were pre-treated with 200 μg salbutamol nebulization for five minutes and 5% saline nebulization for 15 minutes. Then, they were asked to cough out at least 2 mL of sputum in a wide mouthed clean container.[15]

Carbon Content of Airway Macrophages

Airway macrophages were visualized by light microscopy. The area occupied by black material (carbon) in each macrophage was assessed as previously described by an investigator who was blinded to pulmonary function test results. Smears were prepared and stained with hematoxylin and eosin.[16] The smear was analyzed and grading of macrophage was done as follows. Grade 0: no carbon pigments in macrophage; grade 1: few carbon pigments in cytoplasm; grade 2: abundant carbon particles in cytoplasm, but nucleo-cytoplasmic differentiation maintained; and grade 3: macrophage flooded with carbon particles and nucleo-cytoplasmic differentiation lost.

Calculation of Particulate Matters at Different Study Centres

The annual mean level of primary PM_{10}, that is the component of PM emitted directly from local source of combustion was calculated at industrial area and at green zone area by using ambient air

Table 1: Demographic features of the studied children

Parameter	Mean±SD		p* value
	Industrial area (n=300)	Green zone (n=300)	
Age	13.5±2.0	13.6±1.8	0.52
Height (cm)	143.9±10.9	146.7±13.6	0.01
Weight (kg)	33.4±8.8	39.9±10.9	<0.001
BMI (kg/m^2)	15.9±2.8	18.5±4.2	<0.001
Duration of residence in the study area (yrs)	10.4±2.2	10.0±1.1	0.14
Duration of stay at home (hrs)	12.6±1.4	12.9±1.7	0.21

*Unpaired *Student's t* test

quality monitoring instrument (Merck) a week before the subjects were enrolled into the study.

Statistical Analysis

Normality of data distribution was tested by Levene's test. Unpaired *Student's t* test was used to compare demographic features and pulmonary function test parameters. The categorical variables were analyzed by χ^2 test. A $p<0.05$ was considered statistically significant. The data were analyzed by SPSS® 15.0 trial version.

Results

The numbers of children in the four strata were comparable in both groups. The male/female ratio was 65/35 in industrial area group and 62/38 in green area group. The mean±SD age of participants was 13.5±2.0 years for industrial area and 13.6±1.8 years for green zone. The mean annual level of primary PM_{10} concentration in industrial area and green zone was 1403 and 315 µg/m^3, respectively.

BMI of children in industrial zone (15.9±2.8 kg/m^2) was significantly lower than that of green zone students (18.5±4.2 kg/m^2) (Table 1). There was no association between the carbon content of airway macrophages and age, weight, height, BMI, and level of activity. There was also no significant difference in carbon content of airway macrophages between boys and girls. More than 85% of children in

Table 2: Frequency distribution of grades of carbon contents in alveolar macrophages stratified by two study areas.

Area	n	Grade			
		0	1	2	3
Green	300	189 (63.0%)	57(19.0%)	18 (6.0%)	06 (2.0%)
Industrial	300	84 (28.0%)	84 (28.0%)	96 (32.0%)	36 (12.0%)

p<0.001

Table 3: Measured pulmonary function test parameters recorded in study groups

Parameters	Industrial area			Green zone			p* value
	Range	Median	Mean±SD	Range	Median	Mean+/-SD	
FVC	60–116	78	83.4±19.2	66–120	84	84.3±12.7	0.56
FEV_1	74–144	88	97.8±24.0	77–129	100	99.2±13.8	0.35
FEV_1/FVC	108–125.0	115	117.1±5.4	108–125	116	117.8±4.7	0.52
FEF_{25-75}	53–167	105	105.5±24.1	65–176	100	103.1±25.8	0.33

*Unpaired *Student's t* test to determine difference in means

industrial area that aged 8–10 years had carbon particles in their alveolar macrophages; it followed by children aged 11–13 years. Forty-two percent of children aged 14–17 years residing in green zone had more carbon particles in their alveolar macrophages when compared to other age groups.

The mean number of annual episodes of respiratory infections in children in industrial area and green zone was 11.8 and 3.3, respectively. The mean±SD linear height of children was 143.9±10.9 and 146.7±13.6 cm, respectively (Table 1).

Table 4: Number (%) of children stratified by pulmonary function test results and living areas

Parameters	Severity	Industrial	Green zone	p value
FEV_1	>80	172 (57.3)	279 (93.0)	<0.001
	60–80	128 (42.6)	21 (7.0)	
	≤60	0 (0.0)	0 (0.0)	
FEV_1/FVC	≥88	61 (20.3)	18(6.0)	0.08
	70–87	168 (56.0)	276 (92)	
	<69	71 (23.6)	6 (2.0)	
FVC	>80	142(47.3)	219 (73.0)	<0.001
	60–80	145(48.3)	81 (27.0)	
	45–65	13 (4.3)	0 (0.0)	
	<45	0 (0.0)	0 (0.0)	

While 72% of children in industrial area were found positive for carbon particles, 27% of children in green zone were found positive. Grades 2 and 3 carbon particles were seen in alveolar macrophage of 32% and 12% of children in industrial area, respectively. The prevalence rates in green zone children were 6% and 2%, respectively (Table 2).

There was an inverse, dose-dependent association between the carbon content of airway macrophages and pulmonary function: each increase in the grade of carbon content was associated with a reduction of 17% in FEV_1, 12.9% in FVC, and 34.7% in FEF_{25-75}. There was no association between the carbon content of airway macrophages and the FEV_1/FVC ratio (Tables 3, 4). These associations remained significant when lung function was measured 15 minutes after the administration of an inhaled bronchodilator.

Based on pulmonary function test results, 42.6% of children in industrial area had moderate obstructive airway disease; the prevalence was 7% in green zone. Of children in industrial area, 20.3% had restrictive airway disease; the prevalence was 6% in the green zone ($p<0.05$).

Of those children in industrial area who had restrictive airway disease, 22 had grade 2, and 10 had grade 3 carbon

particles in airway macrophages. In green zone children only one had grade 2 carbon particles (Table 5).

In industrial group, airway macrophages carbon particles of grade 2 and grade 3 were observed in 69, and 25, children, respectively—all of them had obstructive airway disease. In green zone group, airway macrophages carbon particles of grade 2 were observed in five and three children who also had obstructive airway disease.

Table 5: Number of children with various grades of alveolar macrophage carbon contents stratified by FEV_1/FVC and study area

FEV_1/FVC	Industrial area			Green zone		
	Grade			Grade		
	1	2	3	1	2	3
≥88	0	22	10	0	3	0
70–87	80	5	1	51	0	0
<70	4	69	25	2	5	2
	p<0.001			p=0.01		

Discussion

In developed countries, modernization has been accompanied by a shift from biomass fuels such as wood to petroleum products and electricity. In developing countries, however, even where cleaner and more sophisticated fuels are available, the small scale industries often continue to use simple biomass fuels. Although the proportion of global energy derived from biomass fuels fell from 50% in 1900 to around 13% in 2000, there is evidence that their use is now increasing among the poor.[17] Poverty is one of the main barriers to the adoption of cleaner fuels. The slow pace of development in India suggests that biomass fuels will continue to be used by the poor for many decades.

Biomass fuel is any material derived from plants or animals which is deliberately burnt by humans. Wood is the most common example, but the use of animal dung and crop residues is also widespread. China, South Africa and some other countries also use coal extensively for domestic needs. Used vehicle tires are burnt which may cause allergy either by direct contact with latex products or inhalation of air-borne allergens; such allergies are frequently termed "latex allergy." Latex is primarily recognized as an indoor allergen. A major component of lorry tires is natural rubber latex. Tire dust in urban air samples has been postulated to contribute to latex sensitization and asthma.[18]

In the present study the mean duration of exposure to outdoor pollutants was similar in both study groups. Earlier studies showed that the predominant source of carbon particles in alveolar macrophages is from combustion of fossil fuels. In our study, we found that the likelihood of detecting carbon particles in alveolar macrophages as well as its grade is higher in children residing in industrial area than green zone.

We also found a statistically significant difference in the mean number of annual respiratory episodes in children in industrial area than in green zone. Similar observations were made in earlier studies conducted in India. However, in contrast to earlier reports, we did not observe any statistically significant difference in PEFR.[19]

Based on pulmonary function test results, 42.6% of children living in industrial area compared to 7% of those in green zone had moderate obstructive airway disease. Twenty percent of children in industrial area compared to 6% in children in green zone had restrictive airway disease. The number of children in industrial area who had grades 2 or 3 alveolar

carbon particles, was significantly higher than those in green zone.

We could not figure out whether the inverse association observed between the grade of carbon content in airway macrophages and lung function parameters represents a short-term or a long-term effect on lung function. Our results have, however, ruled out short-term reversible bronchoconstriction; significant inverse associations remained after bronchodilator therapy.

It is also unclear whether the carbon content of airway macrophages reflects long-term or short-term exposure to PM. Some carbon particles could have been acquired several months before our analysis, since soot has been observed in airway macrophages even 10 months after a brief occupational exposure, and insoluble particles would remain in airway macrophages for up to three months after instillation in experimental studies. Furthermore, the carbon content of airway macrophages may not reflect the content in more distal alveolar cells, since sputum induction samples macrophages from the larger airways.

It has been shown in experimental exposure studies that increased level of activity, lower age, and higher BMI are associated with increased deposition of particles in the lower airway. In the present study, none of these variables were associated with carbon contents of airway macrophages. However, we found that carbon contents in the airway macrophages of children living in an industrial area where primary PM_{10} were abundant was higher than those living in a green zone.

Increased vulnerability to bacterial infection of the lower respiratory tract is a hallmark of COPD.[19] Recent data from human bronchial epithelial cells exposed to cigarette smoke suggest that this may, in part, be due to suppression of antibacterial host defense.[20] Similarly, in children, there is good evidence that exposure to PM increases vulnerability to bacterial infection.[9] This association between PM and bacterial infection in children is important because exposure to PM is ubiquitous and infection is common, with 156 million new episodes of pneumonia per year in young children worldwide (151 million of these are in the developing countries);[21] 10% of these episodes are life-threatening.[21] A recent meta-analysis of studies performed in the developing world estimated that the odds ratio for severe pneumonia in children under five years of age exposed to smoke from biomass and other high PM-emitting fuels is 1.78.[22] Few studies in the developed countries have assessed the association between PM and vulnerability of children to bacterial infection. Even fewer studies of environmental exposures and infection have identified casual pathogens. However, *Streptococcus pneumoniae* is likely to be important, since it is the most common etiologic agent for pneumonia in young children.[23,24]

To conclude, the presence and the amount of carbon particles in airway macrophages can be used as an early marker of altered pulmonary functions. Also the higher the load of carbon particles in airway macrophages, the more severe alteration in pulmonary function test parameters. In this study, those with carbon particles in their alveolar macrophage have shown signs of both obstructive airway disease and restrictive airway disease. It reflects that there is a need for further research to shed light over the correlation between pollution and lung development both *in utero* and post-natal period.

Conflicts of Interest: None declared.

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