Relato de Caso / Case Report

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# Traffic-related air pollution and black carbon in sputum macrophages: a "silent" lung disease?

Poluição do ar relacionada ao tráfego urbano e carbono preto em macrófagos de escarro: uma doenca pulmonar "silenciosa"?

Rodrigo Godinho de Souza<sup>1,5</sup>, Mauro Henrique Moraes Vargas<sup>2,5</sup>, João Paulo Heinzmann Filho<sup>2,5</sup>, Mayara Ferraz Menezes<sup>3,5</sup>, Paulo Márcio Pitrez<sup>4,5</sup>

<sup>1</sup> Biologist; <sup>2</sup> Physiotherapist. Master's Degree in Pediatrics and Child Health, Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS); <sup>3</sup> Graduate student at School of Physiotherapy at PUCRS; <sup>4</sup> Pediatric pulmonologist at Hospital São Lucas da PUCRS, Professor of School of Medicine of PUCRS; <sup>5</sup> Centro Infant, Instituto de Pesquisas Biomédicas da PUCRS, Porto Alegre, RS, Brasil.

#### **ABSTRACT**

Aims: To describe two case reports with significant black carbon deposition in sputum macrophages in healthy young adults from an urban city, showing mild airway obstruction in lung function tests. The role of black carbon deposition by air pollution in the airways and the development of indolent chronic lung disease in populations living in larger cities is not clear and may be a potential world health problem.

Cases description: We report two cases of voluntary adults living in a South American large city who have undergone clinical examination, lung function and analysis of inflammation and black carbon deposition in macrophages from induced sputum. Two Caucasian, previously healthy, nonsmokers, male adults, living in the same city of southern Brazil, with no respiratory symptoms presented with normal physical exams, but with lung function demonstrating mild obstructive lung disorder, with no bronchodilator response. In particular, one of the subjects works daily in delivery services as a motorcycle driver. Induced sputum of both patients showed no signs of cellular inflammation. However, a large number of black carbon content was detected inside macrophages of the sputum sample in both patients.

Conclusions: In summary, significant daily air pollution exposure may play a role in long-term silent disease in adults, potentially leading to clinically relevant chronic lung diseases later in life, particularly in populations of urbanized large cities from developing countries. The development of better, more directly and less invasive, methods for air pollution exposure are required, along with longitudinal studies, in order to measure the real impact of air pollution in chronic lung diseases of susceptible populations.

KEY WORDS: SPUTUM: CHRONIC OBSTRUCTIVE LUNG DISEASE: ASTHMA

## **RESUMO**

Objetivos: descrever dois casos com importante deposição de carbono preto em macrófagos de escarro induzido em adultos jovens saudáveis de uma cidade urbana, mostrando obstrução leve no teste de função pulmonar. A deposição de carbono preto pela poluição do ar nas vias aéreas e no desenvolvimento de doença pulmonar crônica "silenciosa" em populações que vivem em grandes cidades não é clara e pode ser um potencial problema de saúde mundial.

Descrição dos casos: relatamos dois casos de adultos voluntários que vivem em uma grande cidade sul-americana, que se submeteram a exame clínico, função pulmonar e análise de inflamação e deposição de carbono preto em macrófagos de escarro induzido. Os indivíduos eram caucasianos, previamente saudáveis, não-fumantes, adultos, do sexo masculino, moradores da mesma cidade do sul do Brasil, e sem sintomas respiratórios. Apresentaram exames físicos normais, mas com função pulmonar demonstrando doença pulmonar obstrutiva leve, sem resposta ao broncodilatador. Em particular, um dos sujeitos trabalha diariamente em serviços de entrega como motoboy. No exame de escarro ambos não apresentaram sinais de inflamação celular, mas foi detectado um elevado número de carbono preto no interior dos macrófagos.

Conclusões: a exposição contínua à poluição do ar pode desempenhar um papel no desenvolvimento de doenças pulmonares crônicas em longo prazo. O desenvolvimento de um exame melhor, mais direto e menos invasivo para análise da exposição à poluição do ar, juntamente com estudos longitudinais, permitirá medir o real impacto da poluição na etiopatogenia das doenças pulmonares crônicas em populações suscetíveis.

DESCRITORES: ESCARRO; DOENÇA PULMONAR OBSTRUTIVA CRÓNICA; ASMA

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## **INTRODUCTION**

Air pollution has been associated with many human illnesses and has become a major problem in many urbanized cities worldwide. Respiratory system is probably the most vulnerable part of human organism to air pollution. Biomass fuels and motor vehicles are the major sources of air pollution to human health. <sup>1-2</sup> In urbanized cities, air pollution has been associated with respiratory symptoms, asthma and chronic obstructive pulmonary disease (COPD). <sup>3-5</sup> Unfortunately, easy and non-invasive methods for analysis of individual exposure to pollutants have not been properly developed and standardized and the real individual impact of outdoor/indoor air pollution in lung diseases has not been accurately addressed and is still a research challenge.

In the present article, we present two case reports of healthy adults showing macrophages from induced sputum with large amounts of intracellular black carbon. Although asymptomatic, the subjects presented with abnormal lung function. The aim of this article is to help raising relevant questions of how air pollution may play a potential "silent" long-term role in the development of chronic lung diseases.

This article complies with the current rules of the Ethics Committee of our Institution (CEP/PUCRS), related to an approved research project (Protocol # 409.242).

## **CASES REPORT**

We examined two adult Caucasian male healthy volunteers residing in a Southern capital of Brazil, both from a prospective study analyzing inflammation from induced sputum in adults. They were selected through disclosure at the Pontifical Catholic University of Rio Grande do Sul (PUCRS) and the Motoboy's syndicate (Sindimoto-RS).

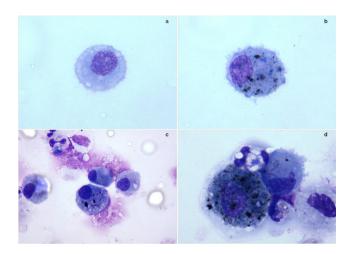
Subjects were tested for lung function (spirometry, with KoKo, nSpire Health Inc., Longmont CO, USA) and induced sputum (20 minutes-protocol with 4.5% saline nebulizations). Differential cell counts (May-Grumwald Giemsa) and the content of carbon from macrophages of sputum were analyzed through optical microscopy. Digital color images from 50 airway macrophages were obtained from each subject using an Olympus digital camera (Q-Color3tm Olympus® America Inc., USA) and microscope (BX43 Olympus® America Inc., USA). The average of surface area on macrophages was calculated through the software Image J (National Institute of Health,

Bethesda, MD, USA), as previously described by Nwokoro et al.<sup>6</sup>

Case #1: A male adult, aged 35 years, physical educator, non-smoker, who performs regular physical activity, previously healthy, asymptomatic for respiratory diseases over the last 4 weeks, was clinically evaluated. He had no history of hospitalization in recent years and no family history of asthma. Spirometry (presented as percentage of predictive values) has shown a mild airway obstruction with forced expiratory volume in one second (FEV<sub>1</sub>) of 80%, with FEV<sub>1</sub>/forced vital capacity (FVC) of 73% and forced expiratory flow at 25 to 75% of the FVC (FEF<sub>25-75%</sub>) of 55%. In induced sputum, a pauci-granulocytic pattern was found (macrophages: 87%, neutrophils: 9%, lymphocytes: 4%).

Case #2: A male adult, aged 33 years, has been a motorcycle courier for eight years, working five days a week, five hours a day. He is a non-smoker, asymptomatic for chronic respiratory symptoms, and no family history of asthma. Spirometry has shown an FEV<sub>1</sub> of 97%, FEV<sub>1</sub>/FVC of 77% and FEF<sub>25-75%</sub> of 77%. Induced sputum also showed a pauci-granulocytic pattern (macrophages: 55%, neutrophils: 43%, and lymphocytes: 2%).

In both patients, black carbon particles inside macrophages from sputum were detected in high amounts. The photomicrographs presented in **Figure 1** illustrate these findings.



**Figure 1.** Selected macrophages from induced sputum from both healthy adults are presented, with presence of black carbon more intense in the sample from the motorcycle driver. **Case #1:** a) Normal macrophage; b) moderate quantity of black carbon. **Case #2:** c) moderate quantity of black carbon; d) high quantity of black carbon.

**See this image in higher resolution:** http://revistaseletronicas.pucrs.br/ojs/index.php/scientiamedica/article/view/16534/11507

## **DISCUSSION**

In clinically healthy adults living in one urbanized city from a developing country in South America, we have detected large amounts of black carbon deposition in sputum macrophages. These findings may reflect the current potential health problems that people living in large cities worldwide are susceptible and that will very likely be a major public health problem in the next decades in these populations.

Deposition of carbon in macrophages from sputum of children and adults exposed to gases of fuel combustion has been reported in previous studies. 6-8 This method of carbon exposure in the lungs is a more direct and individualized method to express this type of exposure, when compared to measurements of particulate matter (PM<sub>10</sub> and PM<sub>2.5</sub>) in the environment. Individual exposure to traffic-related air pollution is highly affected by some factors (type of urban transportation, residential/working proximity to major roads, or place of leisure activities), and hence may vary substantially between subjects. Personalized air pollution exposure measurements are a key factor for improving methods on further studies in this field.

Air pollution has been consistently associated with lung diseases, but the role of exposure to traffic-related air pollution on the development of COPD is controversial.<sup>1-2</sup> A 10-year Swiss cohort of adults has shown that an improvement in air quality may decrease the decline of lung function.<sup>9</sup> A German study focused on women only has found that an increase of PM<sub>10</sub>

was associated with a decrease of FEV, and forced vital capacity (FVC) and an increase of prevalence of COPD.5 In healthy adults, inflammatory markers were acutely increased after a 1-hour exposure to diesel exhaust, under realistic conditions to simulate actual urban cycling conditions. Increased expression of P-selectin and vascular cell adhesion molecule-1 in bronchial mucosal biopsy specimens, as well as unexpected increased eosinophil counts in bronchoalveolar lavage fluid was reported.<sup>10</sup> Both young adults presented in this article had no chronic respiratory symptoms, no sign of cellular inflammation in sputum, but presented with mild obstructive airway abnormalities in lung function tests and high amounts of black carbon deposition in sputum macrophages. These findings raise real concerns regarding to the role of traffic-related air pollution in the "silent" development of chronic obstructive pulmonary diseases in people living in urbanized societies. It is important to stress that young adults working daily with motorcycling services are very common in some developing countries, which are subjects highly exposed to diesel exhaust for many years.

In conclusion, with our brief case reports, we suggest that individual clinical markers of the effect of air pollution (exposure to pollution/oxidative stress measurements in the airways) have to be more accurately developed and as well included in future studies in this field, in order to better understand the relationship between traffic-related air pollution and the development of chronic obstructive pulmonary disease in more susceptible subpopulations worldwide.

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