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Associations between environmental exposures and serum concentrations of Clara cell protein among elderly men in Oslo, Norway ☆, ☆☆

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ABSTRACT

Cardiopulmonary morbidity and mortality is associated with several environmental exposures. Mechanistically, pathophysiological changes in the cardiopulmonary system may lead to the induction of inflammatory responses.

In the present study we explored associations between environmental exposures and serum concentrations of lung Clara cell protein 16 kDa, a biomarker that has recently been used to assess the integrity of the lung epithelium.

Serum Clara cell protein concentrations were associated with both number of cigarettes smoked per day and number of pack-years of smoking. There was no evidence of an association between long-term exposure to ambient air pollution, as assessed at each participant's home address, and serum concentrations of CC16. However, short-term variations in both ambient air pollution and temperature were associated with increases in serum Clara cell concentrations. All findings were robust when other factors were adjusted for.

These findings suggest that acute environmental exposures may compromise the integrity of the lung epithelium and lead to increased epithelial barrier permeability in the lungs of elderly men.

1. Introduction

Cardiopulmonary disease are major causes of morbidity and mortality in most parts of the world and have been associated with several environmental exposures such as ambient temperature (Kim et al., 2003; Marchant et al., 1993), ambient air pollution (Health Effects Institute, 2001; Pope and Dockery, 2006), and tobacco smoke exposure (Ludvig et al., 2005; Steenland et al., 1996).

The exact biological mechanisms underlying the observed adverse health effects are still largely unknown. Biological plausibility is enhanced by the observation of a coherent cascade of cardiopulmonary health effects during an adverse exposure, and by the fact that non-cardiopulmonary health endpoints are not typically associated with the exposure of interest. However, more research on the pathophysiological pathways linking cardiopulmonary morbidity, mortality, and ambient air pollution is still needed. Among other mechanisms, such as altered nervous regulation and direct particle effect on the cardiopulmonary system, animal, and human experimental studies have suggested that ambient air pollutants may induce local inflammation in the lung that may influence the immune system and thereby initiate a systemic inflammatory response (Leonardi et al., 2000; Frampton et al., 2002). Inflammatory processes have been implicated in the pathophysiological pathway from the deposition of particles in the lung to the exacerbation of disorders affecting the cardiopulmonary system. One possible mechanism for how inflammatory processes exacerbate disorders of the cardiopulmonary system is that the secretion of adhesion molecules by pulmonary endothelial cells enhances the binding, and activation of leukocytes, and platelets, the generation of hemostatically active microparticles, and the release of proinflammatory cytokines (Hrachovinova et al., 2003; Van Eeden et al., 2001; Nemmar et al., 2007).

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☆☆ Written consent was obtained for all participants in the Oslo Study II. The names and personal ID numbers of participants were omitted from the data set to ensure confidentiality. The Norwegian Data Inspectorate approved the Oslo Study part II, and the Regional Committee for Medical Research Ethics evaluated the research methodology. This research was conducted in full accordance with the World Medical Association Declaration of Helsinki.

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Clara cell protein16 kDa (CC16) is a lung epithelium-specific protein secreted in the respiratory tract by the non-ciliated Clara cells, known for their vulnerability to toxic exposures (Bernard et al., 1994; Hermans et al., 1999; Steerenberg et al., 2006). The protein seems to serve as anti-microbial and anti-inflammatory agent in the lung lining fluid (Tzouvelekis et al., 2005). However, human and experimental studies have shown that CC16 can also appear in extrapulmonary fluids such as serum and urine (Arsalane et al., 2000; Broeckaert et al., 2000; Halatek et al., 1998). Thus it may serve as a biomarker to assess the integrity of the lung epithelium and lung epithelial permeability. Increases in serum levels of CC16 have been observed after diverse exposures, such as ambient ozone (Broeckaert et al., 2000), smoke inhalation among firefighters, (Bernard et al., 1997), and trichloroamine among regular attendees of chlorinated indoor swimming pools (Lagerkvist et al., 2004). Decrease in serum levels have been reported after chronic exposures to air pollutants, such as cigarette smoke (Van Miert et al., 2005) and in occupational settings to nitric oxides (Halatek et al., 2005). Therefore, serum concentrations of CC16 have been proposed as non-invasive biomarkers to assess the integrity of the respiratory epithelium and lung epithelial permeability (Hermans et al., 1999).

The aim of the present study was to assess associations between serum concentrations of CC16 and environmental exposures. We used data from 1004 men aged 67–77 years from the city of Oslo that participated in the health screening of the Oslo Health Study part II (HUBRO), to investigate the relationship between serum CC16 levels, and environmental exposures, including ambient air pollution.

2. Materials and methods

2.1. Study population

The present study used data from a cross-sectional study, the Oslo Study part II, which was carried out in the city of Oslo, Norway, from January to June 2000 (Haheim et al., 2006; Madsen et al., 2007). A total of 6531 men aged 67–77 years participated in this study (Slørdal et al., 2003).

The data collection included a main questionnaire, one supplementary questionnaire, and a clinical examination. A unit of trained health workers performed the examination and the examinations were performed in one central screening station in the city. All the examinations were conducted during the weekdays (Monday–Friday) between 08:00 and 18:30.

A total of 1200 subjects were selected based on mean concentrations of ambient nitrogen dioxide (NO_2) as proxy for air pollution at their home address during the last year before the clinical examination. This would give contrasts in exposure: 1/3 were randomly picked from the bottom 25% (low exposed), 1/3 from the 25–75% (medium exposed), and 1/3 from the top 25% (high exposed). Exposure at home address was highly correlated for all pollutants (Spearman correlation: 0.82–0.96).

We limited our analyses to cases with complete information on the attendance variables (year, month, day and hour) and the exposure variables ($n = 1004$).

2.2. Health measurements

The clinical examination included a venous non-fasting blood sample which was analyzed for serum total cholesterol, HDL cholesterol, glucose, and triglycerides. An automatic device (DINAMAP®) measured pulse recordings, systolic- and diastolic blood pressures (Haheim et al., 2006). C-reactive protein (CRP) was measured in samples with enough serum using a high-sensitive latex-enhanced CRP assay, detection limit below 0.1 mg/l, with reagents from Roche adapted for analysis in a Hitachi 917 automatic analyzer (Hitachi, Tokyo) (Madsen et al., 2007).

Serum concentrations of CC16 were determined by ELISA, essentially according to the recommendations of the supplier (BioVendor; www.biovendor.com). The assay dilution buffer was supplement with 0.5% bovine serum albumin. Antibody concentrations were lowered to 1.0 and 0.2 μg/ml for capture and detection antibody, respectively. Absorbance was measured and quantified using a plate reader (TECAN Sunrise, Phoenix Research Products, Hayward, CA, USA) complete with software (Magellan V 1.10).

2.3. Tobacco smoke exposure

Participants were asked to fill out whether they were current smokers, former smokers or had never smoked. Current smokers and former smokers reported average number of cigarettes per day. Cigarettes smoked per day were grouped into five categories for current smoking status of non-smokers and former smokers 0 per day, current smokers 0–5, 6–10, 11–15, and above 15 per day.

The participants were also asked to report daily exposure to environmental tobacco smoke (ETS) on an everyday basis (“How many hours per day do you spend in a room with tobacco smoke?”). Cumulative lifetime exposure to smoking was computed as number of pack-years (= [number of daily cigarettes/20] × years of smoking) for both current and former smokers.

2.4. Ambient air pollution exposure

The ambient air pollution data were estimated using EPISODE, a dispersion model based on emissions, meteorology, topography, and background air pollution concentrations measured at regional air monitoring stations in the southern Norway (Slørdal et al., 2003; Walker et al., 1999). Concentrations were calculated for each square kilometer (km^2) and at 8009 receptor points with busy traffic by using hourly emissions and meteorological data during the study period (Walker et al., 1999). The major sources of the pollutants (NO_2, $PM_{2.5}$, and PM_{10}) during this period were traffic exhaust, road dust, wood burning, and long-range transport. The capability of this model has been evaluated elsewhere (Oftedal et al., in press).

Indicators of air ambient pollution exposure at each of the participant's home address considered in these analyses were NO_2, particulate matter (PM_{10}), and fine particulate matter ($PM_{2.5}$). The pollutants were given as daily, weekly, monthly, and yearly values for each participant and were analyzed as both continuous and categorical variables in the models. The pollutant variables were divided into categorized exposure levels based on the quartiles of each pollutant given as $\mu g/m^3$.

As a second way of assessing ambient air pollution exposure we used measurements of the same components from a fixed monitoring site located in the center of the city, representative of a populated area.

2.5. Other study variables

The Norwegian Meteorological Institute provided data on temperature in Oslo during the study period. Temperature was measured as hourly values at two stations located in the center of Oslo and given as average degrees Celsius (°C) per day. The ambient temperature was analyzed at lags 0–7 days before the clinical examination took place.

Respiratory disease was recorded based on both self-reported asthma and bronchitis.

Body mass index (BMI) was calculated based on the measurements of height and weight for each participant.

Alcohol drinking habits were recoded into five categories; 4–7 times a week, 2–3 times a week, 2–3 times a month, few times last year, and did not drink alcohol last year.

Education was reported as the total number of years of education and were recoded into three categories; <10, 10–12, and >12 years.

2.6. Statistical methods

Descriptive statistics, bivariate Spearman correlations, logistic regression models, general linear regression models, (GLM) and general additive models (GAM) were used to estimate the effect on the dependent variables in the statistical packages STATA 9.2 software (Stat Corp., USA) and S-Plus 6.2 software (Insightful, Seattle, WA, USA). The distribution of CC16 was markedly skewed to the right. Natural logarithmic-transformed levels of CC16 gave geometrical mean concentrations that resulted in nearly symmetrical distributions that were used in the statistical analysis. The distribution is presented as geometric mean, interquartile range (IQR), and 95% confidence interval (CI). One-way analysis of variance (ANOVA) was used to test for differences in means between groups, and to test for trends across categorical variables.

The GLM analyses were performed with continuous and categorical parameters and were used to assess the simultaneous relation among the various covariates. The log transformed CC16 was used as the dependent variable, whereas the independent variables were represented in the models as indicator variables for age (continuous), alcohol consumption (categorical), smoking status (categorical), hour of examination (continuous), respiratory disease (categorical), BMI (continuous), cigarettes per day (continuous), exposure to passive smoking (categorical), education (categorical), ambient temperature (continuous), and ambient air pollution (continuous).

The measurements of ambient temperature and air pollution were used to calculate individual average of lags from 0 to 7 days before the clinical examination at both city level and at each participant's home address for