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Respiratory inflammatory responses among occupants of a water-damaged office building

Abstract The National Institute for Occupational Safety and Health (NIOSH) received a request for evaluation of a water-damaged office building which housed approximately 1300 employees. Workers reported respiratory conditions that they perceived to be building related. We hypothesized that these symptoms were associated with airways inflammation. To test this hypothesis, we assessed airways inflammation in employees using exhaled breath condensate (EBC) and the fraction of exhaled nitric oxide (FENO). In September 2001, a health questionnaire was offered to all employees. Based on this questionnaire, NIOSH invited 356 symptomatic and asymptomatic employees to participate in a medical survey. In June 2002, these employees were offered questionnaire, spirometry, methacholine challenge test, allergen skin prick testing, EBC and FENO. FENO or EBC were completed by 239 participants. As smoking is highly related to the measurements that we used in this study, we included only the 207 current non-smokers in the analyses. EBC interleukin-8 (IL-8) levels, but not nitrite, were significantly higher among workers with respiratory symptoms and in the physician-diagnosed asthmatic group. Of the analyses assessed, EBC IL-8 showed the most significant relationship with a number of symptoms and physician-diagnosed asthma.

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Key words: Building-related symptoms; Exhaled breath condensate; Indoor air quality; Interleukin-8; Exhaled nitric oxide.

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Practical Implications
Implementation of exhaled breath condensate and exhaled nitric oxide in indoor air quality problems.

Introduction

Building-related symptoms in non-industrial workplaces such as office buildings have been reported since the early 1980s (Arnow et al., 1978; Kreiss, 1989). Seventy per cent of the US workforce is employed in non-industrial, non-agricultural indoor work environments. An estimated 20–30% of these workers have health symptoms that they attribute to indoor environmental quality (Woods, 1989) resulting in an estimated $22 billion impact on the US economy each year (Mendell et al., 2002; Milton et al., 2000). A major, commonplace cause of indoor environmental quality degradation is water damage. The recent Institute of Medicine-sponsored Damp Indoor Spaces and Health report, states that preventing or reducing the incidence of damp indoor environments is an important public health concern, but that specific causal agents have not yet been identified (Institute of Medicine of National Academies of Science, 2004). However, sufficient evidence exists of an association between the presence of mold or other agents in damp buildings and nasal and throat symptoms, cough, wheeze, asthma symptoms in sensitized asthmatic persons, and hypersensitivity pneumonitis (HP) in susceptible persons. Limited or suggestive evidence exists for an association between exposure to damp indoor environments and shortness of breath, asthma development, and lower respiratory disease in otherwise-healthy children. Therefore, taking preventive

measures to improve the indoor environment should be the main target to protect respiratory health of building occupants.

The National Institute for Occupational Safety and Health (NIOSH) received a request for a health hazard evaluation of an office building in the northeastern USA. Workers reported respiratory and dermatological conditions that they perceived to be building related. Post-occupancy onset of asthma, HP, and sarcoidosis had been documented in building occupants. Approximately 1300 people worked in this smoke free building. The facility was a 20-floor building with parking garages on the bottom four floors and a lobby/cafeteria/mezzanine area on the fifth floor. It had a history of water damage, particularly on the upper floors of the building. Since the mid-1990s, the building had incurred water intrusion through the roof, around windows, and through sliding doors of terraces. The upper floors had suffered the most water damage and mold contamination. During investigation of these problems, the building was found to be operating at a negative pressure with respect to the outdoors, which could lead to exacerbation of water incursion through the building envelope. Furthermore, there had been plumbing leaks on many floors which had damaged interior walls. The first major construction activity related to water incursion began in 2000, with repair of roof copings and brick caulking. From 2000 to 2002, cubicle partitions and carpets were cleaned, wetted carpet and stained wallboard replaced, wallpaper and underlying mold removed from bathrooms, upgrades to the air handling system made, and windows caulked. In 2001, NIOSH conducted a questionnaire survey in the building and found that compared with the US adult population, prevalence ratios were 2.2–2.5 for wheezing, lifetime asthma, and current asthma, 3.3 for adult-onset asthma, and 3.4 for symptoms improving away from work ($P < 0.05$). Adult asthma incidence density increased 7.5-fold after building occupancy. In 2002, abnormal lung function and/or breathing medication use was found in 67% of respiratory cases ($P < 0.01$) (Cox-Ganser et al., 2005).

We hypothesized that building-related symptoms reported by occupants were related to airways inflammation. To test this hypothesis, we assessed airways inflammation non-invasively by evaluation of exhaled breath condensate (EBC) and measurement of the fraction of exhaled nitric oxide (FENO) in workers at the office building. EBC and FENO are simple to perform, well tolerated by participants and without adverse side-effects. Use of these techniques has been studied extensively in asthma and studies showed that these techniques can provide objective and accurate methods for detecting airway diseases (Baraldi et al., 2003; Chatkin et al., 1999; Corradi et al., 2003; Gibson et al., 2000; Steerenberg et al., 2004).

Methods

The study population

A site visit was conducted in September 2001 to administer a short voluntary health questionnaire. Medical testing and a more detailed health questionnaire were offered in June 2002 to 356 symptomatic and asymptomatic employees who had been identified from the September 2001 questionnaire results (Cox-Ganser et al., 2005). Only employees who had worked in the building for at least 1 year were eligible for participation. During the site visit, an additional 15 employees asked to take part in the survey and they were included in the analysis. Study procedures were approved by the NIOSH Human Studies Review Board and all subjects provided informed consent using an approved consent form.

Questionnaire

Trained interviewers administered a computer-based comprehensive questionnaire to participants. The questionnaire included sections on demographics, work history, symptoms, physician-diagnosed conditions, medication use, smoking status, home environment, and quality of life. We defined classes of symptoms based on having any of the following symptoms weekly in the previous 4 weeks:

Any lower respiratory symptom: cough, wheezing, shortness of breath, or chest tightness.

Any nasal symptom: itchy nose, blockage of the nose, sneezing, or runny nose.

Any sinus symptom: headache, pain in face, blowing out thick mucus, or post-nasal drip in back of throat.

Work-related symptom: any one of the above symptoms which improves when away from work.

Spirometry

Experienced NIOSH technicians followed American Thoracic Society (ATS) recommendations for spirometry (American Thoracic Society, 1995). The test results were compared with the lower limit of normal (LLN) values from The National Health and Nutrition Examination Survey (NHANES) III reference values (Hankinson et al., 1999) to identify workers with abnormal spirometric patterns of obstruction and low vital capacity (American Thoracic Society, 1991). Airways obstruction was defined as both Forced expiratory volume in 1 s (FEV_1) and FEV_1/Forced vital capacity (FVC)% below the LLN.

Methacholine challenge test

Methacholine challenge test (MCT) was performed using standardized techniques (Crapo et al., 2000) with five different doses of methacholine (0.125, 0.5, 2.0, 8.0,

and 32.0 mg/ml). We reported methacholine dose as PC20, which was defined as the interpolated provocative concentration of methacholine that caused a 20% fall in FEV_1 from the baseline. We defined bronchial hyperresponsiveness as a PC20 of $\leq$ 16 mg/ml.

Bronchodilator administration

In subjects with a baseline FEV_1% $<$70% of the predicted value, a bronchodilator was offered to detect any reversible bronchoconstriction instead of offering a MCT. Two puffs of albuterol were administered via metered dose inhaler and were followed by spirometry. We defined reversibility as a 12% and 200 ml FEV_1 improvement after bronchodilator administration (American Thoracic Society, 1991).

Fraction of exhaled nitric oxide

Fraction of exhaled nitric oxide was measured off-line using standardized techniques with a rapid-response chemiluminescence analyzer (Sievers Instruments model 280, Boulder, CO, USA) according to the ATS 1999 guidelines (American Thoracic Society, 1999). A two-point calibration was performed each day using nitric oxide (NO)-free gas and 45 parts per million NO precision gas. We performed the off-line measurement of FENO by using 10-l Mylar gas-collection balloons and sampling kits (Sievers model 01410). The target backpressure of 13 cm H_2O was marked on the pressure meter, along with the acceptable range (10–15 cm H_2O). We collected two Mylar balloon samples per subject, analyzed each sample within 6 h of collection, and averaged the two readings. Measurements for three workers did not match within 2 ppb, so their FENO collection was repeated and re-analyzed. We have asked pre-test questions to exclude possible confounding factors for ENO measurements such as, smoking within the last hour, any nitrate containing food within the last 2 h, or having a recent respiratory infection. We re-scheduled those who had a positive response to any one of these confounders.

Exhaled breath condensate

Exhaled breath condensate was collected over a 15-min period from subjects using previously published techniques (Mutlu et al., 2001). Each subject was asked to perform normal tidal breathing into a disposable cold trap-collection device consisting of a coil of corrugated respiratory tubing (Corr-A-Flex II, Hudson Respiratory Care, Inc., Temecula, CA, USA) submerged into a -15°C bath of 50% ethylene glycol. Vapor in the exhaled breath condensed in the cold tubing. The 1–3 ml of condensate were collected in a vial and stored at less than -70°C until analysis. Interleukin-8 (IL-8) and nitrite were measured in EBC. Nitrite was measured using a chemiluminescence NO analyzer (Model 280, Sievers). This involved attaching a purge vessel containing 5 ml of 5% potassium iodide/acetic acid; 1 ml of 50 mg/ml iodine/water and 100 μl of 1:30 antifoam: water reagent (NIP 00013, Sievers). All samples were buffered to isotonicity and pH 7.4 using *N*-2-hydreoxyethylpiperazine-*N*′-2-ethanesulfonic acid for immunoassays. EBC was injected into the purge vessel and the resultant NO formed by the reduction of nitrite was purged from the vessel to the analyzer by helium. EBC nitrite was extrapolated from a plot of sodium nitrite standards run in parallel to the samples. IL-8 content was measured using a chemiluminescent immunoassay (QuantiGlo; R & D Systems, Minneapolis, MN, USA) with a 1.6 pg/ml limit of quantification. IL-8 ranges from 0.12 to 0.97 with a mean limit of detection (LOD) of 0.28 pg/ml. Nitrate LOD was 0.02 μM for a 50 μL sample. All assays were performed in duplicate.

Skin prick testing

Skin prick allergy testing was done with seven commercially available extracts of common indoor and outdoor allergens and three mold mixes using the GreerPIK system (Greer Laboratories, Lenoir, NC, USA): dust-mite mix (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*), German cockroach (*Blattella germanica*), cat hair, seven grass mix, ragweed mix, common weed mix, eastern eight tree mix, Dematiaceae mix (outdoor molds: *Alternaria tenuis*, *Cladosporium herbarum*, *Helminthosporium sativum*, *Pullularia pullulans*, *Spondylocladium atrovirens*, and *Curvularia spicifera*), *Aspergillus* mix, and *Penicillium* mix.

The allergens were placed on the forearm of the subject, along with a positive (histamine) and negative (glycerin in water) control. After 15 min, each response wheal length and width were measured to the nearest millimeter and recorded. For each wheal, the mean diameter (average of the length and width) was calculated. A positive reaction to an allergen was defined as an average diameter at least 3 mm larger than the negative control and $>$25% of the average diameter of the positive control (Dreborg et al., 1989). Atopy was defined as at least one positive skin prick test on allergy testing, using a total of seven common non-fungal antigen extracts. We excluded the fungal skin test results from the atopy definition as exposure to molds in the building was of concern and we wanted to look at the fungal skin prick test results independently of the atopy definition.

Statistical methods

We compared adjusted mean values of FENO, nitrite and IL-8 levels by symptoms, other medical test results and physician diagnoses, using General Linear Models.

Log-transformed FENO, IL-8, and nitrite were used for statistical analysis. We adjusted our analyses for associations between symptoms, lung function, and airways inflammation for age, gender, and atopy. Differences were considered significant at a level of $P < 0.05$. To test a possible effect modification of atopy status, we stratified our data by atopy and calculated the estimated mean values. We used Pearson's correlation to compare levels of FENO, nitrite, and IL-8. All analyses were performed using SAS® statistical software (SAS Institute Inc., Cary, NC, USA).

Results

Fraction of exhaled nitric oxide and/or EBC collection was completed by 224 of the 356 invited employees (63% participation) and additionally 15 volunteers. As smoking is highly related to measurements we used in this study, we excluded 32 employees who reported current smoking and we included only the 207 current non-smokers in the analyses. Of these 207 participants, 205 had interpretable FENO results and 199 had results reported for IL-8 and/or nitrite. Nitrite results were all above the LOD. IL-8 levels were all above the LOD, and 189 (95%) were above the limit of quantification.

The non-smoking participants were predominately female (60%), with a mean age of 47 years. Former smokers made up 28% of the group. Over half of these participants (58%) had at least one positive skin prick test indicative of atopy (Table 1).

The Pearson correlation coefficient between FENO and EBC nitrite level in this group was weak (r: 0.162, P: 0.02). There was no relationship found between FENO and IL-8.

In age-, gender-, and atopy-adjusted general linear models, EBC IL-8 levels were slightly, but statistically significantly higher among those with cough and both overall and work-related lower airway symptoms. EBC nitrite levels were statistically significantly higher (by a small amount) in the employees reporting shortness of breath and also reporting at least one of four lower respiratory symptoms (Table 2). In the models on upper airways symptoms, we found that only EBC IL-8 levels were slightly, but statistically significantly higher among those with overall symptoms (Table 3). FENO level was significantly lower in those participants with physician-diagnosed chronic bronchitis. EBC IL-8 was higher among workers with physician-diagnosed asthma (Table 4). FENO was higher in

Table 1 Characteristics of the non-smoking employees (n = 207)

Age (year, mean ± s.d.)	47.0 ± 8.3
Female	124/207 (59.9%)
Smoking status	
Never smoker	149/207 (72.0%)
Former smoker	58/207 (28.0%)
Atopic (per allergen skin prick tests)	109/189 (57.7%)
Airway obstruction by spirometry	22/205 (10.7%)
Positive methacholine or bronchodilator response	30/172 (17.4%)
FENO (ppb) (mean ± s.d.)	7.1 ± 3.6
IL-8 (pg/ml) (mean ± s.d.)	3.5 ± 4.0
Nitrite (μM) (mean ± s.d.)	0.8 ± 1.1

FENO, fraction of exhaled nitric oxide; IL-8, interleukin-8.

Table 2 Age-, gender-, and atopy-adjusted estimated mean FENO, nitrite, and IL-8 levels by selected lower airway symptoms among non-smoking employees

	FENO (ppb)		Nitrite (μM)		IL-8 (pg/ml)	
	Estimated mean (95% CI)	P*	Estimated mean (95% CI)	P*	Estimated mean (95% CI)	P*
Cough						
Yes	6.4 (5.8–7.0)	0.807	0.6 (0.5–0.8)	0.077	3.3 (3.0–3.7)	**0.007**
No	6.5 (5.9–7.0)		0.5 (0.4–0.6)		2.8 (2.6–3.0)	
Shortness of breath						
Yes	6.5 (5.8–7.4)	0.753	0.7 (0.5–0.9)	**0.045**	3.0 (2.7–3.5)	0.892
No	6.4 (5.9–6.8)		0.5 (0.4–0.6)		3.0 (2.8–3.2)	
Any lower airway symptom[a]						
Yes	6.4 (5.9–7.0)	0.946	0.6 (0.5–0.7)	**0.046**	3.3 (3.0–3.6)	**0.007**
No	6.4 (5.9–7.0)		0.5 (0.4–0.6)		2.8 (2.5–3.0)	
Work-related lower airway symptom[b]						
Yes	6.2 (5.6–6.9)	0.480	0.6 (0.5–0.8)	0.116	3.3 (3.0–3.7)	**0.026**
No	6.5 (6.0–7.0)		0.5 (0.4–0.6)		2.9 (2.6–3.1)	

[a]Having any one of the following symptoms in the last 4 weeks: cough, wheezing, shortness of breath, or chest tightness.
[b]Any one of the above symptoms which improves when away from work.
FENO, fraction of exhaled nitric oxide; IL-8, interleukin-8.
*$P = 0.05$.

Table 3 Age-, gender-, and atopy-adjusted estimated mean FENO, nitrite, and IL-8 levels by selected upper airway symptoms among non-smoking employees

	FENO (ppb)		Nitrite (μM)		IL-8 (pg/ml)	
	Estimated mean (95% CI)	P*	Estimated mean (95% CI)	P*	Estimated mean (95% CI)	P*
Sneezing						
Yes	6.4 (5.9–7.0)	0.815	0.5 (0.4–0.6)	0.950	3.3 (3.0–3.6)	**0.003**
No	6.5 (5.9–7.1)		0.5 (0.4–0.7)		2.7 (2.5–3.0)	
Runny nose						
Yes	6.6 (6.1–7.2)	0.481	0.5 (0.5–0.7)	0.864	3.3 (3.0–3.6)	**0.004**
No	6.3 (5.8–6.9)		0.5 (0.4–0.7)		2.7 (2.5–3.0)	
Any nasal symptom[a]						
Yes	6.6 (6.1–7.1)	0.313	0.6 (0.5–0.7)	0.102	3.1 (2.9–3.4)	**0.030**
No	6.1 (5.4–6.9)		0.4 (0.3–0.6)		2.6 (2.3–3.0)	
Work-related nasal symptom[b]						
Yes	6.4 (5.9–7.1)	0.932	0.6 (0.5–0.7)	0.217	3.2 (2.9–3.6)	0.099
No	6.5 (6.0–7.0)		0.5 (0.4–0.6)		2.9 (2.6–3.1)	
Any sinus symptom[c]						
Yes	6.5 (6.1–7.1)	0.608	0.5 (0.4–0.6)	0.617	3.2 (2.9–3.4)	**0.039**
No	6.3 (5.7–7.0)		0.6 (0.4–0.7)		2.7 (2.4–3.1)	
Work-related sinus symptom[b]						
Yes	6.3 (5.7–7.0)	0.496	0.6 (0.4–0.7)	0.495	3.3 (2.9–3.7)	0.054
No	6.6 (6.1–7.1)		0.5 (0.4–0.6)		2.9 (2.7–3.1)	

[a]Having any one of the following symptoms in the last 4 weeks: itchy nose, blockage of the nose, sneezing, or runny nose.
[b]Any one of the above symptoms which improves when away from work.
[c]Having any one of the following symptoms in the last 4 weeks: headache, pain in face, blowing out thick mucus, or post-nasal drip in back of throat.
FENO, fraction of exhaled nitric oxide; IL-8, interleukin-8.
*$P = 0.05$.

Table 4 Age-, gender-, and atopy-adjusted estimated mean FENO, nitrite, and IL-8 levels by physician-diagnosed diseases, and pulmonary function tests among non-smoking employees

	FENO (ppb)		Nitrite (μM)		IL-8 (pg/ml)	
	Estimated mean (95% CI)	P*	Estimated mean (95% CI)	P*	Estimated mean (95% CI)	P*
Physician-diagnosed asthma						
Yes	7.0 (6.2–7.9)	0.106	0.6 (0.5–0.8)	0.237	3.4 (3.0–3.8)	**0.040**
No	6.2 (5.8–6.7)		0.5 (0.4–0.6)		2.9 (2.7–3.1)	
Physician-diagnosed chronic bronchitis						
Yes	5.2 (4.3–6.2)	**0.018**	0.6 (0.4–0.9)	0.646	3.4 (2.8–4.1)	0.163
No	6.6 (6.2–7.0)		0.5 (0.5–0.6)		3.0 (2.8–3.2)	
Airway obstruction by spirometry						
Yes	6.2 (5.2–7.4)	0.681	0.5 (0.3–0.7)	0.586	2.8 (2.3–3.4)	0.398
No	6.5 (6.0–6.9)		0.5 (0.5–0.6)		3.0 (2.8–3.3)	
Positive methacholine or bronchodilator response						
Yes	7.0 (6.0–8.2)	0.153	0.5 (0.3–0.7)	0.726	3.0 (2.6–3.4)	0.684
No	6.2 (5.8–6.7)		0.5 (0.5–0.6)		2.9 (2.7–3.1)	

FENO, fraction of exhaled nitric oxide; IL-8, interleukin-8.
*$P = 0.05$.

those with self-reported hay fever ($P = 0.019$) (data not shown). Calculating estimated mean values after stratifying our data according to atopy status, did not show us any significant change in FENO and EBC nitrate results. However, EBC IL-8 levels among those with nasal symptoms or/and sinus symptoms were significantly higher for non-atopic only. We observed no significant relationship between EBC, ENO, and skin prick test, spirometry, and MCTs.

Discussion

Previous epidemiologic studies have demonstrated a relationship between water-damaged buildings and increased respiratory symptoms among occupants (Chao et al., 2003; Dales et al., 1991b; Dales et al., 1991a; Strachan, 1988; Strachan et al., 1990). Cox-Ganser et al. (2005) showed that occupancy of this office building was associated with respiratory symptoms and diagnoses of asthma and HP (Clini et al., 2000). In this study, we have evaluated a potential association between airways inflammation assessed using objective measures such as FENO and EBC, and whether this was associated with overall and work-related symptoms among office workers.

We found no significant relationship between FENO and symptoms in this group of non-smokers. However, the FENO level was significantly lower in those participants with physician-diagnosed chronic bronchitis. This is consistent with literature reporting reduced FENO in patients with primary pulmonary hypertension, chronic obstructive pulmonary disease (COPD), cystic fibrosis, and ciliary dyskinesia (Chatkin et al., 1999; Clini et al., 2000; Kharitonov and Barnes, 2000). The relative magnitude of the difference in FENO between groups was small. In addition, NO was determined as its metabolite nitrite from EBC in our study. There was a statistically significant, but extremely weak relationship between FENO and EBC nitrite. This suggested that NO was a significant, but not the only source of lung nitrite. Nitrite and nitrate can also originate from various food sources.

The only statistically significant changes in nitrite levels between symptomatic and non-symptomatic participants were for 'shortness of breath' and 'any lower respiratory symptoms'. Purokivi et al. (2002) concluded that because of the wide spectrum of microbial exposure in different indoor environments, NO alone may not be a sufficient marker of airways inflammation (Purokivi et al., 2002). Expression of inducible nitric oxide synthase in macrophages is only detected after exposure to bacterial strains but not after exposure to fungal species isolated from moisture-damaged buildings (Hirvonen et al., 1997; Huttunen et al., 2000). Franklin et al. (2005) investigated methodological issues for measuring NO metabolites in EBC and they found an inherent variability in repeated measurements that cannot be explained by the collection or analytical technique (Franklin et al., 2005).

Exhaled breath condensate IL-8 was statistically related to a number of symptoms and physician-diagnosed asthma cases. In particular, EBC IL-8 levels were higher in subjects reporting work-related lower airways symptoms, suggesting a potential relationship between mild pulmonary inflammation and building-related exposure. It must be noted, however, that the EBC IL-8 concentrations were extremely low and close to the assay limits of quantification. In addition, the differences in IL-8 concentration between groups were small.

As smoking has been found to be associated with airways inflammation, (Garey et al., 2004) a major strength of this study was that we were able to investigate associations between markers of inflammation and health effects in the large non-smoking population. The one-time measurement of inflammatory markers is a limitation of the present cross-sectional study. Single point collection does not allow for evaluation of temporal relationships or intra-participant variability with respect to inflammatory marker production. Furthermore, the inherent difficulty and variability in the detection of low concentrations of IL-8 in EBC, point to the need for further investigation of the association we found in this study.

To our knowledge, this study is the first to use EBC to evaluate relationships between airways inflammatory markers and building-related health problems. In summary, we did not observe a strong relationship between FENO, EBC nitrate, and water-damaged building-related symptoms. However, we suggest that EBC IL-8 should be carefully evaluated to investigate a potential relationship between water-damaged buildings and respiratory inflammation lending support to the phenomenon of respiratory effects of water-damaged environments.

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Disclaimer

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