Higher Environmental Relative Moldiness Index Values Measured in Homes of Adults with Asthma, Rhinitis, or both Conditions

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Abstract

Higher values of the Environmental Relative Moldiness Index (ERMI), a DNA-based method for quantifying indoor molds, have been associated with asthma in children. In this study, settled dust samples were collected from the homes of adults with asthma, rhinitis, or both conditions (n=139 homes) in Northern California. The ERMI values for these samples were compared to those from dust collected in homes from the same geographic region randomly selected as part of the 2006 American Healthy Home Survey (n=44). The median ERMI value in homes of adult with airway disease (6) was significantly greater than median ERMI value (2) in the randomly selected homes (p<0.0001). In this study in northern California, the homes of adults with asthma had ERMI values consistent with a heavier burden of indoor mold than that measured in other homes from the same region.

Keywords

adult; asthma; ERMI; quantitative PCR

1. Introduction

The prevalence of adult asthma in the US was estimated to have climbed from 6.9% in 2001 to 7.7% by 2009 (Zahran et al, 2011) and the annual adult asthma incidence is 4.9 per 1000 person-years among adult females and 2.8 per thousand person years among males (Winer et. al., 2012). A large set of genetic, social, and environmental factors are believed to be potentially associated with adult asthma prevalence or severity (Apter, 2011). Among the

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many environmental factors, exposure to mold in water-damaged buildings is a distinct factor of interest because of its suspected association with disease (WHO, 2009) and the potential to intervene in order to ameliorate symptoms through lowering exposure.

The Environmental Relative Moldiness Index (ERMI) is a method for indoor mold exposure quantification that may be particularly relevant to residential exposure assessment among persons with asthma. The ERMI was developed using quantitative polymerase chain reaction (QPCR) methodology to measure the DNA concentrations of 36 molds (Haugland et al., 2004; Vesper et al., 2007a). The 36 molds were selected to include 26 "Group 1" molds indicative of damp environments consistent with indoor water damage and 10 "Group 2" mold species that are commonly found in homes without the presence of water damage (Vesper, 2011).

The ERMI is calculated (Equation 1), by taking the sum of the logs of the concentrations of the Group 1 fungi (s_1) and subtracting the sum of the logs of the concentrations of Group 2 fungi (s_2), thus weighting the ERMI toward damp environment molds (Vesper et al., 2007a).

$$ERMI = \sum_{i=1}^{26} \log_{10}(s_{1i}) - \sum_{j=1}^{10} \log_{10}(s_{2j})$$

The ERMI was developed using data from the QPCR analysis of a random sample of 1083 U.S. homes that participated in the Department of Housing and Urban Development (HUD) 2006 American Healthy Homes Survey (Vesper et al., 2007a). The ERMI scale ranges from approximately negative 10 (indicative of very little relative mold contamination) to positive values of 20 (consistent with relatively high amounts of indoor dampness-associated mold). The ERMI scale can be divided into quartiles based on the distribution originally observed; the uppermost quartile, based on a national sample of homes, starts at an ERMI value of 5 (Vesper et al., 2007a).

These normative data for homes have not been compared to data obtained for the homes of adults with respiratory tract illness. In this analysis, we compared ERMI values from dust collected in the homes of an adult asthma and rhinitis cohort geographically limited to northern California to data from a subset of the 2006 American Healthy Homes Survey from the same geographic area. We wished to test whether there were systematic differences between these two samples that might be contributing to the health status of the group with asthma, rhinitis, or both conditions.

2. Materials and methods

A group of subjects with asthma was recruited initially through a random sample of pulmonary or allergy specialty practices in northern California beginning in 1992, supplemented by an additional sample of family medicine specialty practices (1996) and, later, a random digit dial recruitment with eligibility based on participant's report of a physician's condition diagnosis of asthma, chronic rhinitis, or both conditions (1999). In 2008, this community-based cohort was combined with another group of participants drawn from a hospital-based cohort of adults with asthma who were members of a regional, integrated health care delivery organization (IHCDO). The details of the recruitment, selection and retention for the merged asthma and rhinitis cohort have been published previously (Chen et al., 2011; Trupin et al., 2012).

All subjects in the asthma- rhinitis cohort interviewed in the study who were in geographic range (n=496) were invited to participate in home visits that included systematic sampling of the indoor environment, including settled house dust. To obtain settled dust samples from the kitchen and living room, the kitchen floor was vacuumed for at least two minutes and the living room floor for at least six minutes, drawing in air through a pre-filter mesh to remove large particles (356 microns) and the dust collected on a back filter; dust was not collected from the vacuum bag. A fixed size in floor area was not set.

Although dust samples were successfully collected for 302 homes at the time of study, other analyses consumed some of this dust. After other investigations, sufficient dust samples remained for possible ERMI analysis from 202 of the homes originally sampled. Samples were kept at room temperature until shipping for the ERMI analysis. Of those 202 samples, 139 were analyzed in an initial set of assays, while 63 others where only analyzed at a later time (mean time from collection until ERMI analysis 17 v. 22 months; p<0.0001). Among the 139 samples analyzed in the first group there was no association between time elapse until analysis and the ERMI value observed (p=0.39) but among the 63 samples analyzed later, each month of elapsed time was until assay associated with a 1.1 point increase in the ERMI value (p=0.002). We therefore excluded those samples from further consideration, limiting this study to the 139 without a time until analysis effect.

Of subjects in the asthma-rhinitis cohort whose homes were included, 82% had both asthma and rhinitis; approximately one fifth had either asthma or chronic rhinitis alone. The time of home sampling was not linked to disease activity, which varied within the cohort. The 139 homes from which samples were analyzed all had addresses that fell in postal zip codes beginning with the initial two digits of 93, 94, or 95, consistent with the northern California study catchment area. The study was approved by the University of California San Francisco Committee on Human Research.

The HUD survey methods have been described previously (Vesper et al., 2007a). All randomly selected HUD survey homes (referred to hereafter as "Random") with addresses that started with the digits 93, 94, or 95 (n=44) were selected for analysis. This subset of Northern CA samples was from a total of 1083 obtained in the entire US. This study was reviewed for human subject involvement by the Westat Institutional Review Board and a Confidentiality Certificate protecting the identity of the survey respondents issued by the National Institute of Environmental Health Sciences.

Dust samples in the HUD study were collected by vacuuming 2 m^2 in the living room and 2 m^2 in a bedroom for 5 min each with a Mitest™ sampler-fitted vacuum, directly adjacent to the sofa or bed, respectively. The dust was sieved through a 300-micron pore size nylon mesh (Gilson Company, Inc. Lewis Center, OH) and shipped frozen on ice packs overnight to the analytical laboratory and maintained frozen at −20°C until analyzed (no more than 3 months storage). (Vesper et al., 2007a).

The specifics of the QPCR assays yielding the calculated ERMI values have been reported (Haugland et al., 2002; Brinkman et al., 2003; Haugland et al., 2004). In brief, each dust sample (5.0 + 0.1 mg) is spiked with 1×10^6 conidia of *Geotrichum candidum* as an external reference. Each extraction tube is shaken in the bead beater (Biospec Products, Bartlesville, OK) for one minute and the DNA purified using the DNA-EZ extraction kit (GeneRite, Cherry Hill, NJ). Each QPCR assay contained 12.5 µl of "Universal Master Mix" (Applied Biosystems Inc., Foster City, CA), 1 µl of a mixture of forward and reverse primers at 25 µM each, 2.5 µl of a 400 nM TaqMan probe (Applied Biosystems Inc.), 2.5 µl of 2 mg/ml fraction V bovine serum albumin (Sigma Chemical, St. Louis, MO) and 2.5 µl of DNA free water (Cepheid, Sunnyvale, CA). To this mix, 5 µl of the DNA extract from the study

sample is added. All primer and probe sequences used in the assays as well as known species comprising the assay groups are at the website: http://www.epa.gov/nerlcwww/moldtech.htm. Primers and probes were synthesized commercially (Applied Biosystems, Inc.).

Reactions were performed with thermal cycling conditions consisting of 2 minutes at 50°C, 10 minutes at 95°C, followed by 40 cycles of 15 seconds at 95°C for template denaturation and 1 min at 60°C for probe and primer annealing and primer extension. The cycle threshold determinations were automatically performed by the instrument ABI 7900 (Applied Biosystems, Inc.) using default parameters. Assays for each target species and the internal reference (*Geotrichum candidum*) were performed in separate tubes of the 96-well plate format.

Differences between the two study groups in the overall ERMI values as well as differences in the concentrations of 36 individual component mold species that contribute to the ERMI were tested using the Wilcoxon rank sum test. Measured numbers of spores were generally highly skewed within any given species. In addition, a large proportion of results were below detection limits in many cases, for example over 50% non-detects among four species, and over 20% in eleven other species. For these reasons, exact rank tests were performed via Monte Carlo simulation. (10,000 iterations performed). For the individual molds, the Holm-Bonferroni procedure was used to adjust for the multiple comparisons such that the critical p-value used is equivalent to the 0.05 alpha level in the absence of multiple comparisons (Holm, 1975). Analyses were performed in SAS version 9.2 (SAS Institute, Cary NC) and R version 2.14 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

As shown in Table 1, the median ERMI value of 6 (interquartile difference [IQ] =7) in the homes from the adult asthma- rhinitis cohort was significantly higher than the median ERMI value of 2 (IQ=6) in the randomly selected homes from the same zipcodes in northern California (p<0.0001). The observed median value for the asthma- rhinitis cohort was above the upper quartile cut-point for the random group. The logarithmically transformed summary data for both the Group 1 molds (those believed to be more indicative of indoor water damage) and the Group 2 molds (those believed to not to be linked to damp indoor environments) were higher in homes from the asthma- rhinitis cohort compared to the random sample (p<0.0001). The larger population of water-damaged associated Group 1 molds primarily accounted for the difference in the average ERMI values (the difference between the summary log values of Group 1 and Group 2) between the asthma-rhinitis and comparison cohorts. Excluding the results from the 9 homes of subjects with rhinitis only had no effect on the median or interquartile ranges shown in Table 1 except for a shift in the median value for the Group 1 molds from 27 to 26. All of the differences remained significant (p<0.0001).

The patterns of differences for individual molds are shown in Table 2. Of 26 Group 1 molds, 14 were present in statistically significantly higher concentrations in dust sampled in the homes from the asthma-rhinitis cohort. The highest concentration in the asthma-rhinitis homes was noted for *Aureobasidium pullulans*, a Group 1 mold whose median concentration in cells per mg of dust was 55,000, compared to 1100 in the random sample of homes, a 50fold ratio. Of note, one of the Group 1 molds (the *Erotium* group) was found in significantly higher concentrations in dust from the random sample homes. Eight of the 10 Group 2 molds were also present in significantly higher concentrations in dust sampled form the homes from the asthma-rhinitis cohort. Indeed, the two molds in the next highest concentrations to *Aureobasidium* were both *Cladosporium* species from Group 2.

4. Discussion

Elevated ERMI findings have been reported in homes of asthmatic children in Detroit, MI (Vesper et al., 2008), Chapel Hill, NC (Vesper et al., 2007b), and Cincinnati, OH (Reponen et al., 2011; Reponen et. al,. 2012). This is the first study of ERMI values in the homes of adults with asthma, rhinitis, or both conditions. It is particularly relevant to questions of airway disease symptoms and control as opposed to etiology, since few if any of the adults we studied would have been living in the same dwelling since the onset of their disease.

Although the random sample was from the same geographic region, other factors unrelated to health status may have contributed to the differences in ERMI values that we observed. This could include systematic differences in settled dust collection protocols, storage, seasonal or secular trends, microclimatic variables within the region, socioeconomic differences even within the same region, or other unmeasured factors that might have acted as confounders. The fact that the dust samples had been stored under differing conditions frozen for various different lengths of time could have resulted in some DNA degradation could have affected the results. We excluded, however, samples from a group where analysis did indicate a storage duration effect. The algorithm yielding the ERMI value, moreover, is designed such that an overall higher total mold level should not yield a different relationship between the ERMI's indoor molds associated with dampness (Group 1) and outdoor components. Our sample was of relatively small size, particularly the 44 samples from the zip code matched national sample, but this did not appear to limit study power to observe statistically significant differences.

There is growing recognition that mold may be a predictor of childhood asthma risk (Rabinovitch, 2012). Further, it has been speculated that the prevention of exposures to or remediation of water-damaged homes might reduce asthma's impact on health (Mudarri and Fisk, 2007; WHO, 2009). Remediation of water-damaged homes resulted in a 10-fold reduction in emergency room visits or hospitalizations for children (Kercsmar et al., 2006). A similar remediation study for the homes of adults with asthma and/or rhinitis would be of interest, although, because of time spent outside the home by adults, mold contamination in other locations may also be relevant (Karvala et al., 2011).

Acknowledgments

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Highlights

- Higher values of the ERMI mold index indicate more abundant indoor mold associated with damp environments.

- Homes of adults with asthma, rhinitis, or both conditions in northern California had higher ERMI values than a comparison sample

- These findings are particularly relevant to potential sources of symptom exacerbation

Table 1

Comparison of Environmental Relative Moldiness Index (ERMI) component and overall values for settled dust samples from 246 northern California homes

ERMI Component	Random Sample n=44		Asthma Rhinitis Cohort n=139		p-value
	Median	IQ	Median	IQ	
Group 1 Mold Value	15	6	27	9	<0.0001
Group 2 Mold Value	14	6	21	5	<0.0001
Overall ERMI Value	2	6	6	7	<0.0001

Group Mold Values = Sum of the logs Group 1 and Group 2 molds, respectively

Overall ERMI = Difference of the sum of logs, Group 1-Gourp 2

IQ = Interquartile range difference between the upper and lower observed quartiles

Differences tested using the Wilcoxon rank sum test

Table 2

Individual molds contributing to the Environmental Relative Mold Index (ERMI) determination in settled dust samples from northern California homes

Mold Species by Group	Individual Mold Species Cells per mg Dust					Holm's adjusted p value
	Random Sample (n=44)		Asthma-Cohort Rhinitis n=139			
	Median	I-Q	I-Q	Median	I-Q	
Group 1						
Aspergillus flavus	0		4	3	7	NS
Aspergillus fumigatus	8		24	21	32	NS
Aspergillus niger	6		11	100	269	< 0.001
Aspergillus ochraceus	0		0	6	17	< 0.001
Aspergillus penicillioides	31		149	7	36	NS
Aspergillus restrictus	0		0	15	78	< 0.001
Aspergillus sclerotiorum	0		0	0	0	NS
Aspergillus sydowii	0		0	0	2	NS
Aspergillus unguis	0		0	0	0	NS
Aspergillus versicolor	0		0	4	11	< 0.001
Aureobasidium pullulans	1100		1512	55000	89000	< 0.001
Chaetomium globosum	0		2	0	3	NS
Cladosporium sphaerospermum	6		20	39	62	< 0.001
Eurotium group	150		929	27	89	0.001
Paecilomyces variotii	0		4	2	5	NS
Penicillium brevicompactum	50		458	370	650	0.001
Penicillium corylophilum	0		4	6	12	< 0.001
Penicillium crustosum	0		0	49	113	< 0.001
Penicillium purpurogenum	0		0	5	14	< 0.001
Penicillium spinulosum	2		5	3	8	NS
Penicillium variabile	0		5	21	50	< 0.001
Scopulariopsis brevicaulis	0		14	3	9	NS
Scopulariopsis chartarum	0		4	6	16	< 0.001
Stachybotrys chartarum	0		13	11	29	0.036
Trichoderma viride	0		0	16	48	< 0.001

Mold Species by Group	Individual Mold Species Cells per mg Dust					
	Random Sample (n=44)	Asthma-Cohort Rhinitis n=139			Holm's adjusted p value
	Median	I-Q	Median	I-Q	
Group 1					
Wallemia sebi	0	29	520	1620	< 0.001
Group 2					
Acremonium strictum	6	13	4	8	NS
Alternaria alternata	37	120	330	730	< 0.001
Aspergillus ustus	0	0	8	20	< 0.001
Cladosporium cladosporioides type 1	538	797	9000	13400	< 0.001
Cladosporium cladosporioides type 2	28	36	370	760	< 0.001
Cladosporium herbarum	257	600	6600	11600	< 0.001
Epicoccum nigrum	146	439	910	1940	< 0.001
Mucor group	15	27	210	469	< 0.001
Penicillium chrysogenum *type 2*	22	73	26	64	NS-
Rhizopus stolonifer	0	2	21	62	< 0.001

I-Q = Interquartile range difference between the upper and lower observed quartiles.

Holm's adjusted p-value = Lowest alpha level at which the respective difference between median values using the Wilcoxon rank sum test would be significant based on the Holm's-Bonferroni test.

NS = adjusted p >0.05