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Short communication

Higher Environmental Relative Moldiness Index (ERMI^{sm}) values measured in Detroit homes of severely asthmatic children☆

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ABSTRACT

Sieved vacuum bag dust from the homes of 143 children in Detroit was analyzed by mold specific quantitative PCR (MSQPCR) and the Environmental Relative Moldiness Index (ERMI^{sm}) was calculated for each home. Children living in these homes were grouped as non-asthmatic ($n=83$), moderately asthmatic ($n=28$) and severely asthmatic ($n=32$) based on prescription medication usage for their asthma management (none, occasional and daily, respectively). The mean ERMI for each group of homes was 6.2 for non-asthmatic, 6.3 for moderately asthmatic and 8.2 for severely asthmatic children. The ERMI values in the homes of severely asthmatic children were significantly greater compared to the non-asthmatics ($p=0.04$ in Wilcoxon Rank-sum test). *Aspergillus niger* and *Aspergillus unguis* were the primary mold species that distinguished severely asthmatic children's homes and non-asthmatic children's homes ($p<0.05$; Wilcoxon Rank-sum test). The determination of the home's ERMI values may aid in prioritizing home remediation efforts, particularly in those children who are at increased risk for asthma exacerbation.

1. Introduction

Asthma is the most common chronic disease of children in the United States (US) (Rudd and Moorman, 2007). It is characterized by inflammation of the air passages resulting in a narrowing of the airways and its complex etiology involves genetic and environmental factors (Platts-Mills et al., 2006). The study reported here is part of the larger Environmental Protection Agency's Mechanistic Indicators of Childhood Asthma (MICA) study. In this segment of the MICA study, the focus was on mold and asthma.

Mold is one of the many well known triggers for asthma exacerbation (IOM, 2004). We developed the Environmental Relative Moldiness Index (ERMI) (Vesper et al., 2007c) to

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quantify the risk associated with mold burden in a home. The ERMI uses a DNA-based technology called mold specific quantitative PCR (MSQPCR), and a standardized method of collecting, processing and analyzing dust samples (Vesper et al., 2007c). The ERMI value is computed by quantifying 36 indicator mold species (Table 1) in a home dust sample that encompass 26 so called Group 1 molds that indicate water intrusion and 10 Group 2 species that are commonly found,

Table 1 – Median concentration and prevalence of each mold species expressed as cell equivalents (CE) per mg in vacuum bag dust from Detroit homes of non-asthmatic (NA) and severely asthmatic (SA) children

Mold species	Median CE/mg dust in SA	Percent detected in SA homes	Median CE/mg dust in NA	Percent detected in NA homes	Wilcoxon Rank-sum test *p*-values
Group 1					
Aspergillus flavus[a]	**	18	1	31	0.848
Aspergillus fumigatus[b]	1	47	2	44	0.386
Aspergillus niger[c]	**67**	**100**	**24**	**96**	**0.007**
Aspergillus ochraceus[d]	40	88	24	81	0.092
Aspergillus penicillioides	52	97	52	100	0.507
Aspergillus restrictus[e]	**	3	**	8	**
Aspergillus sclerotiorum	2	47	2	40	0.281
Aspergillus sydowii	17	71	6	72	0.242
Aspergillus unquis	**3**	**71**	**2**	**52**	**0.024**
Aspergillus versicolor	12	85	14	81	0.372
Aureobasidium pullulans	5400	100	5700	100	0.374
Chaetomium globosum	9	65	11	72	0.769
Cladosporium sphaerospermum	16	100	9	100	0.102
Eurotium Group[f]	68	100	54	99	0.111
Paecilomyces variotii	3	62	3	53	0.103
Penicillium brevicompactum	14	76	17	76	0.725
Penicillium corylophilum	3	35	2	38	0.547
Penicillium Group[g]	16	21	11	22	0.495
Penicillium purpurogenum	**	18	2	25	0.783
Penicillium spinulosum[h]	**	3	**	0	**
Penicillium variabile	27	53	14	64	0.389
Scopulariopsis brevicaulis	3	38	2	39	0.461
Scopulariopsis chartarum	6	50	3	56	0.345
Stachybotrys chartarum	3	79	3	61	0.260
Trichoderma viride[i]	2	62	2	67	0.771
Wallemia sebi	70	100	96	99	0.471
Group 2					
Acremonium strictum	1	44	1	35	0.262
Alternaria alternata	42	100	46	100	0.596
Aspergillus ustus	5	71	3	68	0.094
Cladosporium cladosporioides Type 1	325	100	370	100	0.588
Cladosporium cladosporioides Type 2	7	100	10	96	0.703
Cladosporium herbarum	135	100	160	100	0.780
Epicoccum nigrum	275	100	300	100	0.534
Mucor Group[j]	37	100	30	96	0.295
Penicillium chrysogenum Type 2[k]	6	76	8	81	0.752
Rhizopus stolonifer	7	44	3	56	0.845

The Wilcoxon Rank-sum test results for CE are shown with *p* values adjusted for multiple comparisons. The mold species listed in bold are significantly different ($p<0.05$) when severely asthmatic homes are compared to the homes of non-asthmatic children. Median values were computed using a Kaplan–Meier survival model with data modified to account for left-censoring. Medians and Wilcoxon tests for mold species with fewer than 20% detections (**) were not calculated.

[a]Includes *A. flavus* and *A. oryzae*.

[b]Includes *A. fumigatus* and *Neosartorya fischeri*.

[c]Includes *A. niger*, *A. foetidus* and *A. pheonicis*.

[d]Includes *A. ochraceus* and *A. ostianus*.

[e]Includes *A. restrictus*, *A. caesillus* and *A. conicus*.

[f]Includes *E. amstelodami*, *E. chevalieri*, *E. herbariorum*, *E. rubrum* and *E. repens*.

[g]Includes *P. crustosum*, *P. camembertii*, *P. commune*, *P. echinulatum* and *P. solitum*.

[h]Includes *P. spinulosum*, *P. glabrum*, *P. lividum*, *P. pupurescens* and *P. thomii*.

[i]Includes *T. viride*, *T. atroviride* and *T. koningii*.

[j]Includes *M. amphibiorum*, *M. circinelloides*, *M. hiemalis*, *M. indicus*, *M. mucedo*, *M. racemosus*, *M. ramosissimus*, *R. azygosporus*, *R. homothallicus*, *R. microsporus*, *R. oligosporus* and *R. oryzae*.

[k]This is the dominant subgroup of species.

even without water damage (Vesper et al., 2007c). Since it is not always possible to obtain a standardized dust sample, dust from the vacuum bag has been shown to be a useful screening tool. ERMI values were higher in asthmatic homes as compared to the homes of non-asthmatic children (Vesper et al., 2007a). The current study assesses the relationship between the ERMI values of homes in Detroit Michigan and the status of the children's asthma.

2. Materials and methods

2.1. Recruitment and eligibility

The protocol was approved by the US Environmental Protection Agency, Westat Inc. and the Henry Ford Health System (HFHS) institutional review boards. The study population consisted of children aged 9–12, whose parent or guardian was enrolled in a nonprofit managed care organization in southeastern Michigan. The study was conducted over a 10 week period beginning in October 2006. If a parent or guardian was able to bring in a vacuum bag ($n=143$), participating children were divided into non-asthmatic ($n=83$), moderately asthmatic ($n=28$) and severely asthmatic ($n=32$) based on the parent's description of their child's use of prescribed medication for asthma management (none, occasional and daily, respectively). Using the Global Initiative for Asthma (GINA) classification system (http://www.ginasthma.com), these categories of asthmatics would be best described as non-, intermittent and persistent asthmatics. The mean age of the children ($n=143$) reported here was 11.6 years; boys (56%) and girls (44%). African Americans and Caucasians comprised 89% and 10% of the participants, respectively.

2.2. Dust collection and analysis and ERMI calculation

Vacuum cleaner dust was transferred into a nylon 300-micron sieve (SV-126; Gilson Company, Lewis Center, OH) equipped with a clean polypropylene bottom collection pan and lid. The sieve assembly was placed in a RX-86 Sieve Shaker (W.S. Tyler, Mentor, OH) and following 30–45 min of vibration, the dust collected for fungal DNA analysis.

The Environmental Relative Moldiness Index (ERMI) values were computed based on the analysis of 5 mg of the sieved dust that had been analyzed by MSQPCR, as previously described (Haugland et al., 2002; Brinkman et al., 2003; Haugland et al., 2004; Meklin et al., 2004; Vesper et al., 2004, 2007b). All primer and probe sequences, as well as known species comprising the assay groups, have been published elsewhere: http://www.epa.gov/nerlcwww/moldtech.htm.

The ERMI is calculated by taking the sum of the logs of the concentrations of the Group 1 species and subtracting the sum of the logs of the concentrations of Group 2 species (Vesper et al., 2007c). For all fungal species not detected in the dust sample, their concentration is set to the minimum detection limit of 1 cell equivalent (CE) per mg dust before log transformation. The ERMI is a single numeric value that represents an index of risk consequential to the mold burden in the home (Vesper et al., 2007c). However, the ERMI cannot be related to any measure of absolute moldiness or absolute mold burden in homes.

2.3. Statistical analyses

Two-sample comparisons between the severe asthma and non-asthma groups were tested using the Wilcoxon Rank-sum test. Mold concentration data generated by MSQPCR had a minimum detection limit (MDL) of 1 cell equivalent (CE) per mg of fine dust. Samples detected below the MDL were set to a value less than the MDL. In this manner, all results below detectable limits were tied at the lowest rank and thus had no effect on the computed Wilcoxon test statistic. Median values (reported in Table 1) were computed using a Kaplan–Meier survival model with data modified to account for left-censoring. For a more detailed description on this method, see Helsel (2005). Median values and Wilcoxon test results were not computed for mold species with fewer than 20% detections per sample group (Helsel, 2005).

3. Results

3.1. ERMI analysis

The mean ERMI values for the homes of the non-asthmatic, moderately asthmatic and severely asthmatic groups of children were 6.2, 6.3 and 8.2, respectively (Fig. 1). The average ERMI in the non-asthmatic and severely asthmatic populations' homes were significantly different ($p=0.04$; Wilcoxon Rank-sum test). However, the ERMI values in homes from children with moderate asthma were not significantly different from those in non-asthmatic children's homes (Fig. 1).

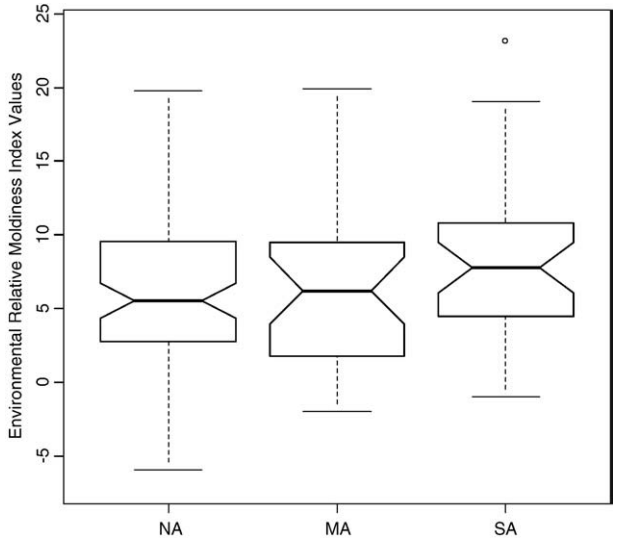

Fig. 1 – Box—plot of mean Environmental Relative Moldiness Index (ERMI) values for vacuum bag dust from homes of non-asthmatic (NA) ($n=83$), moderately asthmatic (MA) ($n=28$) and severely asthmatic (SA) ($n=32$) children in Detroit, Michigan, USA. The average ERMI value in the homes of non-asthmatic and severely asthmatic children were significantly different ($p<0.05$) based on the Wilcoxon Rank-sum test.

Table 1 shows the median cell equivalent concentration of the 36 species of mold as detected in the reservoir dust specimens from the homes of the non-asthmatic and severely asthmatic children's homes, as well as their detection rate in these homes. The CE concentrations of Group 1 species, *Aspergillus niger* and *Aspergillus unguis*, were the only mold species that were statistically elevated in occurrence or concentration in the homes of severely asthmatic children in comparison to those of the non-asthmatic children ($p<0.05$). *A. niger* was detected in nearly all homes (Table 1) but it was measured in about 3.5 times greater numbers in the severely asthmatics' homes. On the other hand, *A. unguis* was detected more often in severely asthmatics homes compared to non-asthmatic's homes (71% versus 52%). However, the median concentrations of the two species were similar (3 versus 2 CE per mg dust). *Penicillium spinulosum*, another Group 1 species, was nearly significantly different in the Wilcoxon Rank-sum test ($p=0.059$). *P. spinulosum* was only detected in the homes of the severely asthmatic children.

4. Discussion

Asthma is a respiratory disease with a complex etiology that is influenced by gene and environment interactions. Sometimes even defining who is an asthmatic can be problematic. In this study we used an easily definable method based on medication use. The GINA system of classification is not the same. However, the GINA guidelines for treatment of "persistent asthma" recommend daily use of anti-inflammatory asthma drugs. So by definition, the "persistent asthma" group would be consistent with our "severe" asthmatic classification. It is this severe or "persistent asthma" group that had higher ERMI values in their homes compared to the non-asthmatic controls.

Mold allergens are among the important environmental triggers for childhood asthma along with dust mite, pet (cat/dog), cockroach and rodent urinary allergens (Park et al., 2006; Zeldin et al., 2006; Kim et al., 2007; Pekkanen et al., 2007). To date, methods for assessing the mold burden have been problematic. Mold burdens measured in the home with a variety of microbiological methods have not correlated with an increased prevalence of asthma (Wood et al., 1988; Huss et al., 2001). The more recent development of the Environmental Relative Moldiness Index, based on the analysis of a standardized dust sample from the living room plus bedroom, has led to a quantitative means of assessing relative mold burdens in homes (Vesper et al., 2007c).

In the present study, significantly higher average ERMI values were measured in the vacuum dust samples collected from Detroit (Wayne County) Michigan homes with children who were severely asthmatic compared to the homes where non-asthmatic children resided. Previously, we have reported higher average ERMI values in homes of asthmatic children in Cleveland, OH (Vesper et al., 2006) and Chapel Hill, NC (Vesper et al., 2007a).

Consistent with these earlier studies, this study demonstrates that Group 1 mold species that indicate water intrusion, contributed to the elevated ERMI values in dust samples from severely asthmatic children's homes. The mold species *A. niger* and *A. unguis* accounted for the differences in the ERMIs between the severe and non-asthmatic children's homes. In Chapel Hill, the species which accounted for the differences in asthmatic homes were *Chaetomium globosum*, *Aspergillus fumigatus* and the *Eurotium* Group (Vesper et al., 2007a); and in Cleveland, *Scopulariopsis brevicaulis* and *Tricoderma viride* (Vesper et al., 2006). The Group 2 molds, which are common mycoflora across the US (Vesper et al., 2007c), have not been associated with higher asthma levels in our studies. The higher ERMI values can be viewed as indicators of water-damage but they are not proof that these molds cause asthma or asthma exacerbation.

Since the costs for treating asthma continue to escalate, prevention of asthma or minimized the exacerbations by minimizing exposure to asthma triggers might reduce the need for direct medical treatment (Mudarri and Fisk, 2007). One important step is remediating water-damaged homes. In Cleveland, such remediation resulted in a ten-fold reduction of emergency room visits or hospitalizations for the asthmatic children (Kercsmar et al., 2006). Our data suggests there may be utility in monitoring of the home for mold contamination using the ERMI analysis. Additional studies in other cities are underway to assess the relationships between the ERMI values in homes and asthma.

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