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Short Communication

The level of submicron fungal fragments in homes with asthmatic children

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ABSTRACT

Objectives: Much scientific evidence indicates a positive association between moldy environments and respiratory illnesses and/or symptoms (e.g., asthma). Recently, submicron fungal fragments (< 1.0 μm) have been suggested as a potential contributor to adverse health effects due to their biological composition (e.g., antigens, mycotoxins, and (1,3)-β-D-glucan) as well as their small size. However, the contribution of exposure to fine fungal particles on adverse health outcomes has been poorly characterized, particularly in homes with asthmatic children. We characterized the airborne level of smaller-sized fungal particles between homes with and without asthmatic children.

Methods: We visited 29 homes with ($n=15$) and without ($n=14$) an asthmatic child and sampled submicron fungal fragments in a living room and child's bedroom, along with outdoor sampling, using the NIOSH two-stage sampler. (1,3)-β-D-glucan of fungal fragments analyzed by *Limulus Amebocyte* lysate assay (LAL) was used for quantifying their exposure.

Results: Overall, the geometric mean (GM) concentration of (1,3)-β-D-glucan in submicron fungal fragments in indoor air was two-fold higher in homes with asthmatic children (50.9 pg/m^3) compared to homes with non-asthmatic children (26.7 pg/m^3) ($P<0.001$). The GM concentration of these particles in child's bedroom in homes with an asthmatic child (66.1 pg/m^3) was about three times higher than that in homes with non-asthmatic children (23.0 pg/m^3) ($P<0.001$). The relative humidity had a negative correlation with the concentration of (1,3)-β-D-glucan in submicron fungal fragments (Pearson coefficient $=-0.257$, $P=0.046$).

Conclusions: Our findings indicate that homes with asthmatic children have a higher concentration of submicron fungal fragments compared to homes with non-asthmatic children. A greater exposure to smaller-sized fungal particles may occur in homes with an asthmatic child as relative humidity decreases. The very careful control of relative humidity in indoor air is necessary for reducing exposure to fine fungal particles and inhibiting the growth of microorganisms in homes with allergic diseases.

1. Introduction

Many epidemiological studies and a recent review by the WHO showed that increased exposure to mold attributed to water-damaged or damp conditions in buildings could causally contribute to exacerbation of asthma symptoms (Afshari et al., 2009; Antova et al., 2008; Carpenter, 2004; Fisk et al., 2007; Institute of Medicine, 2004). The development of asthma could be attributed to allergic responses linked with exposure to mold (Fung and Hughson, 2003; Robbins et al., 2000), but the causal mechanism is still unclear. Therefore, a proper exposure assessment for mold in indoor environments is necessary, and a tailor-made strategy for prevention and alleviation of asthma symptoms should be prepared based on the results of a proper assessment of indoor mold.

The culture method followed by air sampling for indoor mold has been used in many previous studies to investigate the association of exposure to mold with the development or exacerbation of asthma (Afshari et al., 2009; Institute of Medicine, 2004). However, the limitations of this method due to short sampling times, the different growth rates by types of mold, and culturability dependence on media make it difficult to determine the cause-and-effect relationship of mold exposure on disease outcomes (Reponen et al., 2011; Vesper et al., 2009, 2007). In recent years, fine fungal particles of less than 1 μm (hereinafter 'submicron fungal fragments'), which contain fungal allergens, mycotoxins, and (1,3)-β-D-glucan, have been suggested as a potential

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factor affecting the exacerbation of disease symptoms (Reponen et al., 2007; Seo et al., 2008, 2009). The submicron fungal fragments can stay airborne longer than fungal spores, which are larger, and can penetrate deeply into lungs and be deposited due to their small aerodynamic diameter. A previous study has shown that submicron particles attribute to broken spores and hyphae of *Stachybotrys chartarum* may be deposited at a rate 230-fold higher than intact airborne spores (Cho et al., 2005). In addition, exposure to airborne fine particles has been linked with adverse health effects on the respiratory and cardiac responses. In particular, the number concentrations of ultrafine particles (<0.1 μm), rather than the mass concentrations of these particles, have been strongly associated with adverse health effects (Penttinen et al., 2001; Peters et al., 1997; Von Klot et al., 2002). For this reason, a greater health impact from submicron fungal fragments might be expected due to their smaller size and higher number concentration. However, the contribution of submicron fungal fragments including debris of spores and hyphae on health are poorly characterized.

In this study, we evaluated and compared the level of submicron fungal fragments expressed as the concentration of (1,3)-β-D-glucan between homes with and without asthmatic children. In addition, the effect of physical factors in indoor air such as temperature and humidity on the concentration of submicron fungal fragments was evaluated.

2. Methods

2.1. Study subjects

We selected 15 homes with asthmatic children shown to be sensitized to mold only by skin prick tests among the childhood asthmatics registered at the Environmental Health Center for Asthma, Korea University General Hospital, during 2010 and 2011. We also chose 14 homes with non-asthmatic children determined by a physician after visiting the Pediatrics Clinic, Korea University General Hospital, selected to have similar ages, genders, the number of people residing, and size of dwellings as shown in Table 1. None of the non-asthmatic children were also sensitized with any allergen of 18 common aeroallergens used for the skin prick test. This study was approved by the institutional review board of the Korea University Anam Hospital (No. ED07111).

2.2. Exposure assessment

We used a questionnaire for collecting demographic information of children and data of characteristics of dwellings: types and size of dwellings, living level (ground floor or higher), house age, and the presence of visible mold or water stains on the wall or ceilings. Indoor investigations of dwellings were also performed by trained researchers, and the procedures of exposure assessment are described below in detail.

2.2.1. Air sampling for submicron fungal fragments and analysis

Air samples for submicron fungal fragments were collected using the NIOSH two-stage sampler (Lindsley et al., 2006) as described in detail elsewhere (Seo et al., 2008). Briefly, each NIOSH two-stage sampler was loaded with a 37 mm gamma-irradiated polycarbonate filter with a pore size of 0.8 μm (SKC Inc., Eighty Four, PA, USA) and connected to a pump (Gillian 5000; Sensidyne, FL, USA). The samplers were placed in the living room and child's bedroom (indoor) and balcony (outdoor) of the visited homes (total number of samples: 3 samples (indoor+outdoor)/home × 29 homes=87 samples). Sampling was performed for about 7–8 h depending on the overall concentrations of airborne particles determined by an optical particle counter (Model 1.108, Grimm Technologies, Inc., Douglasville, GA, USA) to avoid spores to bounce into the filter (Seo et al., 2007). For quality control field blank samples were installed in the living room and balcony. After the sampling, the filter cassette was covered with aluminum foil, placed on ice, and then stored at 4 °C until analysis. To minimize the effect of external factors such as rain and anthropogenic disturbances, the sampling was carried out approximately from 8:00 am to 4:00 pm during September and October of 2012 under empty house and closed conditions of all the windows. Also, all parents were asked not to clean their home the day before and on the day of sampling to avoid the release of fungal spore and fine particles into indoor air attributed to different cleaning practices.

Table 1
Characteristics of dwellings and demographic information of asthmatic and non-asthmatic children.

	Group with asthmatic children (case)	Group with non-asthmatic children (control)
Number of subjects	15	14
Gender (boy/girl)	8/7	6/8
Age (years) (mean ± SD[a])	8.1 ± 1.6	8.5 ± 1.3
Size of dwellings (mean ± SD[a]) (m^2)	79.2 ± 9.2	83.1 ± 7.3
House age		
< 1990	4	2
> 1990	11	12
Living level		
Ground floor	6	3
Next floor	9	11
Types of house		
Apartment	14	12
Row house	1	2
Visible mold or water stains		
Yes	3	5
No	12	9

[a] SD; standard deviation.

The concentration of submicron fungal fragments was estimated by analyzing (1,3)-β-D-glucan, which exists in fungal cell walls and is known to cause severe inflammation in the respiratory system. The kinetic chromogenic LAL (*Limulus Amebocyte* lysate) analysis (Glucatel; Associates of Cape Cod, East Falmouth, MA, USA) for measuring (1,3)-β-D-glucan has been used in many previous studies (Iossifova et al., 2009; Reponen et al., 2007; Seo et al., 2007, 2008, 2009). Briefly, the filter from the cassette was transferred to a 15 ml conical tube (Fisher Scientific, Pittsburgh, PA, USA), and submicron fungal fragments were extracted using 5 ml of PBS solution (Fisher Scientific, Pittsburgh, PA, USA) with the use of a vortexer (Model 231; Fisher Scientific, Pittsburgh, PA, USA) and a sonicator (Model FS20; Fisher Scientific Inc., Pittsburgh, PA, USA). A 1.0 ml-aliquot of the extracted solution was used to make stained glass slides for microscopic examination using a light microscope (Eclipse Ci; Nikon Corporation, Tokyo, Japan) for checking the absence of fungal spores. The field sampling was performed again if the fungal spores were observed on the stained filter, and the solution without fungal spores only was used for the (1,3)-β-D-glucan analysis. To make (1,3)-β-D-glucan with a tertiary structure water-soluble, 0.5 ml of 0.6 M NaOH was added to 0.5 ml of the extracted solution, and it was agitated for 1 h using a mechanical agitator (Model 75 Wrist-action shaker; Burrell Scientific, Pittsburgh, PA, USA). Following the agitation, 50 μl of (1,3)-β-D-glucan lysate was added to 25 μl of the solution mixed with NaOH, and the concentration of (1,3)-β-D-glucan was measured for about 150 min using an absorbance microplate reader (ELx808TM; Bio-Tek Instruments Inc., Winooski, VT, USA). The analysis result was expressed in pg/ml, and by estimating the total sampling time, it was converted to pg/m^3.

2.2.2. Sampling for airborne mold and analysis

We sampled airborne mold in the living room and child's bedroom separately using a one-stage Andersen sampler (Andersen Instruments, Atlanta, GA, USA). Air sampling was performed for 10 min using a vacuum pump (Model no. 1531-107B-G289X, Gast Manufacturing, Benton Harbor, MI, USA) at a flow rate of 28.98 LPM. We used malt extract agar supplemented with cycloheximide (0.5 g/L) (Sigma-Aldrich, St. Louis, MO, USA) to inhibit the growth of bacteria from homes. The colony numbers were counted on the plates to determine the concentrations of airborne mold after a 4-day incubation at 20–25 °C. Each field blank for mold at each home was taken and tested for growth. Nearly all of the blank samples had no or one colony, so sample counts were adjusted for the reading of blank samples. The concentrations were finally expressed as the corrected colony numbers followed by the positive-hole correction per air volume (CFU/m^3) (Macher, 1989). In addition, the temperature and relative humidity in each living room and child's bedroom were monitored using a portable thermo-hygrometer (GrayWolf Sensing Solution, Tuamgraney, Co., Clare, Ireland).

2.3. Statistical analysis

The concentration of (1,3)-β-D-glucan in submicron fungal fragments showed a right-skewed distribution in normality tests. The geometric mean (GM) was used for reporting the results in this study. The Wilcoxon signed-rank test was performed to compare the indoor and outdoor concentrations of (1,3)-β-D-glucan in submicron fungal fragments between homes with and without asthmatic children. Also, the Wilcoxon matched-paired signed-rank test was performed to compare the concentrations between the living room and child's bedroom. The