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Nocturnal Asthma and Domestic Exposure to Fungi

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Key Words

Nocturnal asthma · Fungi · NAHA · Exposure

Abstract

Fungal growth indoors is a common problem both in older and modern buildings. Numerous studies demonstrate a deterioration of asthma in environments with fungal growth but precise estimates of exposure levels have not been reported. This study was carried out to investigate whether levels of airborne fungi were higher in homes of subjects with nocturnal asthma as compared to controls. Subjects with nocturnal asthma ($n=42$) and non-nocturnal asthma ($n=14$) were recruited from an outpatient department. Subjects without disease or respiratory symptoms ($n=24$) served as controls. Among the asthmatics, determinations were made of pulmonary function before and after treatment with bronchodilators. The subjects were equipped with a pump and a filter and instructed to sample air in their bedroom. The filters were analysed for the content of N-acetylhexosaminidase (NAHA) as a marker of fungal cell biomass. In some houses the number of colony forming units was also determined. Patients with asthma had significantly

higher activities of NAHA in their bedrooms as compared to controls (31.2 vs. 12.5 U m^{-3} , $p=0.004$). When the material was divided according to the 2nd quartile of NAHA (14.0 U m^{-3}), 29 out of 42 subjects with nocturnal asthma had high values compared to 5 out of 24 for the controls ($p<0.0001$). The results support the hypothesis that patients with nocturnal asthma have high levels of fungi in their homes. This provides a basis for treatment and exposure diminution. Whether that exposure is causative for their disease; or if it aggravates already existing airway symptoms remains to be elucidated.

Introduction

Fungal growth indoors due to water leakage, condensation of humidity or flooding is a common problem both in developing and developed countries [1]. Traditionally, the health effects of fungal exposure were related to the development of allergic reactions, often referred to as asthma [2]. Initially, it was believed that the pathogenesis of asthma was due to fungal antigens which induced the formation of IgE antibodies. These antibodies induced

clinical symptoms through effector cells such as mast cells. It is now realised that asthma is a complex disease with several different phenotypes [3,4]. A number of fungal cell wall agents (FCWA) such as β -glucan, chitin, and peptides may induce an inflammatory response through effects on the innate immune system. β -glucan may induce both Th1 and Th2 derived reactions [5,6] and chitin induces the secretion of different inflammatory mediators [7]. Moulds can also produce mycotoxins during growth in buildings, causing health risks [8,9].

Under normal conditions the levels of fungi indoors are similar to or even lower than levels outside. Increased levels in relation to outdoor values are present in buildings with moisture damage or in spaces where dust is accumulated due to inadequate cleaning [1]. In terms of domestic exposure the longest exposure time is in the bedroom. Nocturnal asthma would thus be the asthma disease entity with the closest relation to fungal exposure.

Regarding the exposure assessment, FCWA retain their biological activity also when the cell is dead. Determinations of fungal cell mass are thus a more precise measure of the potential exposure than viable counts or spore determinations. Furthermore the latter do not comprise mould cell fragments which may comprise up to half the total FCWA content in airborne samples [10,11].

Several studies have measured the activity of the enzyme N-acetylhexosaminidase (NAHA) as an indicator of mould cell mass [12,13]. Although it is not specific for fungi, several studies in complex environments have demonstrated a good correlation with fungal biomass, mould biomarkers (ergosterol and phospholipid fatty acid) [14]. Significant correlations to total spore counts were found in air samples and in dust generated from biomass in a bio fuel plant [15]. Strong correlations were found between fungal biomass (gravimetric weight) and NAHA in fungal species grown on nutrient agar and between ergosterol and NAHA activity on gypsum boards [16].

The present study was undertaken to evaluate if the activity of NAHA, as an indicator of fungal cell mass, was related to the presence of nocturnal asthma.

Methods

Subjects

Subjects were recruited among patients at the Clinic of Respiratory Diseases and Allergy at the University Medical Centre, Ljubljana and the Bolnisična Sezana hospital, Slovenia. Inclusion criteria for nocturnal asthma were similar to those used in previous studies [17,18] and

comprised respiratory symptoms, dyspnoea early in the morning, use of bronchodilators during the night or at wake up, and a bronchodilator induced increase in forced expiratory volume in one second (FEV₁) or forced vital capacity (FVC) by more than 12% and 200 mL. Atopy was tested by skin-prick methodology including mould, grass, pollen, milk, ambrosia, birch, chestnut, willow, egg, cat, and dog allergens. Cases of asthma without night related symptoms were also included. Controls were members of the staff and other contacts who were healthy, non-smoking and without respiratory symptoms. The study was approved by the ethical committee at the University Medical Center.

Air Sampling and Analysis of NAHA

For the sampling of NAHA the participants were supplied with a portable pump and filter holders preloaded with cellulose acetate filters (Mixed Cellulose Esters, 25 mm PCM Cassettes, 0.8 μ m pore size, Zefon International, Inc., Ocala, FL, USA). The subjects turned on the pump (15 L min⁻¹) and about 2.5 m³ of indoor air were drawn through the filter. Each measurement comprised sampling in the bedroom. The duration was about 3 h, either at night or early in the morning after wake-up. The exact air volume sampled was read from a gas volume meter attached to the pump. All subjects slept in the room where sampling was done.

For the analysis of NAHA activity, 1 mL of NAHA substrate (Mycometer ApS, Copenhagen, Denmark) was added to the filter and incubated for about 30 min. After incubation, usually 30 min depending on room temperature, 2 mL developer was added. The liquid was then sucked out with a syringe through the filter and placed in a cuvette. The fluorescence in the cuvette was read in a fluorimeter (Picofluor, Turner Designs, Sunnyvale, CA, USA). One count is equal to 2.3 ng *Aspergillus oryzae* NAHA. To avoid random scatter, the counts read in the fluorimeter were divided by 10 and given as a round figure to express the NAHA activity in units (NAHA U m⁻³) [13].

Air Sampling of Viable Fungi

Samples for viable airborne fungi determination were collected using the SAS Super 180 (Surface air system) sampler (pbi International) with a flow rate of 180 L min⁻¹. 0.48 m³ of air were collected on Sabouraud agar plates, which were incubated aerobically at 37°C for 7 days. The same volume of air was collected on DG 18 (Dichloran glycerol 18% agar) agar plates which were incubated aerobically at 25°C for 7 days. The instrument was calibrated regularly according to the manufacturer's

instructions. Viable counts were adjusted using the positive hole correction factor [19] and reported as colony forming units per cubic metre (c.f.u. m⁻³) of air sampled. Sampling was made in parallel to the NAHA sampling.

Statistical Analysis

Differences between groups were evaluated for statistical significance using the Mann-Whitney and the chi²-tests. Relationships were calculated using Spearman's test. A p-value of 0.05 was considered significant.

Results

Table 1 shows the background characteristics of the controls and subjects with nocturnal and non-nocturnal asthma.

The subjects with asthma responded as expected to the bronchodilator whereas there were no changes among controls. There were a slightly higher proportion of skin positive tests among the subjects with asthma.

Table 2 shows the levels of NAHA and number of c.f.u. in the bedrooms of controls, subjects with nocturnal asthma and non-nocturnal asthma.

Table 1. Characteristics of controls and subjects with nocturnal and non-nocturnal asthma. Pulmonary function before and after bronchodilatation. Mean and SEM.

	Controls	Nocturnal asthma	Non-nocturnal asthma
N	24	42	14
Age	48.3 (3.2)	49.9 (2.4)	41.5 (4.4)
Females %	42	69	57
Smokers	4	3	1
Skin prick test pos	5	14	7
FVC before	107 (4.3)	86.6 (2.8)	92.8 (4.3)
after	105 (3.2)	103 (2.2)	97.5 (3.7)
FEV ₁ before	98.3 (3.9)	71.4 (2.8)	86.5 (4.5)
after	97.4 (1.4)	92.3 (2.6)	93.2 (4.1)

Table 2. NAHA activity (U/m³) and number of c.f.u. in homes of controls and subjects with and without nocturnal asthma. Mean and SEM.

	NAHA			c.f.u.		
	n	mean	SEM	n	mean	SEM
Controls	24	12.5	3.6	12	106	52
Nocturnal asthma	42	31.2	6.1	11	698	340
Non-nocturnal asthma	14	14.4	2.6	-	-	-

There was a significant difference between the NAHA activity in homes of controls and those with nocturnal asthma (p<0.001, Mann-Whitney). The c.f.u values were higher in the bedrooms of subjects with nocturnal asthma (p=0.002). Overall there was no correlation between NAHA and c.f.u. values.

For the further analysis, the subjects were divided into those with a home exposure to NAHA lower or higher than the 2nd quartile (14 U m⁻³). The results are shown in Table 3.

There was a significantly larger number of subjects with nocturnal asthma exposed to the higher activities of NAHA (p<0.001, chi²-test). There was no significant difference among subjects with non-nocturnal asthma.

Discussion

The major finding of this study is the high exposure to fungal cell biomass in the home environment of subjects with nocturnal asthma.

There are some shortcomings that need to be addressed. The exposure determination was made at one time only. It is known that levels of airborne fungi vary over different seasons [20], but the same seasonal variation would have been present in the houses of controls. Good relationships between indoor measures at different occasions have also been reported [13,21]. Also the major source of fungi indoors is active growth due to humidity problems in the building, which would be a specific factor that is different between control homes and those of asthmatics.

The analysis of airborne samples comprised the determination of NAHA as a measure of fungal biomass. Among fungal species, NAHA is produced mainly by filamentous fungi such as *Aspergillus* and *Penicillium* species [12]. The enzyme is also produced by a range of organisms including bacteria, fungi, protozoa, and mammalian cells and is thus not specific for fungi. On the other hand when fungi grow indoors such as in humid conditions, there is no reason to believe that other sources of

Table 3. Number of controls and subjects with and without nocturnal asthma exposed to low (<14 U/m³) and high (>14 U/m³) activities of NAHA.

Subjects	n	Low		High	
		n	%	n	%
Control	24	19	79	5	21
Nocturnal asthma	42	13	31	29	69
Non-nocturnal asthma	14	8	57	6	43

NAHA such as pollen or mammalian cells would also multiply. The specificity for detection of buildings with fungi is very high [13]. From a practical point of view NAHA can thus be considered specific for fungi in indoor conditions with increased humidity.

Regarding c.f.u. there was a significant difference in exposure levels between controls and subjects with nocturnal asthma but no relation between c.f.u and NAHA. This is not unexpected as viable colonies are a poor marker of risk due to exposure to fungi. All bioactive agents such as β -glucan and chitin retain their activity when the cell is dead and in fragments of cells [22]. One study demonstrated that desiccation of the fungal spores increased the availability and thus bioactivity of β -glucan [10].

The results confirm previously reported relationships between the risk of asthma and exposure to fungi [2,23,24]. Seemingly contradictory results were reported in a study comprising 198 children with asthmatic and allergic symptoms and 202 controls [25]. In that study no association was found between asthma and the spore concentrations or visible signs of dampness in the homes. It is, however, rather unlikely that such a vast range of diagnoses are all related to fungal exposure, hence the lack of significant relationships. In the present study a particular form of asthma was selected where the characteristics of its occurrence suggested a bedroom related exposure. Most of the subjects were unaware of the mould problems in their homes. There was no visible mould growth on walls or in the ceiling but when beds or other furniture were removed, colonies of mould growth were discovered.

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An important support for the importance of fungal exposure in asthma comes from an extensive study where antifungal medication was found to improve the clinical status of asthmatic subjects [26]. Patients with severe asthma sensitized to at least one species of fungus, using a skin prick test, were treated with itraconazole for 32 weeks. The quality of life score and pulmonary function used to assess the effects of the treatment decreased in the intervention group as compared to the placebo group.

The high activities of NAHA found in homes of persons with nocturnal asthma also indicate that an efficient treatment of the disease should include remediation of fungi at home. Such schemes have previously been found to be efficient in the prevention of asthma among children [27].

Conclusion

In conclusion, the results from the study suggest that exposure to high levels of fungal cell mass during night-time is related to nocturnal asthma. The treatment of subjects with nocturnal asthma should include inquiries about possible mould problems in their homes and suggestions for remedial actions.

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