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Outdoor (1→3)-β-D-glucan Levels and Related Climatic Factors

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Objectives: To evaluate the monthly variation in the airborne (1→3)-β-D-glucan level throughout one year and its relationship with climatic factors (temperature, relative humidity, wind speed, hours of daylight, cloud cover, and pollen counts).

Methods: A total of 106 samples were collected using a two-stage cyclone sampler at five outdoor sampling locations (on top of 5 university buildings). The kinetic limulus amebocyte lysate assay was used to obtain (1→3)-β-D-glucan levels.

Results: Airborne (1→3)-β-D-glucan levels were significantly higher in the spring, particularly in April, and temperature was significantly related to (1→3)-β-D-glucan levels ($r=0.339$, $p<0.05$).

Conclusions: (1→3)-β-D-glucan levels may be highest in the spring, and outdoor temperature may influence (1→3)-β-D-glucan levels.

Key words: (1→3)-β-D-glucan, Indicators, Climatic factors, Temperature, Pollen

INTRODUCTION

Exposure to airborne fungi has been widely recognized as a plausible cause of dampness-related respiratory morbidity [1]. In particular, exposure to fungal (1→3)-β-D-glucans has been associated with non-allergic responses. (1→3)-β-D-glucans also has potent biological properties and may adversely affect airway inflammation [2].

Until now, most of the studies on airborne (1→3)-β-D-glucans has been conducted in workplace environments such as metal industry plants, wastewater treatment plants, and greenhouses [3-5]. Limited data have been collected in outdoor environments while considering various climatic factors and sampling periods, and the follow-up duration of these studies has been limited [6,7].

Therefore, we aimed to first characterize the monthly levels of (1→3)-β-D-glucan throughout one year to understand how (1→3)-β-D-glucan levels fluctuate in the outdoor environment and if these changes are potential indicators of climate change. Second, we evaluated the relationship between (1→3)-β-D-glucan level and various climatic factors such as outdoor temperature, relative humidity, wind speed, hours of daylight, cloud cover, and pollen counts.

METHODS

From February 2011 to January 2012 (winter, spring, summer, fall, and winter), five outdoor locations on the roofs of five university buildings, were used to sample the air. These locations included (A) the Graduate School of Public Health, (B) the Genetic Engineering building, (C) the School of Chemical Engineering, (D) the School of Biological Engineering, and (E) the College of Pharmacy.

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In total, 106 air samples were collected from all of the outdoor stations. The samples were collected 100 to 150 cm above the ground. The sampler uses a two-stage cyclone to collect (1→3)-β-D-glucan, and the diameters of the first-stage inlet and second-stage inlet are 2.0 and 2.5 mm, respectively. The two-stage cyclone sampler collects aerosols in two 1.5-mL tubes as well as inside a backup tube. At an airflow rate of 2 L/min, the collection efficiency for each tube was determined. At 2 L/min, the first tube had a 50% cutoff diameter of 2.7 μm with a sharpness of 1.43, and the second tube had a 50% cutoff diameter of 1.5 μm with a sharpness of 1.75. The Korea Meteorological Administration provided data on the outdoor temperature, relative humidity, wind speed, hours of daylight, cloud cover, and pollen counts for each month during the study period.

The samples were stored at 4±2°C and then sent to an analytical laboratory within a week of sampling. All samples were analyzed within one month of collection. (1→3)-β-D-glucan extraction from each filter was carried out using a kinetic chromogenic limulus amebocyte lysate assay kit (Glucatell; Associates of Cape Cod, Falmouth, MA, USA), which included a (1→3)-β-D-glucan-specific reagent, reagent-grade water, and a (1→3)-β-D-glucan standard. The (1→3)-β-D-glucan-specific reagent used in this kit is a lysate of *Limulus polyphemus* blood cells from which factor C has been removed. Thus, this reagent prevents any cross-reactivity with endotoxin. The concentration of (1→3)-β-D-glucan is presented as pg/m^3. The assay limit of detection was 3.125 pg/mL for the extract.

All statistical analyses were conducted using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Parametric statistics were used to test for significant differences among the level of (1→3)-β-D-glucan at each outdoor location; the measured levels of (1→3)-β-D-glucan were found to be normally or log-normally distributed. Pearson correlation analysis was performed to investigate the relationship between the (1→3)-β-D-glucan level and each climatic factor.

RESULTS

The airborne (1→3)-β-D-glucan levels were evaluated based on the 50% cutoff diameters of stage one (2.7 μm) and stage two (1.5 μm). The value of (1→3)-β-D-glucan at 2.7 μm was higher than that at 1.5 μm when tested in all of the laboratories. At the 2.7 μm 50% cutoff diameter, the highest geometric mean (GM) was 378 pg/m^3 (27 to 7143 pg/m^3) observed at location E, and the lowest was 195 pg/m^3 (14 to 2299 pg/m^3) at location A. At the 1.5 μm 50% cutoff diameter, the highest GM was 27 pg/m^3 (6 to 88 pg/m^3) observed at location C, and the lowest was 12 pg/m^3 (<3.3 to 55 pg/m^3) at location D (Table 1).

Table 1 also shows the average temperature, relative humidity, wind speed, hours of daylight, and cloud cover measured at each of the five outdoor stations monthly. At all stations, the total (1→3)-β-D-glucan levels ranged from <3.2 to 7143 pg/m^3 with a GM of 312 pg/m^3. The highest GM level, 429 pg/m^3, was observed at station E. However, the lowest GM was 255 pg/m^3 at location D.

The scatter plot in Figure 1 depicts the correlation between the GM of (1→3)-β-D-glucan levels and outdoor temperature and indicates that (1→3)-β-D-glucan tended to have higher levels between the spring and the summer months.

The results of the Pearson correlation analysis for the relationship of (1→3)-β-D-glucan level with temperature, relative humidity, wind speed, hours of daylight, cloud cover, and pollen levels are shown in Table 2. The outdoor (1→3)-β-D-glucan levels we measured were significantly correlated with temperature (r=0.301, p<0.05).

DISCUSSION

Of all five sampling locations, location E had the highest GM levels (1→3)-β-D-glucan (geometric standard deviation [GM]: 429 [5.9] pg/m^3) (Table 1). Location E is surrounded by a forest. The level of (1→3)-β-D-glucan could have been higher in this sampling location because (1→3)-β-D-glucans have a non-allergenic water-insoluble structural cell wall that is a component of most fungi, higher plants, and many lower plants [8].

Our results suggest that temperature is correlated with (1→3)-β-D-glucan level and pollen counts. Previous studies have also showed that temperature is significantly correlated with pollen counts [9]. In addition, the advancement of flowering was found to be correlated with an increase in minimum temperature; therefore, minimum temperature may influence pollen production [10].

High levels of (1→3)-β-D-glucan were found in the spring (April and May), and low levels were found in the fall (November) (Table 1). Higher pollen levels during the spring compared to the other seasons may explain the high level of (1→3)-β-D-glucan in April and May; however, the differences between the spring and fall were not statistically significant. Pollen is usually carried by the wind in the spring; thus, it may represent a

Table 1. (1→3)-*β*-*D*-glucan level (pg/m^3) and climatic factors by sampling location

Sampling location	Calendar month	Levels (pg/m^3)					Average level of climatic factors[1]				
		n	Stage 1	n	Stage 2	Total	Temperature (°C)	RH (%)	Wind (m/s)	Daylight (h)	Cloud cover
A	1	1	37	1	23	60	0.9	64.6	2.5	2.9	3.4
	2	1	60	1	<3.3	60	1.4	50.8	2.2	8.3	3.4
	3	1	2299	1	22	2321	5.7	38.3	3.5	10.5	2.9
	4	1	1229	1	27	1256	12.2	77.1	3.5	2.1	7.5
	5	1	119	1	26	145	20.7	60.4	4.6	0.9	8.3
	6	1	654	1	19	673	24.9	43.0	2.6	6.2	7.1
	7	NA									
	8	1	209	1	53	262	24.4	75.6	2.2	1.0	8.8
	9	1	406	1	13	419	25.6	68.1	1.7	3.6	6.3
	10	1	189	1	5	195	17.3	53.1	2.6	5.0	8.3
	11	1	14	1	12	26	14.2	81.0	3.1	1.7	9.8
	12	1	144	1	43	187	7.1	39.1	3.9	9.0	0.1
B	1	1	104	1	4	108	4.7	48.9	3.1	1.9	7.5
	2	1	627	1	14	641	6.2	48.1	1.9	9.5	0.5
	3	1	1390	1	36	1425	2.2	43.9	4.4	10.7	1.1
	4	1	2125	1	32	2157	8.9	83.9	3.3	0.0	9.1
	5	1	1906	1	24	1931	17.5	89.8	3.0	0.0	10.0
	6	NA									
	7	1	1296	1	52	1348	24.1	71.0	2.1	0.0	9.3
	8	1	26	1	<3.3	26	27.1	70.0	1.9	1.5	8.0
	9	1	94	1	12	107	18.1	44.4	2.2	7.9	2.0
	10	1	58	1	21	80	9.4	37.4	1.7	10.5	0.0
	11	NA									
	12	1	85	1	24	109	7.1	29.1	2.6	9.0	0.0
C	1	1	78	1	6	84	2.9	54.5	2.5	7.5	4.3
	2	1	395	1	38	395	5.6	52.1	2.3	9.7	0.9
	3	1	876	1	32	876	2.2	-	3.7	11.2	0.9
	4	1	2476	1	31	2476	11.2	49.3	3.5	10.5	1.3
	5	1	2094	1	33	2094	20.7	47.3	1.9	0.1	9.5
	6	NA									
	7	1	927	1	88	1012	24.8	81.0	2.7	0.3	8.6
	8	1	400	1	29	400	23.5	71.4	2.2	2.2	6.5
	9	1	58	1	43	58	21.8	41.1	2.1	11.0	0.0
	10	1	49	1	12	49	7.6	36.9	2.9	9.9	0.0
	11	1	29	1	42	29	5.6	53.1	2.6	0.0	9.0
	12	1	72	1	15	78	5.4	42.8	2.2	7.8	3.4
D	1	1	68	1	22	89	5.6	22.4	2.8	9.5	0.3
	2	1	334	1	5	340	6.1	57.4	2.5	5.6	4.9
	3	1	852	1	53	905	7.3	61.5	3.2	9.6	0.4
	4	1	1861	1	15	1876	13.2	47.4	2.5	0.8	8.9
	5	1	2798	1	41	2839	20.1	53.5	2.0	6.1	3.6
	6	NA									
	7	1	718	1	55	773	26.2	81.6	2.5	2.4	8.1
	8	1	158	1	19	177	26.4	57.5	2.8	7.6	6.8
	9	1	37	1	13	50	19.8	52.6	2.5	0.2	7.1
	10	1	23	1	9	32	9.5	42.8	1.5	9.5	3.3
	11	NA									
	12	1	36	1	<3.3	36	4.0	37.4	1.8	0.0	9.8
E	1	1	54	1	52	107	1.2	52.8	2.5	6.7	3.3
	2	1	60	1	4	65	2.3	59.1	1.9	0.5	6.9
	3	1	1375	1	69	1375	9.2	54.6	2.8	10.5	0.4
	4	1	7143	1	52	7143	11.3	61.0	4.6	6.5	7.1
	5	1	3656	1	21	3656	21.5	39.5	2.0	9.7	5.5
	6	1	2077	1	42	2077	24.5	52.5	2.6	7.5	4.1
	7	NA									
	8	1	938	1	50	988	25.2	73.3	1.9	4.8	6.6
	9	1	148	1	7	154	17.7	44.5	3.6	5.6	4.5
	10	1	269	1	5	274	12.8	44.0	1.7	10.0	1.1
	11	1	27	1	14	41	1.6	39.1	2.0	9.4	0.0
	12	1	91	1	12	103	5.5	33.9	4.8	8.9	1.0
Total		53		53							
GM (GSD)			286 (5.4)		19 (2.9)	312(5.0)					

RH, relative humidity; NA, not applicable; GM, geometric mean; GSD, geometric standard deviation.
[1]Total mean of the number of sample at each location.

Table 2. Pearson correlation analysis between the total level of (1→3)-β-D-glucan and climatic factors

	(1→3)-β-D-glucan level	Temperature	Relative humidity	Wind speed	Hours of daylight	Cloud cover	Pollen count
(1→3)-β-D-glucan level	1.000						
Temperature	0.339*	1.000					
Relative humidity	0.255	0.396**	1.000				
Wind speed	0.255	-0.177	0.067	1.000			
Hours of daylight	0.048	-0.292*	-0.599**	0.091	1.000		
Cloud cover	0.143	0.404**	0.618**	-0.017	-0.889**	1.000	
Pollen count	0.258	0.373**	0.223	-0.128	0.060	0.010	1.000

*$p<0.05$, **$p<0.001$, n=53.

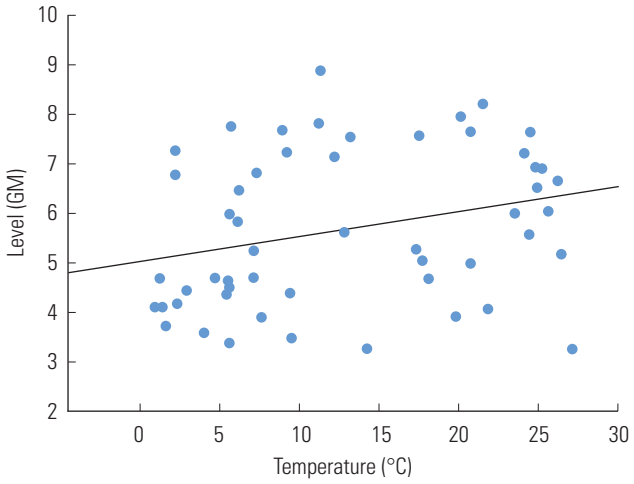

Figure 1. Scatter plot of the geometric mean (GM) of (1→3)-β-D-glucan level and outdoor temperature ($r=0.339$, $p=0.013$).

major source of (1→3)-β-D-glucan. A previous study found tree pollen counts to fluctuate seasonally, and counts were highest from March to May when about 95% of the total yearly tree pollen count is in the air [9]. Moreover, (1→3)-β-D-glucan may account for up to 60% of the dry weight of the cell wall of fungi [11], and pollen allergens may exist in smaller particles than intact pollen grains [12]. These findings suggest that fragments of biological particles may adversely affect health [6]. In this study, high (1→3)-β-D-glucan levels were significantly related to high temperature (Table 2), and fungal growth tends to favors high temperatures [13].

Nevertheless, (1→3)-β-D-glucan levels were collected on different days across the A to E locations throughout the spring, and pollen counts may have differed on the days levels were measured; therefore, this could be the main reason for differences among locations during the spring (Table 1).

When comparing indoor and outdoor levels of (1→3)-β-D-glucan, the total GM level in the outdoor environments measured in our study year (312 pg/m^3) was lower than that of data collected in studies on indoor environments. For example, (1→3)-β-D-glucan levels were reported to be 5700 pg/m^3 in day care centers, 3200 pg/m^3 in office buildings, and 3700 pg/m^3 in homes [14].

This study has important limitations. First, data were not collected for each outdoor location on the same day due to issues of accessibility, a lack of resources, and limitations in our approved permits. Furthermore, average levels of climatic factors may not have accurately reflected the levels in our small sampling areas. However, there are limited data on the relationship between outdoor climatic factors and (1→3)-β-D-glucan. In most previous studies on airborne (1→3)-β-D-glucan, measurements were taken over a short period and in an indoor environment with simulated exposures to airborne microorganisms. However, a strength of this study is that we evaluated the monthly variance of airborne (1→3)-β-D-glucan over one year and found significant differences in (1→3)-β-D-glucan levels throughout the year, especially in April.

The outdoor levels of (1→3)-β-D-glucan measured on the roofs of five university buildings varied throughout the study year. The highest level of (1→3)-β-D-glucan occurred in the spring and (1→3)-β-D-glucan was significantly correlated to outdoor temperature. This study provides basic information on the monthly variations of (1→3)-β-D-glucan levels and its relation with other outdoor factors. In Korea, (1→3)-β-D-glucan might be considered the main indicator of pollen in outdoor environments.

CONFLICT OF INTEREST

The authors have no conflicts of interest with the material presented in this paper.

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