보건학석사 학위논문

Quantity, Size Distribution and Behavior Characteristics of Cough-generated Aerosol Produced by Cold Patients

감기 환자의 호흡기에서 발생하는

에어로졸의 특성

2017년 2월

서울대학교 보건대학원

환경보건학과 산업보건전공

이 진 호

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이 논문을 보건학석사 학위논문으로 제출함

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ABSTRACT

Quantity, Size Distribution and Behavior Characteristics of Cough-generated Aerosol Produced by Cold Patients

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Objective It is generally recognized that most nosocomial infections are spread by expelled particles at close range, usually within 1 m from the site of generation, and occasionally through contact. Although the World Health Organization (WHO) established a cut-off to classify droplets (> 5 μm) and airborne particles (< 5 μm), and a 2 m cut-off for patients during the Middle East respiratory syndrome outbreak in Korea in 2015, questions have been raised regarding the efficacy of a single cut-off delineation and possible infection by aerosol transmission beyond a distance of 2 m. The purpose of this study was to characterize cough-generated

aerosol emissions from cold patients, and to determine their behavioral characteristics (particle transmission distance) in indoor air.

Methods This study was carried out with 10 subjects who were diagnosed with acute upper respiratory infections at medical institutions. Patients participated in two experiments. The first experiment was conducted using a stainless steel chamber and the second was conducted in a clean room. The number and size distribution of particles generated from each cough were measured in the stainless steel chamber. Tests were repeated three times by each patient. In the clean room, participants coughed and total particle concentration before, during, and after the cough was measured once for each patient at 0.5 m and 3 m. A scanning mobility particle sizer and an optical particle spectrometer were used to measure the particles, and an ultrasonic spirometer was used to measure pulmonary function of the lungs, mean cough aerosol volume, and peak airflow during coughing. All measurements were performed in the same way after patients recovered and differences between infections were compared.

Results The number of particles from a cough by participants with a cold increased by 560 ± 513% compared to those after recovery (while ill: 4,995,000 ± 6,090,000, after recovery: 1,376,000 ± 1,459,000) ($p < 0.001$). The proportion of expelled particles with a diameter < 5 μm (particles that can be transmitted through the air) was 99.9 ± 0.3% of the total number and 90.2 ± 12.2% of total surface area while the subjects had a cold. Most of the particles propagated to the far field (3 m) and the near field (0.5 m) in the air, regardless of the subject's infection status. The number of particles was significantly higher ($p < 0.001$) than the background concentration when the patient was coughing even in the far field, which exceeded

the WHO recommended isolation distance of 2 m.

Conclusion The results show that the number of aerosol particles expelled during coughing by patients with a cold was significantly higher ($p < 0.001$) than that after recovery from a cold. We confirmed that aerosols generated during coughing, regardless of symptoms, were transferred to the far field (3 m) and near field (0.5 m). These results suggest that the < 5 μm dichotomous cut-off for a droplet and aerosol criterion of the WHO, and the 2 m cut-off for possible airborne infection of the Korea Centers for Disease Control, should be reconsidered for effective prevention of airborne infections.

Keywords: Cough aerosol, airborne transmission, respiratory infections, disease transmission, droplet nuclei

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1. Introduction

Transmission of an infection can be classified as either droplet transmission or airborne transmission. Droplet transmission is defined as transmission of diseases by particles expelled at close range, usually within 1 m from the site of generation, and occasionally through contact. The World Health Organization (2007) recommended a 5 μm aerosol diameter cut-off to classify droplet (> 5 μm) and airborne (< 5 μm) transmission. This relation between droplet and airborne transmission is underpinned by the studies of Wells (1934) and Hamburger and Robertson (1946).

It is generally recognized that most nosocomial infections are spread by contact (Beggs, 2003). However, several studies on airborne transmission of infectious pathogens in indoor environments using this framework of single cut-off delineation failed to acknowledge the size of particles, or that they did not exclusively disperse by airborne or droplet transmission, but instead used both ways simultaneously (Gralton et al., 2011). Although many nosocomial infections are associated with direct person-to-person contact in indoor environments, there is a strong association between the transmission of many pathogens, such as measles, smallpox, tuberculosis, and severe acute respiratory syndrome (SARS), and indoor air movement (Li et al., 2007). Hence, determining aerosol diameter and indoor airflow is important to understand pathogen-containing aerosol movement. Nevertheless, there is a lack of evidence to suggest that infections are airborne-transmitted among humans in healthcare settings, because epidemic diseases, such as influenza, are believed to have a relationship with respiratory airborne transmission (Roy and Milton 2004), and human coughing seems to promote the

spread of cough-generated aerosols by producing more airborne aerosols than vocalizing or breathing (Morawska et al., 2009). According to Blachere et al. (2009), viral RNA of seasonal influenza was detected in the emergency room of a hospital and aerosol transmission was implicated (Wong et al. 2010).

Inadequate categorization of close contact from the Korea Centers for Disease Control and Prevention (KCDC) was suggested as the key factor in the spread of Middle East respiratory syndrome (MERS) in South Korea in 2015. The "Guidelines for the Management of Middle East Respiratory Syndrome (MERS)", published by the KCDC in December 2014, refers to the contact person as "a person who has had physical contact with a confirmed or suspected patient (or within 2-m)", and did not consider the possibility of airborne transmission of aerosols. Consequently, the response guidelines of the Korea Ministry of Health and Welfare and the KCDC for MERS outbreak, pertaining to close contact, are considered inadequate due to insufficient data (Choi et al., 2015).

Due to the wide-ranging and potentially long-term transmission of cough-generated airborne particles, it is important to understand the dynamics of coughed particles of different sizes. Coughing can release particles with a higher concentration than breathing or talking, as coughing discharges a large quantity of airborne particles at a high discharge velocity. An experimental study demonstrated that the influenza A virus remains infectious in small particle aerosols and can transit across rooms (Noti et al. 2012). Influenza virus and viral RNA can be detected in droplets > 5 μm and nuclei < 5 μm (Milton et al., 2013, Lindsley et al., 2010). Lindsley et al. (2012) reported that the mean number of particles expelled by each cough is 900–302,200. It is very likely that a cough jet from an influenza-

infected subject contains pathogens and spreads airborne diseases that can be inhaled into the respiratory tracts of other individuals. Furthermore, coughed particles have a wide range of sizes and different transport characteristics. Lindsley et al. (2012) observed that coughed particles have a size range of 0.35–10 μm and Yang et al. (2007) reported a mean size distribution of coughed droplets of 0.62–15.9 μm, with nuclei sizes of 0.58–5.42 μm. A review conducted by Gralton et al. (2011) summarized the size range of coughed particles from a large number of studies and concluded that the size of cough-generated particles ranges from 0.1–100 μm. However, no study has been performed on transmission of cough-generated airborne nanosized particles (< 0.1 μm) at a distance greater than the direct contact distance.

This study focused on the emission and lingering of airborne phase particles before sedimentation and their potential for long-range transmission. Hence, the objectives of this study were to characterize cough-generated aerosol emissions and to determine their behavioral characteristics in indoor air under the relative humidity and temperature conditions of a healthcare facility.

2. Materials and Methods

2.1. Study outline

This study consisted of two experiments; one was conducted in a cylindrical exposure chamber and the other was conducted in a clean room. The exposure chamber was used to estimate the quantity and size distribution of cough-generated aerosols. We observed cough-generated aerosol diffusion in the near field (< 1 m) and far field (> 2 m) in the clean room, as well as variations in concentration by size and distance. The two experiments were repeated after the subjects recovered, to assess differences between pre- and post-recovery.

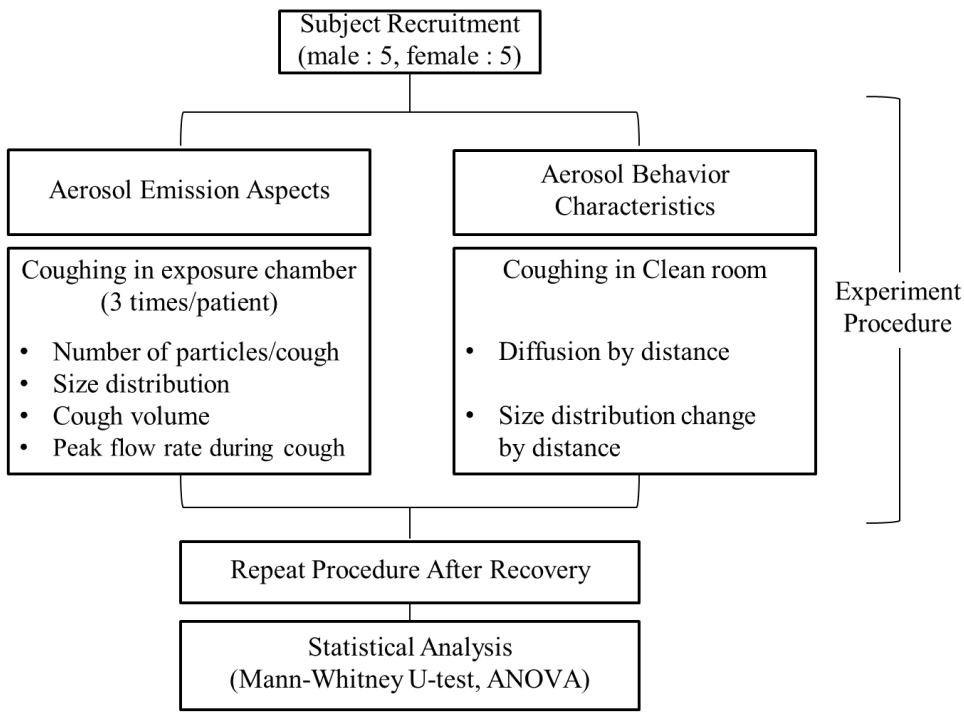

Figure 1. Study outline.

2.2. Recruitment

Patients with cold symptoms were recruited from August 2016 to November 2016, and all participants were clinically diagnosed with a cold. For inclusion in the study, all subjects were required to be 18–39 years of age, of male sex (or non-pregnant female), to have no other health problems, to have received no vaccination against influenza within the last 6 months, and to be a lifetime non-smoker. Subjects were asked a few questions about their illness and current symptoms. Twelve subjects were recruited to the study. Of these, 10 (5 males and 5 females; mean age, 22–33 years) were confirmed as having a cold on their first visit to the experimental room; they returned for the second test session after their symptoms had resolved.

All recruitment and study processes were approved prior to the start of the study by the Seoul National University Institutional Review Board.

2.3. Monitoring procedure

Instrumentation

A scanning mobility particle sizer (SMPS) (NanoScan Model 3910; TSI Inc., Shoreview, MN, USA) and an optical particle spectrometer (OPS) (Model 3330; TSI Inc.) were used and a 1 min interval was applied to measure the particle concentration and size distribution in real-time. The detectable size ranges of the instruments were 10–420 nm for the SMPS and 300–10,000 nm for the OPS. An ultrasonic spirometer (EasyOne; Medical Technologies, Andover, MA, USA) was used to measure mean coughed aerosol volume and peak air flow during coughing, and a 40-L stainless steel cylinder served as a collection chamber for coughed aerosols. The collection chamber was fitted with an inlet port for the spirometer and an outlet linked with the SMPS and OPS.

Aerosol Emission Aspects - Chamber

The subjects participated in two. The 40-L stainless steel chamber was used to evaluate aerosol emissions. To evaluate emissions of cough-generated aerosols, the participant was seated in front of the steel chamber and asked to breathe normally for 5 min to remove background aerosols from their respiratory tract. An air pump was used to remove background particles from the chamber. After breathing for 5 min, the air pump was turned off, and the subject was asked to inhale as deeply as possible, and to cough with maximum force through the spirometer mouthpiece, which was connected to the chamber. After coughing, the participant exhaled the aerosol that remained in their respiratory tract, and the aerosol was collected and analyzed. After analysis, the chamber was evacuated for 10 min using the air pump, and the subject was asked to repeat the coughing procedure two more times for a total of three coughs. After each participant finished the procedure, the spirometer mouthpieces and equipment, including the chamber, were cleaned with disinfectant and UV light.

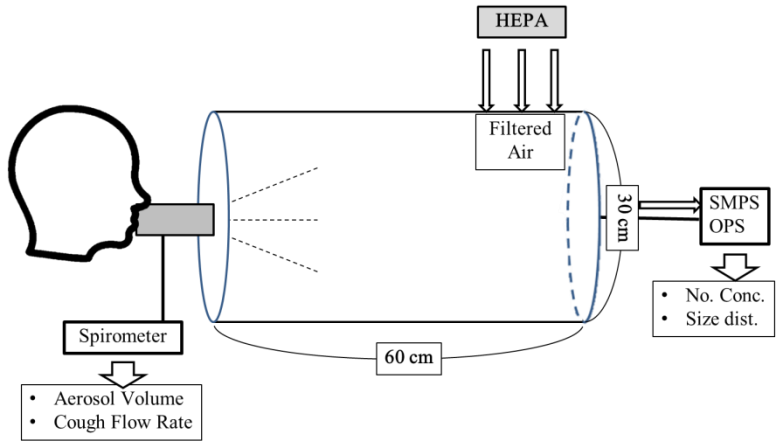

Figure 2. Experimental scheme for the exposure chamber.

Aerosol Behavior Characteristics - Clean room

The experiment to evaluate the behavioral characteristics of the cough-generated aerosol in an indoor environment was conducted in the clean room, which controlled background particulates to < 10 particles/cc using a high efficiency particulate air (HEPA) filter-equipped ventilation system. The volume of the clean room was 40.32 m^3 (7.0 m [W] × 2.4 m [L] × 2.4 m [H]). The participant was asked to put on dustproof clothing, and to take an air shower to exclude the possibility of there being any particulate matter from other sources, such as dust dispersion. The temperature and relative humidity were consistently monitored by a real-time thermo-hygrometer (Model TR-72U; T&D Inc., Redmond, WA, USA) to maintain the room conditions.

Figure 3 shows the sampling diagram. The direct reading instruments for measuring particle concentration and size distribution were located in each sampling location. According to the concept of influenza transmission of previous studies, we divided the clean room area into a near field (< 1 m) and a far field (> 2 m). The SMPS-1 was located 0.5 m from the participant to evaluate the aerosol emissions in the direct contact transmission range. The SMPS-2 and OPS were located 3 m from the participant to identify the particle dispersion and the exposure at the direct contact range.

Experiment Condition

Relative humidity and temperature were maintained at 30–50% and 21–25℃, respectively, to represent indoor air conditions in a hospital or emergency room (American National Standards Institute/American Society of Heating, Refrigeration, and Air Conditioning/American Society of Healthcare Engineering, 2008). When the subjects had a cold, the mean temperature in the clean room was 24.0℃ (standard deviation [SD] = 0.59), and mean relative humidity was 38.3% (SD = 3.42). After the subjects had recovered, the mean temperature in the clean room was 23.8℃ (SD = 0.36), and mean relative humidity was 37.2% (SD = 1.28).

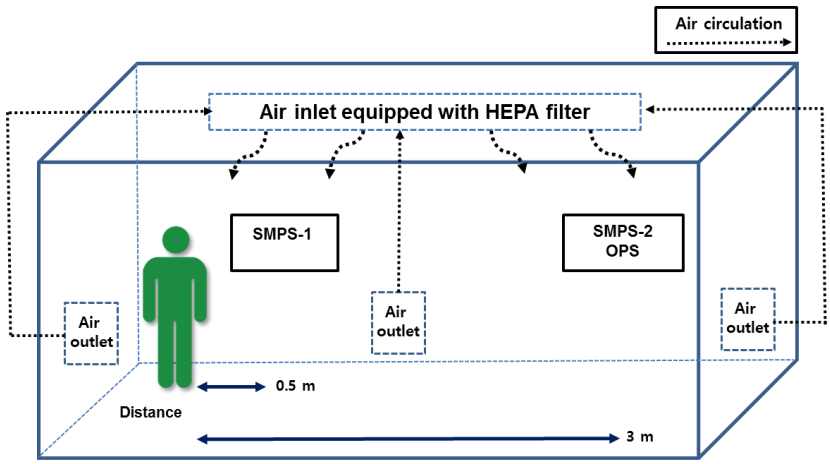

Figure 3. Experimental scheme conducted in the clean room. Filtered air was circulated about 60 min before the experiment to lower the background aerosol concentration, and circulation was suspended during the experiment.

Each experiment was divided into three phases, as follows:

1) Before the cough

   The HEPA-filtered air circulation system was operated for at least 60 min to remove contaminants from the clean room. After the particulate concentration level was stabilized, the ventilation system was stopped and 30 min of sampling was started to obtain a background aerosol concentration level.

2) During the cough

   This phase comprised the coughing and rest periods. The participant was asked to cough continuously for 1 min, and to rest for 5 min to exhale the aerosol remaining in the respiratory tract. This cough cycle was repeated five times for 30 min of cough-generated aerosol emissions.

3) After the cough

   Real-time monitoring was continued for 30 min to monitor the residence and diffusion of cough-generated aerosols after the cough.

2.4. Calculation and data analysis

The concentration and size distribution data measured by the SMPS and OPS were used to estimate particle number concentrations and the size distribution. The SMPS provided aerosol particle counts in 13 size bins and the OPS provided 5 bins. The particle concentrations of 18 optical bins 10 nm to 10 μm in diameter were monitored. Data from the SMPS and OPS channels were merged using Multi Instrument Manager software (MIM-2 ver. 2.0; TSI Inc.) provided by the manufacturer.

All data acquired from real-time monitoring were analyzed statistically. Descriptive statistics were recorded to compare the aerosol concentration during versus after cough. The number and volume of aerosol particles per cough are presented as arithmetic means (AM) ± SD because the results were acquired from experiments repeated three times. The particle concentrations at each sampling location are shown as AMs ± SD.

The results of each subject's coughing while ill were compared to coughs done after recovery using the Mann–Whitney U test. One-way analysis of variance was conducted to compare the particle concentration according to elapsed time (before, during, and after the cough) and Tukey's test was applied to determine the difference in particle concentration by time elapsed. A result was considered significant when $p \leq 0.05$. All analyses were conducted using SAS software (9.4; SAS Institute, Cary, NC, USA). SigmaPlot software (ver. 10; Systat Software, San Jose, CA, USA) was used to visualize the results.

The particulate concentrations in the chamber were assumed to be the same everywhere and it was also assumed that the aerosol dispersed equally when the concentration was highest 5 min after the cough. Equation 1 was used to estimate the aerosol emissions for each subject:

$$Number\ of\ aerosol\ per\ cough = (C_{particle,max} - \overline{C}_{particle,bg}) \times V_{chamber}$$

Equation 1.

Where $C_{particle,max}$ is the particle concentration inside the chamber 5 min after the cough, V is chamber volume (m^3), and $\overline{C}_{particle,bg}$ is the mean background concentration inside the chamber beginning 5 min before the test. Several assumptions exist for Equation 1 that may lead to inaccuracies when estimating aerosol emissions. Size-resolved particle dynamics, coagulation, and constant particle loss rates were ignored.

3. Results

3.1. Individual characteristics of subjects

The mean time from the first to the second visit was 32.1 ± 12.1 days. Cough volume and cough peak flow rate were measured during illness and after recovery, while the forced vital capacity (FVC), forced expiratory volume in 1 sec (FEV1), and peak expiratory flow (PEF) of subjects were measured when the patients were ill.

The general characteristics of the participants, including cough volume, cough flow rate, FVC, FEV1, and PEF, are summarized in Table 1.

The air volume of each cough, and the peak cough flow rate, increased slightly after recovery compared to during the cold (cough volume, $p = 0.57$; peak cough flow rate, $p = 0.27$). Mean air volume and peak cough flow rate during the cold were 1.68 ±h1.19 L and 6.01 ±.1.45 liters/sec (LPS), respectively, and increased to 1.96 ±1.02 L and 6.59 ±.1.98 LPS after recovery.

FVC, FEV1, and PEF were significantly higher in males than in females (all p-values = 0.01). Mean FVC, FEV1, and PEF of female subjects were 2.92 ± 0.24 L, 2.52 ± 0.25 L and 4.59 ± 1.02 L, respectively, whereas they were 4.54 ± 0.65 L, 3.88 ± 0.40 L, and 9.07 ± 1.07 L in males, respectively. The peak cough flow rate and cough volume of each cough during a cold were also significantly higher in males than in females (all p values = 0.04) but the difference disappeared after recovery, although peak cough flow rate and cough volume were still higher in males than in females ($p = 0.09$ and $p = 0.06$, respectively).

Table 1. Individual characteristics of the participated subjects

ID	Gender	Age	Height (cm)	Weight (kg)	FVC(L)	FEV1(L)	PEF(LPS)	Cough Volume(L)		Cough Peak Flow Rate(LPS*)	
								While ill	After Recovery	While ill	After Recovery
1	F	25	158	49	2.75	2.61	4.00	0.81	1.33	4.56	2.83
2	F	24	160	49	2.52	2.30	4.59	0.45	0.44	3.58	3.90
3	F	24	158	46	3.03	2.45	4.22	0.73	0.95	5.84	6.37
4	F	29	162	52	3.10	2.28	3.59	1.12	1.60	5.35	6.02
5	F	22	158	63	3.18	2.97	6.53	1.53	2.37	5.92	6.45
Sub-total	F	24.8±2.3	159.2±1.6	51.8±5.9	2.92±0.24	2.52±0.25	4.59±1.02	0.93±0.36	1.34±0.64	5.05±0.88	5.11±1.47
6	M	26	177	77	4.75	4.26	9.29	1.23	3.39	6.28	8.25
7	M	25	173	75	4.77	4.09	10.37	3.87	3.30	8.11	9.19
8	M	30	174	77	3.24	3.12	7.10	1.61	1.50	6.12	8.43
9	M	26	182	90	4.97	3.85	9.18	1.44	1.37	5.52	5.88
10	M	33	174	77	4.95	4.07	9.40	4.02	3.39	8.79	8.58
Sub-total	M	28±3.0	176±3.2	79.2±5.4	4.54±0.65	3.88±0.40	9.07±1.07	2.43±1.24	2.59±0.94	6.96±1.25	8.07±1.13
Total	-	26.4±3.1	167.6±8.8	65.6±14.8	3.73±0.94	3.20±0.75	6.83±2.47	1.69±1.18	1.96±1.02	6.01±1.44	6.59±1.98
P-value		0.07	0.01	0.01	0.01	0.01	0.01	0.04	0.09	0.04	0.06

* Litters per sec; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 sec, PEF, peak expiratory flow

3.2. Aerosol Emission Aspects

Quantity comparison between subjects

The number of particles expelled per cough while the subjects had a cold ranged from 731,000 to 18,756,000 (mean, 4,914,600 particles/cough). The number of particles expelled per cough after the subjects recovered ranged from 200,900 to 450,000. When the patients had a cold, the mean number of particles per cough was higher than that after they recovered ($p < 0.001$). However the mean was not significantly different between the sexes, either when patients were ill or had recovered ($p = 0.68$ and $p = 0.21$, respectively).

The surface area of particles expelled per cough while the subjects had a cold varied from 156,000 to 66,824,000 μm^2 (mean, 7,210,000 μm^2/cough). The surface area of particles expelled per cough after the subjects recovered ranged from 39,000 to 2,681,000 μm^2 (mean, 521,000 μm^2). When the patients had a cold, the mean surface area of particles per cough was higher than that after they recovered ($p = 0.002$). However, the mean was not different between the sexes, either when the patients were ill or had recovered ($p = 0.40$ and $p = 0.30$, respectively).

Table 2. Number and surface area of particles expelled per cough - Chamber (n=3)

ID.	Gender	Number of Particles / Cough			Surface Area of Particles / Cough (μm²)		
		While ill	After Recovery	P-value	While ill	After Recovery	P-value
1	F	4,443,000 ± 2,300,000	661,000 ± 421,000	0.081	438,000 ± 210,000	66,000 ± 53,000	0.081
2	F	774,000 ± 477,000	600,000 ± 180,000	0.190	748,000 ± 786,000	55,000 ± 14,000	0.190
3	F	2,542,000 ± 959,000	546,000 ± 291,000	0.383	160,000 ± 87,000	106,000 ± 109,000	0.383
4	F	4,674,000 ± 1,857,000	566,000 ± 292,000	0.383	818,000 ± 306,000	444,000 ± 531,000	0.383
5	F	18,806,000 ± 6,984,000	1,039,000 ± 604,000	0.081	66,825,000 ± 33,647,000	121,000 ± 114,000	0.081
Sub-total		6,248,000 ± 7,291,000	683,000 ± 182,000	<0.001	13,805,000 ± 30,498,000	159,000 ± 145,000	<0.001
6	M	3,226,000 ± 1,525,000	1,178,000 ± 440,000	1.00	127,000 ± 136,000	714,000 ± 863,000	1.00
7	M	1,596,000 ± 1,145,000	4,229,000 ± 2,728,000	0.383	1,212,000 ± 481,000	842,000 ± 629,000	0.383
8	M	3,522,000 ± 2,057,000	363,000 ± 108,000	0.081	492,000 ± 343,000	39,000 ± 17,000	0.081
9	M	1,043,000 ± 490,000	983,000 ± 651,000	0.663	156,000 ± 44,000	113,000 ± 87,000	0.663
10	M	9,326,000 ± 6,181,000	2,940,000 ± 1,910,000	1.00	550,000 ± 330,000	2,681,000 ± 3,132,000	1.00
Sub-total		3,742,000 ± 4,235,000	1,939,000 ± 1,430,000	0.147	512,000 ± 498,000	882,000 ± 958,000	0.481
Total		4,995,000 ± 6,090,000	1,376,000 ± 1,459,000	<0.001	7,210,000 ± 19,901,000	521,000 ± 774,000	0.002

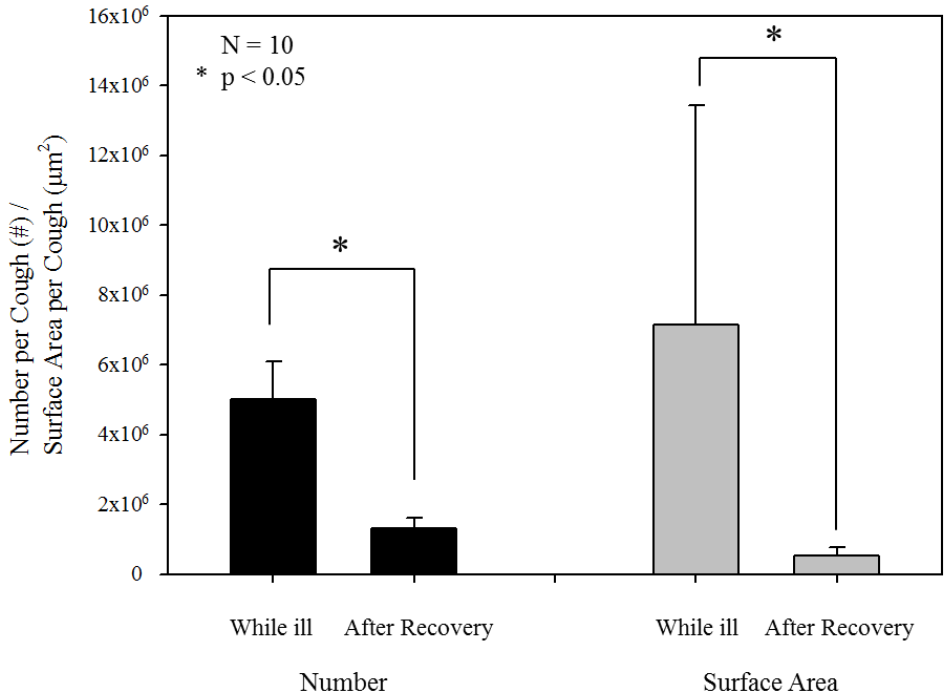

Figure 4. Number and surface area of particles per cough while ill and after recovery. Results were derived from chamber experiment. Each bar shows the average of total three coughs, and the error bars show the standard error.

Size distribution of cough-generated aerosol

Figure 5 shows a plot of the number of aerosol particles expelled per cough, as detected in each size bin, and Figure 6 shows a plot of the surface area of aerosol particles per cough in each size bin. About 99.9 ± 0.3% of all expelled particles had diameters < 5 μm (airborne transmission) when the subjects had a cold, which was 90.2 ± 12.2% of the total surface area. The particle number concentration was decreased respectively in each size channel of instruments.

Mean number of particles per cough and mean surface area of particles per cough were higher within a certain diameter range (< 100 nm, 100–300 nm, 420–1,000 nm, 1.0–2.5 μm) when the patients had a cold versus after they had recovered ($p < 0.001$).

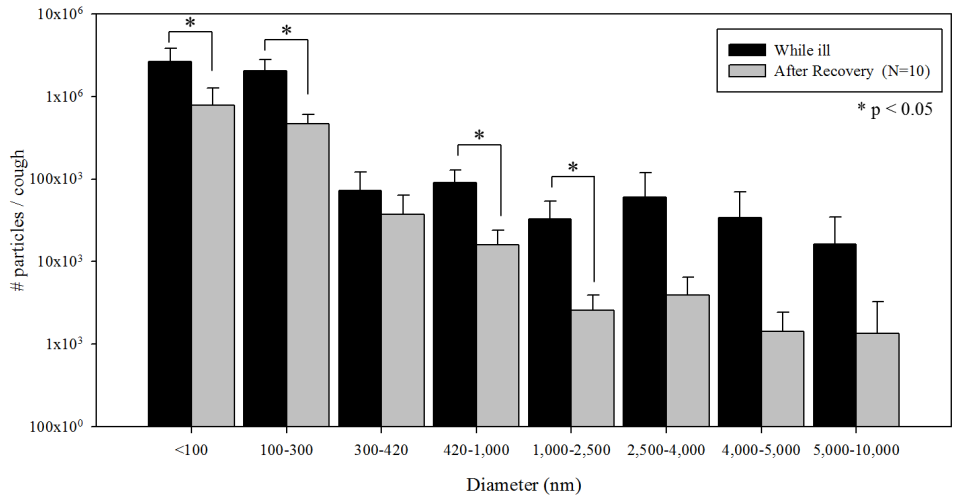

Figure 5. Number of particles per cough in each size - Chamber.

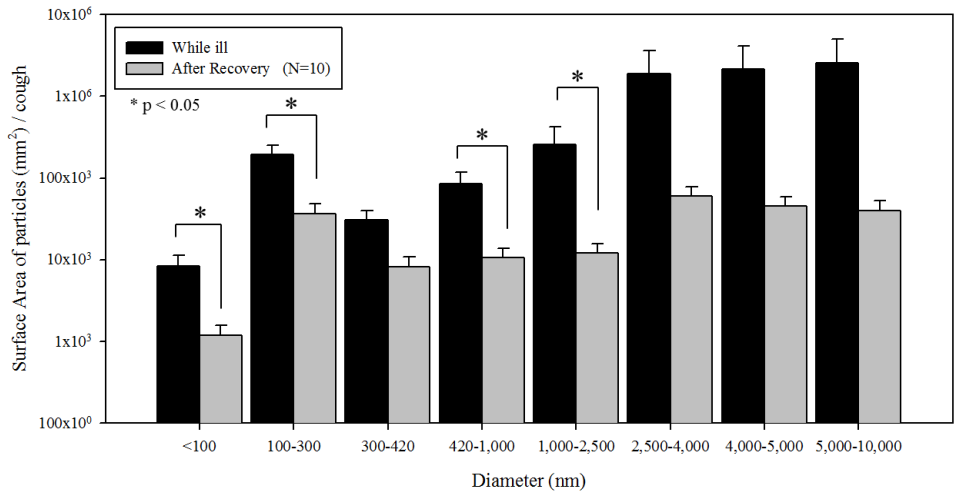

Figure 6. Surface area of particles per cough in each size - Chamber.

3.3. Aerosol behavior characteristics

Spatial diffusion of aerosol

Table 3 shows a summary of particle concentrations in the background, during cough and after cough in the 0.5 m (near field) and 3 m (far field) from the source (participant). Particle concentrations during cough of the patients with a cold were considerably higher than those of the background level and after cough, but after patients were recoved, Particle concentrations during cough was not significantly higher than those of the background level and after cough.

Nine of ten subjects showed increased particle concentrations in the far field compared to the background concentration while they were ill. The AM of particle concentration in the far field increased during coughing by infected subjects compared to the AM of the background concentration. The difference in particle number concentration in clean room between background and cough was varied from 65 to 710 particles/cm^3 and the difference was significant ($p < 0.001$).

The AM of the particle concentration in the near field was higher compared to the AM of the background concentration in The difference in particle number concentration in clean room between background and cough was varied from 8 to 448 particles/cm^3 and the difference was not significant ($p = 0.22$).

Table 3. Particle number concentration by experiment phases – clean room (# / cc, SMPS only)

		0.5 m			3.0 m		
ID	Diagnosis	Background (N=30) AM ± SD	During Cough (N=30) AM ± SD	After Cough (N=30) AM ± SD	Background (N=30) AM ± SD	During Cough (N=30) AM ± SD	After Cough (N=30) AM ± SD
1	While ill	1,163± 173	1,443 ± 330	1,346 ± 90	1,098 ± 56	1,503 ± 192	1,200 ± 103
	After Recovery	2,619 ± 202	2,605 ± 162	2,891 ± 171	2,437 ± 173	2,281 ± 130	2,596 ± 145
2	While ill	2,019 ± 110	2,159 ± 113	2,100 ± 98	1,730 ± 96	1,797 ± 96	1,754 ± 77
	After Recovery	4,044 ± 163	3,920 ± 161	3,708 ± 173	3,275 ± 160	3,501 ± 139	3,251 ± 395
3	While ill	-	-	-	1,154 ± 100	1,460 ± 141	1,147 ± 74
	After Recovery	884 ± 81	944 ± 86	1,037 ± 79	690 ± 63	755 ± 80	789 ± 65
4	While ill	-	-	-	1,282 ± 69	1,488 ± 117	1,202 ± 213
	After Recovery	3,732 ± 294	4,001 ± 223	3,917 ± 180	3,494 ± 252	3,758 ± 266	3,605 ± 187
5	While ill	-	-	-	2,277 ± 198	2,987 ± 628	2,260 ± 172
	After Recovery	1,627 ± 85	2,011 ± 239	1,752 ± 177	1,270 ± 70	1,270 ± 70	1,454 ± 92
6	While ill	-	-	-	3,329 ± 131	3,955 ± 236	3,162 ± 227
	After Recovery	2,137 ± 125	2,401 ± 155	2,645 ± 283	2,032 ± 96	2,102 ± 148	2,314 ± 140
7	While ill	1,968 ± 134	2,141 ± 121	2,314 ± 159	1,611 ± 87	1,881 ± 141	2,061 ± 115
	After Recovery	2,786 ± 169	2,815 ± 132	2,542 ± 147	2,547 ± 166	2,661 ± 141	2,350 ± 146
8	While ill	2,753 ± 106	2,761 ± 160	2,730 ± 207	2,515 ± 101	2,231 ± 153	2,429 ± 171
	After Recovery	3,980 ± 316	4,006 ± 302	3,655 ± 221	3,548 ± 287	3,430 ± 284	3,150 ± 259
9	While ill	-	-	-	3,244 ± 206	3,873 ± 220	3,928 ± 335
	After Recovery	3,881 ± 204	3,997 ± 203	3,978 ± 160	3,421 ± 177	3,534 ± 152	3,382 ± 117
10	While ill	2,893 ± 238	3,341 ± 274	2,559 ± 129	3,495 ± 248	3,800 ± 357	3,124 ± 200
	After Recovery	3,622 ± 482	3,747 ± 412	3,703 ± 447	3,293 ± 548	3,830 ± 310	3,397 ± 444

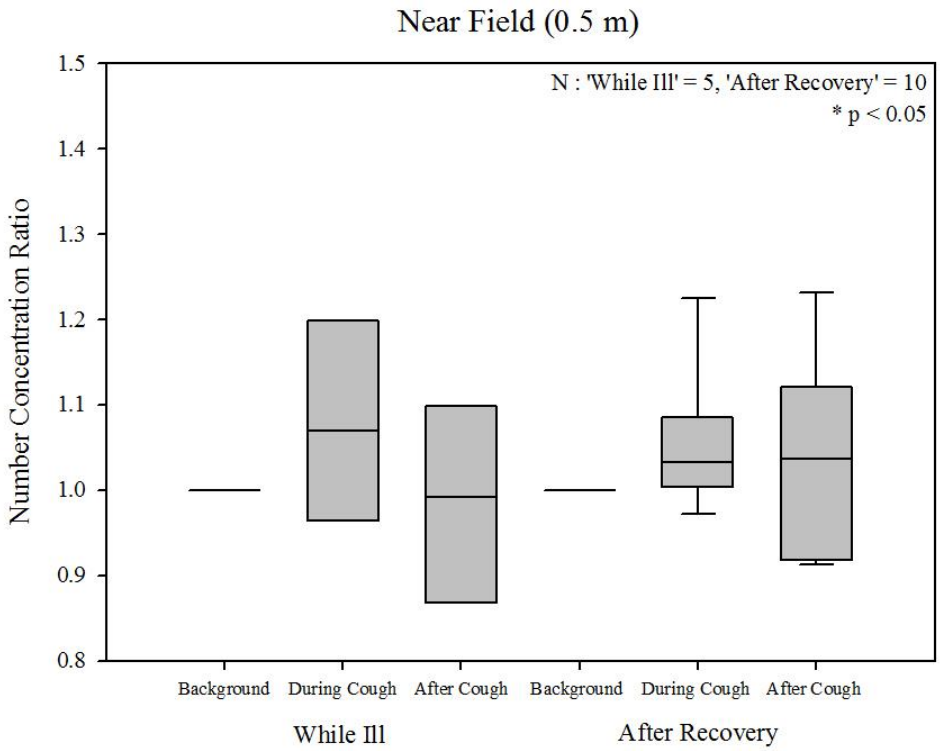

Figure 7. Particle number concentration ratio at near field. The mean number concentration of each phase was normalized by dividing by mean number concentration of background. Values shown are median (line within box), 25th and 75th percentiles (bottom and top of box, respectively), 10th and 90th percentiles (lower and upper bars on whisker, respectively).

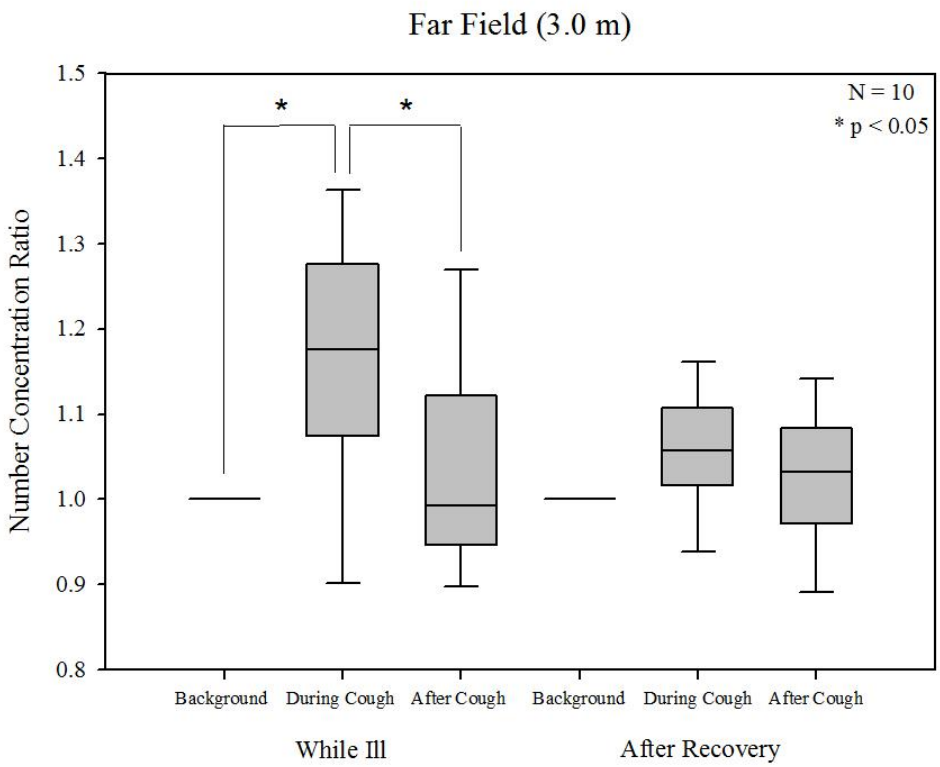

Figure 8. Particle number concentration ratio at near field. The mean number concentration of each phase was normalized by dividing by mean number concentration of background. Values shown are median (line within box), 25th and 75th percentiles (bottom and top of box, respectively), 10th and 90th percentiles (lower and upper bars on whisker, respectively).

Size distribution by distance

Figure 9 shows the comparison of particle concentrations by distance. The distribution of particle concentrations during coughing were not different in the 13 different-sized bins, which ranged from 10 nm to 420 nm (p = 1.000).

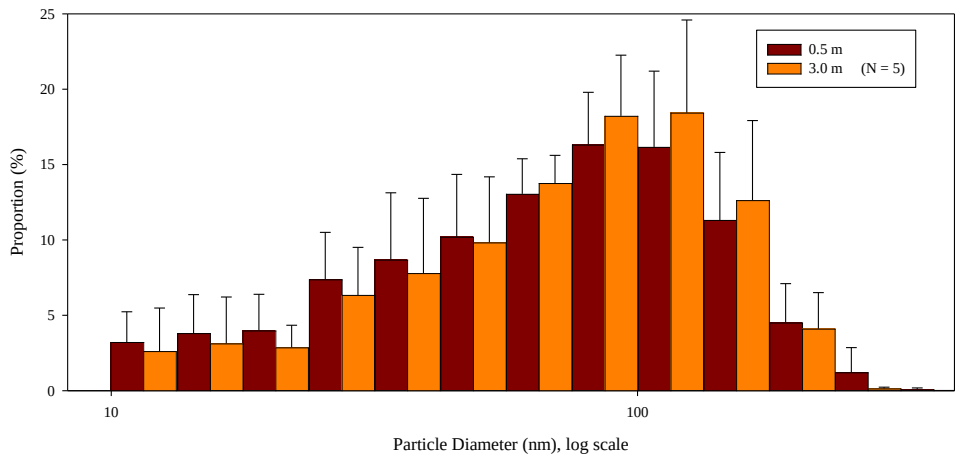

Figure 9. Proportion of particle number concentration in each size – clean room.

Correlation between momentum of cough and particle concentration

In exposure chamber experiments, when patients have cold symptom, number of aerosol generated from single cough has no statistically significant correlation with FVC(0.93), FEV1 (0.90), PEF (0.65), volume of cough (0.28), cough peak flowrate (0.43), body mass index (0.57), and sex (0.63). However, after patients have recovered, number of aerosol generated from single cough has statistically significant correlation with FVC (0.04, r = 0.65), FEV1 (0.03, r = 0.68), PEF (0.01, r = 0.78) and volume of cough (0.02, r = 0.72) and it did not have statistically significant correlation with cough peak flowrate (0.19), body mass index (0.33), and sex (0.19).

In clean room experiments, when patients have cold symptom, ratio of aerosol number concentration during cough to background has statistically significant correlation with number of cough (0.04, r = 0.65). However, it has no statistically significant correlation with FVC(0.73), FEV1 (0.96), PEF (0.47), volume of cough (0.49), cough peak flowrate (0.58), body mass index (0.56), and sex (0.27). After patients have recovered, ratio of aerosol number concentration during cough to background has no statistically significant correlation with number of cough (0.65), FVC(0.75), FEV1 (0.85), PEF (0.65), volume of cough (0.38), cough peak flowrate (0.38), body mass index (0.99), and sex (0.38).

4. Discussion

The possibility of airborne transmission of pathogen-containing aerosols is a critical issue for the public health community. However, many questions remain about potentially infectious aerosols produced by ill people. Many recent studies were focused on generation of aerosol expelled from respiratory, and its transmission possibility was usually studied by using models like suggested by Xie et al.(2007), Redrow et al. (2011) and Wei et al. (2015), not an experimental method. The present results show that people produce ultrafine aerosols < 5 μm, as well as aerosols with a greater number of particles, when they are sick with influenza compared to after they have recovered.Similar to several studies on the quantity of cough-generated aerosols, the number of cough-generated aerosol particles expelled by our subjects varied tremendously from person-to-person (Stelzer-Braid et al,. 2009, Fabian et al., 2008, Lindsley et al., 2010). The range of generated particles was 731,000 particles/cough to 18,756,000 particles/cough while subjects had an infection. These results suggest a “superspreader” effect; if an individual expels greater quantities of infectious particles, they may spread virus or other infectious agent to others at a much higher rate. When calculating the number of particles per cough, the maximum concentration within 5 min after cough was assumed to represent the state which aerosol were completly diffused. This may have led to overestimation of the particles emitted compared with the result the result calculated by using average of number concentration within 5 min after cough. However, considering wide indivisual variation of high emissions, it is reasonable to calculate the concentration of particles conservatively.

The fraction of small particles less than 5 μm in this study was 99.9% of total number and 90.2% of total surface area. As seen in Figure 5, most aerosols were less than 5 μm , which meant they could enter and deposit at the alverolar region rather than upper respiratory tract and branchiloles. In other words, most of cough generated aerosols are belong to the respirable particle mass rather than inhalable or throracic particle mass. So, when considering infection by aerosols, the respirable fraction of cough-generated aerosol is of great concern. Comparing with the particles depositing in the nasal region, particles depositing into lungs have considerably lower dose for infection (Tellier, R. 2006).

Moreover, because of the capacity to reach the alveoli, the respirable fraction of cough-generated aerosols is of great concern. Compared with particles deposited in the nasal region, particles deposited in the lungs have a considerably lower infection dose for infection (Tellier, 2006). Approximately 99.9% of the total number of particles expelled by the subjects in this study were < 5 μm in diameter (airborne transmitted particles), and 90.2% of the total surface area. As seen in Figure 5, most aerosols were less than 5 μm , which meant they could enter and deposit at the alverolar region rather than upper respiratory tract and branchiloles. In other words, most of cough generated aerosols are belong to the respirable particle mass rather than inhalable or throracic particle mass.

Compared from previous studies, dome differences were existed. From the result of Yang S et al. (2007), many small droplet nuclei have the total average size distribution of 0.58-5.42 μm and 82% of droplet nuclei centered in 0.74–2.12 μm. It was contrast to this study has modal diameter of < 100 nm. The process where the authors of that study transferred respiratory-origin warm and water vapor

saturated cough aerosol to a dry bag at room temperature. This would have involved a disturbance to the original equilibrium size of the aerosol because of the saturation and occurred on the walls of the bag. Therefore, the size distribution would have been larger than that of the aerosol in the respiratory tract. According to another study of Johnson et al. (2009), particles with sizes of 8–10 μm are the most common type The size of the droplets varied from 0.1 to 16 μm and the number concentration was also varied, from 0.001 to 5.5 #/cc. These differences were supposedly due to differences among monitoring devices. The lower diameter limit of SMPS was much smaller than device(APS) used in Johnson's study, which used devices with a lower diameter limit of 0.5 μm. (SMPS:10 nm, APS:0.5 μm). From the wide range of detection we obtained the numeric proportion of nanosized particles, and this made the large difference of aerosol number concentration. The size of pathogens may be informative regarding the size of the particles that carry them. For example, larger pathogens, such as bacteria(1-2 μm), are found in larger particles (Wainwright et al., 2009), whereas smaller pathogens, such as viruses(20-30 nm), are found in smaller particles (Fabian et al., 2008, Hersen et al. 2008). Hence, detecting aerosols with instruments capable of detecting a wide range of particle sizes may be effective to determine the particles that can induce a viral infection.

From the exposure chamber experiments result, when patients have cold, number of aerosol generated from single cough has no statistically significant correlation with FVC(0.93), FEV1 (0.90), PEF (0.65), volume of cough (0.28). However, after patients have recovered, number of aerosol generated from single cough has statistically significant correlation with FVC (0.04, r = 0.65), FEV1

(0.03, r = 0.68), PEF (0.01, r = 0.78) and volume of cough (0.02, r = 0.72). Through this, a question can be raised that infection of disease can affect the emission characteristics of cough aerosols. From the clean room experiments result obtained from cold patient, we found that there was correlation between ratio of aerosol number concentration during cough to background and number of cough (0.04, r = 0.65). However, other factors has no correlation with aerosol emission of cold patient.

Our correlation analysis results were similar to those of Zayas et al. (2012). They reported that the concentration of droplets was not related to age, sex, weight, height, or BMI in 45 patients. However, Yang et al. (2007) found a significant difference in concentration depending on sex in three age groups. Their 30–50 years age group showed the highest aerosol concentration, and there was also a higher airborne droplet concentration in males than in females. Johnson et al. (2009) reported a significant correlation between the droplet concentration and age of 15 individuals, and the concentration differed markedly by particle size. In our study, the participants were all young and healthy adults; thus, our results may not be representative of the entire population. Furthermore, the number of subjects was small, and the aerosol production results varied significantly from person to person. This study suggests that within the same age groups, number concencentration could be varied signifantly.According to several studies, relative humidity may also play a role in affecting particle trajectory. Yang et al. (2011) showed that the total concentration of influenza A virus contained in aerosol particles decreased with increasing relative humidity across all particle sizes. Generally, evaporation is predicted as a function of droplet size. This makes fine droplets evaporate faster

than large droplets. Higher relative humidities also slow the evaporation process. Yang et al. (2011) showed that the total concentration of influenza A virus contained in aerosol particles decreased with increasing relative humidity across all particle sizes.

There were some limitations in this study. First, number of participants were small so that this study could not reflect all age groups. But we found that within the same age group and same gender were wide variations of aerosols per cough. Also, we measured lung fuction like FVC, FEV1, PEF and cough volume as well as number of aerosol so that we could linkage these two measurements. Second, all subjects were infected in cold at the time of initial test, but their illnesses were in different stages; some subjects were more ill than others, though all participants were diagnosed at the hopistal. Third, relatively large particles, i.e., more than 10 ums, have been likely deposited by impaction on the wall in exposure chamber and sedimented in clean room and could be more underestimated than smaller ones. In exposure chamber experiment, lengh from partipants mouth to the measuring instrument is less than 60 cm and the emitted cought was propagated as conical shape which might to contribute less impaction on the wall of the chamber though some large particles were inevitably deposited. In clean room experiment, also, large droplets might be sedimented before moving far distance. But the purpose of this study was to test the possibility of cough generated aerosol transmission to the far field as well as near field. So, the finding of far distance transmission of cough generated aerosol in this study suggest that it was wrong assumption that most cough generated aerosol was large droplet and could be deposited in near field less than 2 m.

Although these limitations could lead to underestimate the result of the study, we found available airborne particles small enough for transmission and a temporary increase of in the particle number concentration at the far distance by airborne transmission. . We found no difference in the size distribution between the direct contact range (0.5 m) and the airborne transmission range (3.0 m), which may have been due to a lack of monitoring according to distance. We used OPS only at the distance of 3.0 m, thus in near field, the upper limit of the SMPS (420 nm) was too small to detect the diameter distribution change of large particles like droplets or larger droplet nuclei at near field. However, as shown at Figure 5. Most of aeorosol expelled from cough were droplet nuclei. Thus, this result may not be different with result from simultaneous use of monitoring devices with wide particle size detection ranges.

5. Conclusions

Individuals infected with influenza release potentially infectious aerosols when they cough, sneeze, and speak. Coughing is the most important, and most common, source of transmission of infectious agents. The present results show that patients with a cold can release cough-generated airborne transmission-available particles; transmission was detected at a distance of 3 m, which is not considered as the contact transmission distance. Furthermore, we found a decrease in the number of particles expelled by patients' coughing, and most of the particles generated from coughing were < 5 μm in size; such particles can float in the air for at least 1 hour. These results suggest that the airborne spread of pathogens may be possible even at a distance > 3 m from a patient with a respiratory disease. Hence, more attention should be paid to airborne infections to prevent the spread of disease.

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국문초록

감기 환자의 호흡기에서 발생하는 입자의 특성

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연구목적 : 대부분의 감염은 환자로부터 1m 이내의 가까운 범위에서 기침, 재채기 등의 활동으로 인해 배출된 비말에 의한 것이거나 접촉을 통해 전파된다고 여겨져 왔다. 그러나 최근 실내 환경에서 다양한 병원체의 전염이 실내 공기의 흐름과 밀접한 관계가 있음이 밝혀졌다. 하지만 환자로부터 발생한 비말과 비말핵의 공기 중 거동에 대해서는 아직 명확하게 밝혀진 바가 없어 이에 대한 연구가 필요한 실정이다. 본 연구의 목적은 급성 상기도 감염 증상을 보이는 환자의 기침으로 인해 발생하는 입자의 발생량과 공기 중 거동 특성을 파악함으로써, 그로 인한 공기 중 전파 가능성이 존재하는지를 확인하고자 하는 것이다.

연구방법 : 본 연구는 의료기관에서 급성 상기도 감염 진단을 받은 사람을 대상으로 진행되었다. 실험참여자는 클린 룸에서 일정한 시간 동안 기침을 실시하였으며, 기침 전, 중, 후의 클린 룸 내 총 입자 수 농도를 환자로부터 0.5 m와 3 m 떨어진 지점에서 측정하였다. 또한 스테인레스 스틸 챔버에서 실험대상자가 한 번 기침하였을 때 발생하는 입자의 수와 직경 분포를 측정하였다. 입자의 측정에는 Scanning Mobility Particle Sizer (SMPS)와 Optical Particle Spectrometer (OPS)가 이용되었으며, 기본적인 폐기능과 기침의 부피와 최대 기류 속도를 측정하기 위해서 초음파식 폐활량계가 이용되었다. 모든 측정은 환자가 완치된 후 같은 방법으로 다시 시행하여 감염여부가 주는 차이를 비교하였다.

연구결과 : 노출 챔버에서의 실험 결과, 기침 시에 발생하는 입자들은 실험대상자에 따라 그 발생량이 기침당 18,756,000개에서 731,000개, 완치 후에는 200,900에서 450,000 개로 개개인의 차이가 매우 컸다. 실험대상자의 감염여부에 무관하게 공기 중 전파가 가능한 5μm 이하 크기의 입자가 대부분이었으며, 이는 환자가 감염되었을 때 숫자로는 전체의 99.9 ± 0.3 %, 표면적으로는 전체의 90.2 ± 12.2 %였다. 환자들이 감염되었을 때에는 기침으로 인해 발생하는 입자의 수가 완치 후에 비해 유의하게 높았다.

클린 룸에서의 실험 결과, WHO에서 권고하는 격리 거리를 초과한 3.0 m 지점에서도 환자가 기침하였을 때, 클린 룸 내 입자의 수 농도가 기침하기 전에 비해 유의하게 증가하였다. 실험대상자가 완치된 이후에도 입자

가 증가하였으나 이는 통계적으로 유의하지 아니하였다. 또한 10-420 nm 사이의 입자들의 경우 0.5 m 지점과 3.0 m 지점에서 직경 분포의 차이를 볼 수 없었다.

결론 : 본 연구를 통해 급성 상기도 감염 증상을 가진 환자가 기침을 하는 동안 공기 중으로 전파가 가능한 입자가 발생함을 알 수 있었다. 대부분의 비말이 침강하는 거리에서도 기침으로 인해 발생한 입자상 물질이 전파되었으며, 이는 곧 급성 상기도 감염 또는 그와 유사한 증상을 보이는 질병의 경우 공기 중으로 감염이 이루어 질 가능성이 있음을 시사한다.

주요어: 공기 중 전파, 기침 에어로졸, 입자상 물질, 호흡기 감염, 비말핵

학번: 2015-24063

Appendix

Appendix 1. Number of particles per cough in each size bin and each subject - Chamber

ID	Diag.	10-100 nm AM ± SD	100-300 nm AM ± SD	300-420 nm AM ± SD	420-1000 nm AM ± SD	1.0-2.5 µm AM ± SD	2.5-4 µm AM ± SD	4-5 µm AM ± SD	5-10 µm AM ± SD
1	Ill *	370,449 ± 523,865	4,023,331 ± 1,748,340	N / D	48,238 ± 40,717	788 ± 891	55 ± 48	N / D	N / D
	Rec.*	279,677 ± 236,718	306,996 ± 92,194	74,574 ± 105,434	N / D	N / D	N / D	N / D	N / D
2	Ill	328,563 ± 240,094	291,525 ± 171,141	3,990 ± 5,614	138,977 ± 92,988	2,371 ± 353	1,769 ± 2,270	6,168 ± 8,693	797 ± 1,034
	Rec.	84,014 ± 118,785	515,313 ± 137,729	N / D	665 ± 858	84 ± 51	N / D	N / D	N / D
3	Ill	1,770,491 ± 844,480	611,380 ± 448,653	127,100 ± 179,717	32,531 ± 5,685	696 ± 555	68 ± 67	N / D	60 ± 55
	Rec.	209,621 ± 218,590	319,851 ± 342,514	10,766 ± 15,196	4,000 ± 2,652	830 ± 664	1,090 ± 1,384	158 ± 194	107 ± 121
4	Ill	1,963,423 ± 1,245,312	2,581,446 ± 847,881	47,483 ± 106,780	1,092 ± 434	61,588 ± 29,981	1,289 ± 352	231 ± 178	N / D
	Rec.	193,596 ± 191,410	305,065 ± 147,008	56,264 ± 79,539	1,804 ± 909	1,780 ± 1,314	5,164 ± 6,546	2,318 ± 3,141	432 ± 582
5	Ill	10,543,713 ± 4,412,848	6,510,767 ± 2,776,994	97,766 ± 138,232	353,577 ± 83,072	228,311 ± 28,487	585,048 ± 183,580	326,546 ± 188,270	160,049 ± 102,946
	Rec.	727,203 ± 614,637	291,445 ± 350,419	N / D	16,059 ± 16,985	2,075 ± 1,217	1,838 ± 2,570	N / D	N / D
Female	Ill	2,995,328 ± 4,372,963	2,803,690 ± 2,760,956	58,802 ± 120,499	114,883 ± 140,688	58,751 ± 89,865	117,646 ± 247,705	66,598 ± 154,928	32,189 ± 78,783
Sub-total	Rec.	298,822 ± 395,320	347,734 ± 254,832	28,329 ± 67,088	4,510 ± 9,726	958 ± 1,202	1,627 ± 3,725	507 ± 1,674	120 ± 310

* : 'Ill' means 'While ill' and 'Rec' means 'After Recovery'

† : 'N / D' is none detected, the value under limit of detection

Appendix 1. Continued

ID	Diag.	10-100 nm AM ± SD	100-300 nm AM ± SD	300-420 nm AM ± SD	420-1000 nm AM ± SD	1.0-2.5 μm AM ± SD	2.5-4 μm AM ± SD	4-5 μm AM ± SD	5-10 μm AM ± SD
6	Ill*	600,398 ± 278,079	514,106 ± 405,965	12,956 ± 18,294	35,299 ± 13,504	4,643 ± 2,358	6,509 ± 8,501	3,365 ± 4,729	1,111 ± 1,542
	Rec.*	2,274,669 ± 1,215,449	942,953 ± 944,083	N / D†	7,073 ± 1,491	569 ± 377	514 ± 626	N / D	51 ± 42
7	Ill	963,474 ± 1,100,352	459,422 ± 253,961	119,005 ± 168,269	25,152 ± 9,406	9,604 ± 3,693	11,577 ± 3,369	5,287 ± 2,047	2,046 ± 2,292
	Rec.	3,062,216 ± 3,030,203	1,086,687 ± 535,082	19,125 ± 13,523	35,160 ± 457,94	8,309 ± 9,503	14,677 ± 16,631	2,138 ± 653	239 ± 164
8	Ill	1,752,817 ± 1,207,558	1,603,207 ± 821,536	N / D	147,829 ± 104,435	16,281 ± 15,189	1,193 ± 1,340	440 ± 345	51 ± 42
	Rec.	124,196 ± 26,102	214,103 ± 61,374	21,501 ± 30,378	3,444 ± 3,038	182 ± 228	N / D	N / D	N / D
9	Ill	452,625 ± 640,080	484,155 ± 314,112	94,239 ± 133,245	7,471 ± 5,269	4,024 ± 4,394	562 ± 766	158 ± 194	N / D
	Rec.	562,798 ± 318,755	357,611 ± 363,198	43,838 ± 61,967	16,103 ± 14,376	1,577 ± 1,757	408 ± 547	230 ± 296	37 ± 22
10	Ill	5,936,636 ± 4,181,286	3,022,467 ± 1,842,573	219,794 ± 290,675	144,562 ± 93,637	2,244 ± 2,476	164 ± 203	N / D	N / D
	Rec.	1,979,990 ± 2,020,550	744,064 ± 737,497	136,691 ± 161,524	47,156 ± 32,349	5,967 ± 2,843	9,108 ± 7,344	5,894 ± 5,983	11,497 ± 15,706
Male	Ill	2,276,044 ± 2,855,189	1,302,441 ± 1,391,611	86,616 ± 181,329	66,418 ± 90,804	6,545 ± 9,323	2,802 ± 4,711	1,185 ± 2,258	438 ± 1,303
Sub-total	Rec.	1,265,919 ± 1,970,876	583,314 ± 566,032	46,822 ± 91,663	27,432 ± 30,842	4,136 ± 5,482	6,144 ± 10,537	2,329 ± 4,055	2,581 ± 8,357
Total	Ill	2,635,687 ± 1,173,320	2,053,006 ± 730,970	72,710 ± 48,881	90,651 ± 38,218	32,648 ± 21,824	60,224 ± 58,299	33,892 ± 36,158	16,314 ± 18,320
	Rec.	782,371 ± 474,777	465,524 ± 143,715	37,576 ± 25,568	15,972 ± 8,089	2,547 ± 1,352	3,886 ± 2,599	1,419 ± 1,023	1,351 ± 1,910

* : 'Ill' means 'While ill' and 'Rec' means 'After Recovery'

† : 'N / D' is none detected, the value under limit of detection

Appendix 2. Surface area of particles per cough in each size bin and each subject – Chamber (μm^2)

ID	Diag.	10-100 nm AM±SD	100-300 nm AM±SD	300-420 nm AM±SD	420-1000 nm AM±SD	1.0-2.5 µm AM±SD	2.5-4 µm AM±SD	4-5 µm AM±SD	5-10 µm AM±SD
1	Ill *	1,163±950	379,189±95,134	N/D †	45,462±22,155	6,191±4,041	1,733±876	N/D	N/D
	Rec.*	878±429	28,933±5,016	31,239±25,499	N/D	N/D	N/D	N/D	N/D
2	Ill	1,032±435	27,475±9,312	1,671±1,357	130,981±50,598	18,623±1,602	55,592±41,186	387,557±315,362	125,289±93,854
	Rec.	263±215	48,567±7,494	N/D	627±467	663±234	N/D	N/D	N/D
3	Ill	5,562±1,531	57,621±24,413	53,243±43,465	30,659±3,093	5,470±2,519	2,163±1,227	N/D	9,504±5066
	Rec.	658±396	30,145±18,637	4,510±3,675	3,770±1,443	6,520±3,013	34,264±25,105	9,956±7,052	16,809±11,031
4	Ill	6,168±2,258	243,295±46,136	27,285±22,271	1,029±236	483,713±135,952	40,508±6,401	14,962±6,133	N/D
	Rec.	608±347	28,751±7,999	23,569±19,237	1,700±494	13,981±5,959	162,255±118,743	145,671±113,976	67,946±52,785
5	Ill	33,124±8,004	613,625±151,107	40,954±33,432	333,236±45,202	1,793,158±129,174	18,379,850±332,9778	20,517,537±6,829,693	25,140,474±9,336,203
	Rec.	2,284±1,114	27,468±19,067	N/D	15,135±9,242	16,302±,5518	57,755±46,618	N/D	N/D
Female	Ill	9,409±12,048	264,241±216,550	24,632±21,097	108,273±120,494	461,431±690,683	3,695,969±7,341,970	4,184,538±8,167,838	5,056,372±10,042,158
Sub-total	Rec.	938±701	32,772±7,942	11,866±13,020	4,250±5,590	7,526±6,648	51,118±59,618	31,916±56,975	18,929±25,060

* : 'Ill' means 'While ill' and 'Rec' means 'After Recovery'

† : 'N / D' is none detected, the value under limit of detection

Appendix 2. Continued

ID	Diag.	10-100 nm	100-300 nm	300-420 nm	420-1000 nm	1.0-2.5 μm	2.5-4 μm	4-5 μm	5-10 μm
		AM ± SD	AM ± SD	AM ± SD	AM ± SD	AM ± SD	AM ± SD	AM ± SD	AM ± SD
6	Ill*	7,146 ± 2,204	88,871 ± 51,371	N / D	6,666 ± 811	4,476 ± 1,714	16,164 ± 11,371	N / D	8,043 ± 3,874
	Rec.*	1,886 ± 504	48,453 ± 22,090	5,427 ± 4,424	33,268 ± 7,348	36,473 ± 10,694	204,488 ± 154,194	211,466 ± 171,584	174,604 ± 139,870
7	Ill	3,026 ± 1,995	43,299 ± 13,819	49,852 ± 40,696	23,705 ± 5,118	75,433 ± 16,748	363,718 ± 61,116	332,216 ± 74,275	321,433 ± 207,905
	Rec.	9,620 ± 5,496	102,417 ± 29,115	8,011 ± 3,270	33,137 ± 24,918	65,265 ± 43,093	461,112 ± 301,662	134,338 ± 23,716	37,626 ± 14,935
8	Ill	5,506 ± 2,190	151,098 ± 44,703	N / D	139,324 ± 56,827	127,877 ± 68,875	37,492 ± 24,309	27,670 ± 12,527	8,043 ± 3,874
	Rec.	390 ± 47	20,178 ± 3,339	9,007 ± 7,347	3,246 ± 1,653	1,436 ± 1,038	N / D	N / D	N / D
9	Ill	1,421 ± 1,160	45,630 ± 17,092	39,477 ± 32,226	7,041 ± 2,867	31,610 ± 19,929	17,677 ± 13,895	9,956 ± 7,052	N / D
	Rec.	1,768 ± 578	33,704 ± 19,762	18,364 ± 14,987	15,177 ± 7,822	12,392 ± 7,971	12,829 ± 9,936	14,495 ± 10,757	5,851 ± 2,084
10	Ill	18,650 ± 7,584	284,860 ± 100,261	92,072 ± 70,301	136,245 ± 50,951	17,628 ± 11,228	5,180 ± 3,690	N / D	N / D
	Rec.	6,220 ± 3,664	70,126 ± 40,130	57,260 ± 39,065	44,442 ± 17,601	46,862 ± 12,892	286,148 ± 133,196	370,339 ± 217,043	1,805,871 ± 3,874
Male	Ill	7,149 ± 6,079	122,751 ± 89,990	36,283 ± 34,462	62,596 ± 61,706	51,404 ± 45,078	88,046 ± 138,229	74,496 ± 129,218	68,823 ± 126,322
Sub-total	Rec.	3,976 ± 3,434	54,975 ± 28,934	19,613 ± 19,324	25,854 ± 14,687	32,485 ± 23,086	193,047 ± 173,291	146,391 ± 136,363	405,450 ± 703,017
Total	Ill	8,279 ± 9,609	193,496 ± 180,280	30,457 ± 29,160	85,434 ± 98,411	256,417 ± 530,629	1,892,007 ± 5,496,917	2,129,517 ± 6,130,926	2,562,597 ± 7,526,577
	Rec.	2,457 ± 2,907	43,874 ± 23,945	15,740 ± 16,925	15,052 ± 15,497	20,005 ± 21,079	122,082 ± 147,743	891,54 ± 119,150	212,189 ± 533,648

* : 'Ill' means 'While ill' and 'Rec' means 'After Recovery'

† : 'N / D' is none detected, the value under limit of detection