

Fractional Exhaled Nitric Oxide (FeNO) and Spirometry as Indicators of Inhalation Exposure to Chemical Agents in Pathology Workers

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Objective: The objective of this study was to examine whether fractional exhaled nitric oxide (FeNO) and spirometry can be used as indices to evaluate adverse health effects of low-concentrated chemical inhalation exposure, mainly to formaldehyde. **Methods:** Thirty-three subjects (pathology technicians) and 30 controls (workers without handling any chemicals in the same hospitals) participated in this study. All participants underwent FeNO measurement and spirometry before and after 5 days of work. **Results:** FeNO significantly increased in the subjects with a history of asthma ($P < 0.05$), whereas forced vital capacity (FVC) and forced expiratory volume in the first second (FEV1) decreased in the subjects ($P < 0.05$). Furthermore, work duration and pre-work levels of FEV1 in the subjects had a significant association. **Conclusion:** The results suggest that FeNO, FVC, and FEV1 represent effective health-effect indices of low-concentrated chemical inhalation exposure.

BACKGROUND

Formaldehyde (FA) is a useful and important chemical in industry. In the medical field, it is used as formalin for disinfection and preservation. On the contrary, FA is recognized as a respiratory sensitizer and environmental pollutant. Chronic exposure to FA may cause bronchial asthma (BA)^{1,2} upper respiratory tract inflammation,³ decreased pulmonary function,⁴ and chemical sensitivity (CS).⁵

Physicians and laboratory technicians who work in pathology departments of general hospitals have occupational exposure to chemical substances, mainly to FA. In Japan, work environment measures require treatment of chemical substances in work areas by law, most of which are rated as being good environments. However, these results do not necessarily mean that adverse health effects do not occur in workers. There are some reports on occupational asthma due to FA^{6–9} and chronic or repeated FA exposure affecting lung function in healthy adults.^{10,11}

Occupational exposure limits (OELs) were recommended by the Japan Society for Occupational Health (JSOH) and American Conference of Governmental Industrial Hygienists (ACGIH) reference values for preventing adverse health effects of occupational exposure to chemical substances in workers. It is not necessarily correct to conclude that work environments below OELs guarantee complete absence of health impairment in workers. Safe and harmless work environments are only guaranteed with appropriate implementation of local exhaust ventilation and work management.

Fractional exhaled nitric oxide (FeNO) is a useful biomarker to assess airway inflammation.¹² It has been shown to be elevated in patients with BA^{12,13} and is used as a diagnostic test for BA in clinical practice.^{12–14}

It has been suggested that FeNO is a useful marker for adverse respiratory health effects of occupational exposure, for example, exposure to chemical agents,^{15–17} dusts,¹⁸ and air pollution.¹⁹ Moreover, it has also been reported that FeNO levels are significantly elevated in children living in homes with average FA levels of ≥ 50 ppb.²⁰ This suggests that long-term exposure to low-dose FA may invoke a subclinical inflammatory response in the airways of healthy children. We hypothesized that FeNO might be a possible index for the detection of adverse health effects caused by occupational inhalation exposure to low-dose FA in workers.

Spirometry is one of the basic pulmonary function tests, and the indices, vital capacity (VC), forced VC (FVC), and forced expiratory volume in the first second (FEV1), have been used frequently in investigations about occupational chemical inhalational exposure and the diagnosis of the occupational asthma. Meo et al¹⁷ reported that FEV1 and FEV1/FVC in the workers of oil refineries who were exposed to some chemicals, for example, benzene, toluene, xylene, and so on, were significantly decreased as compared with controls.

Peak expiratory flow (PEF) is used for monitoring of airway inside diameter in asthmatic patients, and is considered to be the most useful method by a diagnosis of the occupational asthma in Japanese Guideline for Diagnosis and Management of Occupational Allergic Diseases. PEF is also used as one of the evaluation methods of the effect of chemical exposure for airway. However, as for the past reports about health effect evaluation of the chemical exposure with spirometry or the PEF, cases with high-concentrated chemical exposure workers were often found, but there were few reports about the measurement of low-concentrated chemical exposure worker.

The objectives of this study have two points, one is whether low-concentration chemicals that mainly include FA evoke an airway inflammation or an airway functional change, and the other is to verify whether FeNO, spirometry, and PEF are useful as indicators of exposure to low-concentration chemicals. In an attempt to clarify these objectives, this study was performed to compare the physiological examinations before and after 5 days work between chemical-exposed and unexposed workers.

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METHODS

Study Population

This study was performed in six general hospitals in Sendai, Japan, from November 2014 to April 2016. The study population consisted of an exposed subject group (39 participants) and an unexposed control group (32 participants). The subjects were physicians and laboratory technicians who worked in the pathology departments of six general hospitals and always handled several chemical substances, including FA, through their work. The controls were workers who did not handle any chemical substances through their work in other departments, for example, office workers, physiological examination technicians, or physical therapists of the same hospitals. All participants worked for 8 hours a day, for 5 days a week.

Study Protocol

Before the study, all subjects and controls filled out a questionnaire on their chemical-handling history (work duration in months), medical history (including BA, rhinitis, and other allergies), and smoking habits. The investigation period was set for 5 consecutive weekdays, reflecting a usual work situation, and FeNO measurements and spirometry were conducted at the beginning and end of this period. In addition, the subjects monitored and recorded PEF three times a day and wore two individual chemical exposure monitors during the work time of this period. The protocol summary is shown in Figure 1.

The study protocol was approved by the Ethics Committee of the Ethics Committee of Tohoku University School of Medicine, and written informed consent was obtained from all participants before study participation.

Exposure Monitoring of Chemical Substances

To measure occupational exposure concentrations, the subjects wore two kinds of individual exposure monitors during their work time. The DSD-DNPH Diffusive Sampling Device (Sigma-Aldrich Co., Tokyo, Japan) was used for monitoring FA exposure concentration, and the 3M Organic Vapors Monitor 3500 or 3520 (OVM; 3M Japan CO Ltd, Tokyo, Japan) was used for monitoring the concentration of other volatile organic compounds (VOCs) handled during work. The subjects wore these monitors on the neck close to the breathing zone in order to evaluate inhalation exposure. They handled the monitors, kept them frozen or refrigerated according to each manual, and, after removal, they were stored in a refrigerator until analysis. OVM data extraction and analysis were performed, as previously described,²¹ at the chemical laboratory of Environment Conservation Center in Tohoku University.

FeNO Measurement

FeNO levels were measured using NIOX MINO (Aerocrine, Solna, Sweden), according to standardized procedures issued by the

American Thoracic Society (ATS) and European Respiratory Society (ERS).¹² Measurements were conducted on Monday morning and Friday evening of the investigation period. FeNO measurements were performed before spirometry.

Spirometry

Spirometry was performed on Monday morning and Friday evening of the investigation period using the Multi-Functional Spirometer HI-801 (Chest M.I., Tokyo, Japan). VC, FVC, FEV1, and forced expiratory ratio (FEV1/FVC) were recorded as respiratory function parameters. Measurements were based on standardization of ATS/ERS.²²

Monitoring PEF

PEF was measured using Asthma Check (Chest M.I., Tokyo, Japan) to observe the circadian variability of respiratory function. We explained how to use the PEF monitor and record each value to the subjects. During the investigation period, the subjects measured and recorded PEF by themselves three times a day (before work, before lunch, and soon after work). The controls did not perform the PEF monitor.

Statistical Analysis

Statistical analysis was performed with JMP ver. 12 for Windows (SAS Institute Inc., Cary, NC). The general characteristics of the subject and control groups were compared using *t* test (about age) and Pearson χ^2 test [about gender, degree of having history of BA or allergic rhinitis (AR), and smoking habit]. For comparison of FeNO, VC, FVC, FEV1, and FEV1/FVC in the subject and control groups, the Wilcoxon signed-rank test (paired groups) or the Mann-Whitney *U* test (unpaired groups) were used. A single linear regression model was used to test the association between pulmonary function and work duration or individual FA exposure concentrations. In addition, a multiple linear regression model was used to test the effects of work duration and age for pulmonary function level. A *P* value of less than 0.05 was considered to be significant.

RESULTS

This study was conducted in order to evaluate whether FeNO, spirometry, or PEF showed the adverse health effects caused by occupational chemical inhalation exposure in workers. The characteristics of the subjects and controls are summarized in Table 1. It is already known that current smoking and steroid use are significant factors that influence FeNO levels and spirometry; therefore, six subjects and two controls who were smokers or using inhaled steroids were excluded, so finally, 33 subjects and 30 controls were investigated. In each group, there were six subjects (18%) and seven controls (23%) with a current or past history of BA. None of the subjects or controls had a current or past history of respiratory disease other than BA. There were no statistically significant differences in age, gender, and ratio with AR between two groups.

Table 2 summarizes the level of chemicals detected by individual exposure measurement in the subject group. FA, xylene, ethyl benzene, and acetone were detected, and the mean concentrations in both groups were less than the OEL-Mean (OEL-M) recommended by the JSOH.

The FeNO measurements are presented in Table 3. There was no significant change in the 5-day investigation period in the whole controls, with or without history of BA (Fig. 2A, Table 3). Pre (Monday morning)-work FeNO levels were not significantly different between the subjects and controls overall (Fig. 2A), or between the subjects with and without a history of BA (Fig. 2B). However, post (Friday evening)-work, FeNO levels were significantly higher than pre-work levels in the subject group with a history of BA [pre: 29.8 ± 16.8 (25.5) ppb (mean $\pm$ SD (median)), post: 47.5 ± 39.7 (30.5) ppb, $P=0.028$], and conversely, significantly lower in the

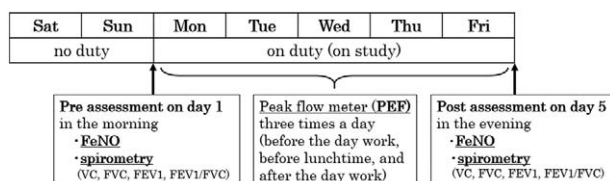


FIGURE 1. The protocol of study. The period was 5 consecutive weekdays. Subjects and controls performed FeNO measurement and spirometry at the beginning (pre) and the end (post) of this study period. Also, the subjects measured their PEF levels by themselves at three times a day during the study period.

TABLE 1. General Characteristics of Study Participants

Number of Participants	Subjects (<i>n</i> = 39)	Controls (<i>n</i> = 32)	
Current Smoker, <i>n</i>	6	1	
Corticosteroid User, <i>n</i> (%)	0	1	
Surveyed Number	Subjects (<i>n</i> = 33)	Controls (<i>n</i> = 30)	
Age	23–67 (median; 33)	23–62 (median; 31.5)	n.s.
Male, <i>n</i> (%)	11 (33.3)	13 (43.3)	
Female, <i>n</i> (%)	22 (66.7)	17 (56.7)	n.s.
Bronchial asthma*, <i>n</i> (%)	6 (18.2)	7 (23.3)	n.s.
Allergic rhinitis*, <i>n</i> (%)	19 (57.6)	15 (50.0)	n.s.
Smoking habit			
Never smokers, <i>n</i> (%)	30 (90.9)	22 (73.3)	n.s.
Ex-smokers, <i>n</i> (%)	3 (9.1)	8 (26.7)	
Working duration, months	6–505 (median; 97)	—	

No significant difference was found between subjects and controls about age, gender, degree of having history of bronchial asthma or allergic rhinitis, and smoking habit. n.s., not significant.

*Current or past history.

TABLE 2. Results of Chemical Exposure Measurement During the Investigation Period

Chemical	<i>n</i>	OELs, ppm		TWA 8 hours, ppm			
		JSOH (OEL-M)	ACGIH (TLVs)	Median	IQR	Min	Max
Formaldehyde	33	0.1	0.3	0.024	0.012–0.054	0.004	0.079
Xylene	33	50	100	0.647	0.325–1.234	0.000	5.739
Ethyl benzene	30	50	20	0.120	0.057–0.185	0.000	0.757
Acetone	11	200	250	0.091	0.056–0.213	0.000	0.721

There was no subject who exposed any chemicals over OELs

ACGIH, American Conference of Governmental Industrial Hygienists; IQR, interquartile range; JSOH, Japan Society for Occupational Health; *n*, number of whom we detected the chemicals from; OEL-M, Occupational Exposure Limit-Mean; OELs, Occupational Exposure Limits; TLVs, Threshold Limit Values; TWA, time-weighted average concentration.

group without a history of BA [pre: 21.3 ± 15.5 (17.0) ppb, post: 17.5 ± 11.0 (14.0) ppb, $P = 0.007$] (Fig. 2B, Table 3).

For spirometry, there were no significant differences in VC, FVC, FEV1, or FEV1/FVC pre-work levels between the subjects and controls. In the controls, there were no significant differences for all indices between Monday morning and Friday evening. In the overall subjects, FVC [pre: 3.54 ± 0.74 (3.40) L, post: 3.48 ± 0.77 (3.31) L, $P = 0.013$, Fig. 3A, Table 4] and FEV1 [pre: 3.03 ± 0.71 (2.83) L, post: 2.97 ± 0.71 (2.78) L,

$P = 0.004$, Fig. 4A, Table 4)] were significantly decreased after 5 days of work. These results were similar in the subjects group without history of BA [FVC: pre: 3.66 ± 0.73 (3.40) L, post: 3.57 ± 0.76 (3.35) L, $P = 0.003$, Fig. 3B], FEV1: pre: 3.14 ± 0.72 (2.90) L, post: 3.07 ± 0.72 (2.81) L, $P = 0.001$, Fig. 4B. There was no significant difference for VC or FEV1/FVC in the subjects. For PEF, there was no daily variation of more than 20% or significant decrease from morning to evening in the subjects (Table 5).

TABLE 3. FeNO Levels and Rate of Change in Overall or With/Without History of Bronchial Asthma

	Subjects			Controls		
	Overall	BA +	BA –	Overall	BA +	BA –
<i>n</i>	33	6	27	30	7	23
Prelevel, ppb	22.9 ± 15.8 18 (12–29)	29.8 ± 16.8 25.5 (19–44.8)	21.3 ± 15.5 17 (12–22.5)	19.7 ± 15.6 16 (10.3–21.8)	36.7 ± 23.1 33 (20–50.5)	14.5 ± 7.3 13 (9–19)
Postlevel, ppb	22.9 ± 22.0 15 (11–26)	47.5 ± 39.7 30.5 (22.3–68)	17.5 ± 11.0 14 (11–20.5)	18.0 ± 11.8 15.5 (10–21.3)	30.9 ± 15.3 26 (22–39)	14.0 ± 7.2 13 (8.5–18)
Δ% [%]	0.0 (–23.1 to 12.5)	26.6 (13.1–49.8)	–18.8 (–27.1 to 8.3)	0.0 (–17.8 to 18.6)	0.0 (–22.8 to 10.2)	0.0 (–15.5 to 19.4)

The number of pre and postlevel in the table shows the mean $\pm$ SD (upper rank), median, and IQR (in the parenthesis) (lower rank).

The number of Δ% in the table shows the median, and in the parenthesis shows IQR.

Prelevel and rate of change of FeNO were not significantly different between overall subjects and controls. Comparing subjects with controls in the group with or without BA history, both were no significant differences in pre FeNO levels.

Δ%, rate of change = (post FeNO – pre FeNO)/pre FeNO $\times$ 100; post FeNO, FeNO level measured on the evening of Friday; pre FeNO, FeNO level measured on the morning of Monday.

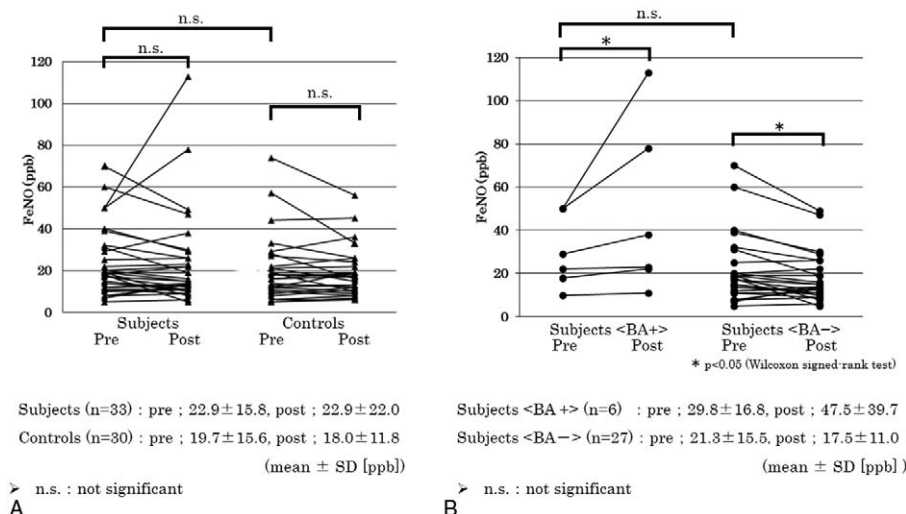


FIGURE 2. Pre and post levels of FeNO in overall subjects and controls (A), and in subjects with and without BA history (B). (A) In both overall subjects and controls, there were no significant differences between pre and post-FeNO levels. Also, pre-FeNO levels were not significantly different between subjects and controls. (B) FeNO showed significant increase ($P < 0.05$) in the group with BA history in subjects after 5 days chemical-treated work, whereas FeNO decreased significantly ($P < 0.05$) in the group without BA history in subjects.

TABLE 4. Spirometry and the Rate of Change in Each Indicator

	Subjects			Controls		
	Prelevel	Postlevel	$\Delta\%$	Prelevel	Postlevel	$\Delta\%$
VC	3.51 ± 0.73 3.39 (3.01–3.84)	3.48 ± 0.73 3.28 (3.00–3.98)	-0.41 (–4.83 to 3.03)	3.95 ± 0.98 3.91 (3.15–4.48)	3.95 ± 0.97 3.79 (3.24–4.46)	-0.09 (–2.28 to 3.38)
FVC	3.54 ± 0.74 3.40 (3.02–3.80)	3.48 ± 0.77 3.31 (2.95–3.91)	-2.31 (–5.40 to 0.00)	3.96 ± 0.91 4.04 (3.16–4.53)	3.96 ± 0.94 4.08 (3.19–4.53)	0.58 (–0.89 to 1.96)
FEV1	3.03 ± 0.71 2.83 (2.51–3.35)	2.97 ± 0.71 2.78 (2.46–3.21)	-2.49 (–4.18 to 0.00)	3.33 ± 0.81 3.27 (2.66–3.96)	3.33 ± 0.81 3.28 (2.64–3.98)	-0.37 (–1.99 to 2.13)
FEV1/FVC	85.4 ± 7.9 85.4 (79.8–91.3)	85.0 ± 6.8 85.7 (81.4–89.8)	-0.09 (–2.86 to 2.75)	84.1 ± 6.3 82.5 (80.1–88.9)	84.0 ± 5.8 85.0 (80.6–88.2)	-0.88 (–2.09 to 1.42)

The number of pre and postlevel in the table shows the mean $\pm$ SD (upper rank), median and IQR (in the parenthesis) (lower rank).

The number of $\Delta\%$ in the table shows the median, and in the parenthesis shows IQR.

In either indicator (VC, FVC, FEV1, FEV1/FVC), the significant differences were not found in pre levels between the subjects and controls.

$\Delta\%$, rate of change = (post level – pre level)/pre level $\times$ 100; post level, the level measured on the evening of Friday; pre level, the level measured on the morning of Monday.

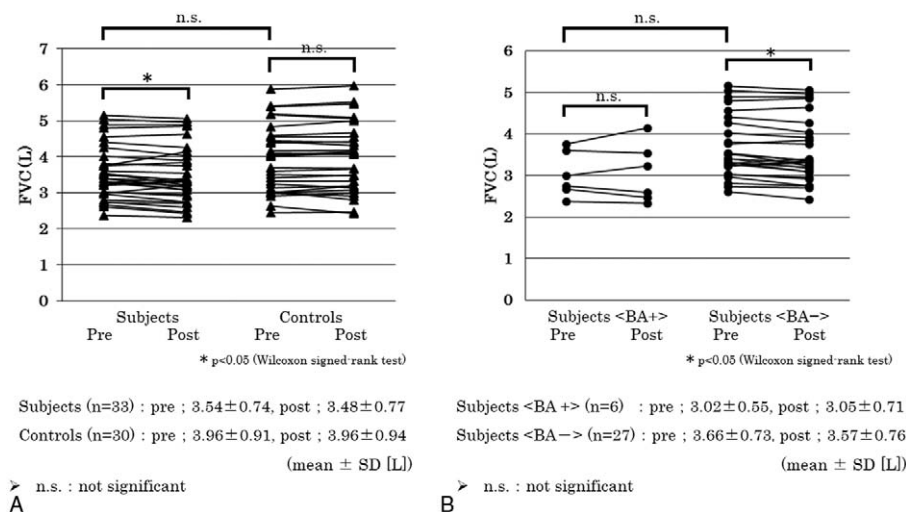


FIGURE 3. Pre and postlevels of FVC in overall subjects and controls (A), and in subjects with and without BA history (B). (A) FVC was significantly decreased before and after the study period in overall subjects ($P < 0.05$). (B) In subjects without a history of BA, FVC was significantly decreased after 5 days of work ($P < 0.05$).

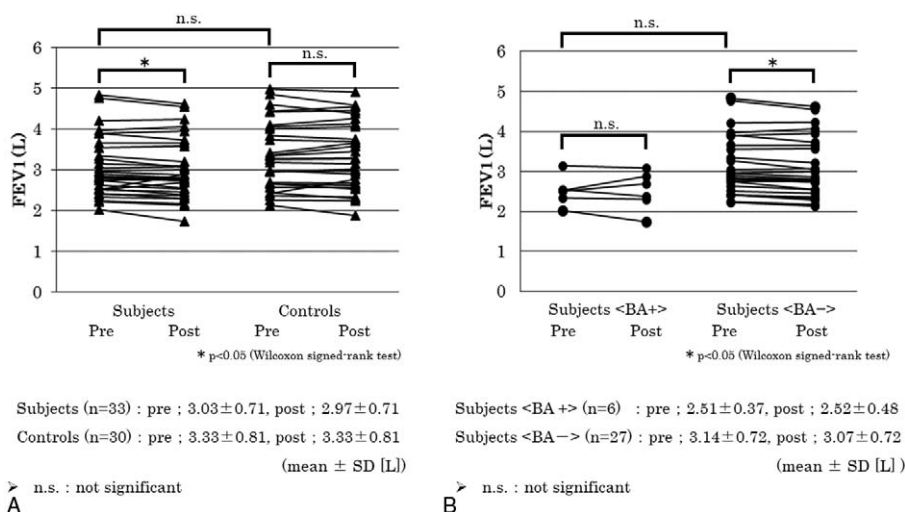


FIGURE 4. Pre and post levels of FEV1 in overall subjects and controls (A), and in subjects with and without BA history (B). (A) FEV1 was significantly decreased before and after the study period in overall subjects ($P < 0.05$). (B) In subjects without a history of BA, FEV1 was significantly decreased after 5 days of work ($P < 0.05$).

The relationship between work duration (work period of chemical exposure) and the test results was examined, and a strong correlation was seen between work duration and pre-work FEV1 levels in the subjects without a history of BA (Spearman product-moment correlation coefficient = -0.728, $P < 0.01$, Fig. 5A). However, VC, FVC, and FEV1 values are known to decrease with age; therefore, not only work duration but also subject age might have influenced decrease in FEV1. To confirm this, we performed step-wise multiple regression analysis to compare the contribution ratio of work duration and age to pre-work FEV1 level. Only work duration was found to contribute significantly to the pre-work FEV1 level ($\beta = -0.658$, $P < 0.001$), and, therefore, work duration had a greater influence than age. Furthermore, in the groups without history of BA, the degree of the decrease of pre-work FEV1 levels by aging of the subjects was bigger than that of the controls (Fig. 5B). About the correlations between FA individual exposure concentrations and the rate of change of each test value measured, no significant correlations were found.

DISCUSSION

FeNO, spirometry, and PEF measurement were performed on workers who were exposed to chemical agents, mainly FA, in university or general hospital pathology departments, and it was revealed that low concentrations of chemical substances had an effect on inflammation and respiratory function of the respiratory tract. Furthermore, taking these data into account, FeNO and spirometry could be applied as an indicator to detect adverse health effects on workers of chronic low concentration chemical exposure.

We calculated the sample size required for this study with using the [sample size/power] platform in JMP Pro12. In order to

evaluate the effect of FA exposure, for example, when FeNO was used as an effect indicator, and the level of significance was assumed to be 5%, the power to be 0.8 and the effect size to 5ppb, the sample size combined with controls and subjects was 304. But there are few hospitals with their own pathology department in our city except for university hospital, and even that hospitals had only a few exclusive staff. So, it was difficult for us to investigate a lot of samples in a limited period. And the funds did not allow us to study such a number of people. These affected the limit of our study.

The work environment measurements were conducted in the hospitals and the assessments were considered to be suitable for the work environment within regulation. Individual chemical-exposure measurements of the subjects were carried out during work hours, and no workers had OELs exceeding the recommended JSOH²³ or ACGIH guidelines. The JSOH defines OEL-M as “the reference value for mean exposure concentration at or below which adverse health effects caused by the substance do not appear in most workers working for eight hours a day, forty hours a week under a moderate workload.” In addition, the recommendations of OEL by the JSOH state that “occupational disease may be induced at levels of exposure below the OELs in workers.” These recommendations suggest that even if the work environment is judged to be appropriate within regulation, the risk of adverse health effects on workers remains. Therefore, direct evaluation of the health effects in individual workers and identification of indicators that can estimate the risk are important.

FeNO is a biomarker of airway inflammation and is applied as a diagnostic tool for BA. In past reports on occupational exposure to toxic substances, FeNO levels in shoe- and leather-factory workers over 10 years, who handled toluene, xylene, and methyl-ethyl ketone, were significantly increased by 40% after 8 hours/day of work.¹⁶ In a study by Ljubičić et al,¹⁸ swine farm workers exposed to dust and ammonia gas were found to have higher levels of FeNO at 7.5 ppb than controls. Furthermore, Franklin et al²⁰ reported FeNO levels of healthy children living in houses in which FA concentrations were more than 50 ppb were significantly higher than in children whose home environment had FA levels of 50 ppb or less. This suggests that FeNO levels may be increased even at low-concentration FA exposure. In the present study, pre-work FeNO levels were not significantly different between the subjects and controls. These data disagree with that of the study by Franklin et al²⁰ in that low-concentration FA exposure increasing FeNO levels. The cause of this difference may be whether the survey target is an adult or a child.

TABLE 5. The Summary of PEF Records in Subjects

	Overall Subjects	BA- Subjects
% of the standard value		
Before work time (%)	83.6 ± 10.5	85.6 ± 10.2
Before having lunch (%)	84.3 ± 11.4	86.2 ± 11.4
Soon after finished work (%)	85.3 ± 11.7	87.4 ± 11.5
Daily variation (%)	6.3 ± 2.9	6.5 ± 3.0

Data are presented as arithmetic mean ± SD. Any subjects had neither daily variation of more than 20% nor significant decrease from morning to the evening.

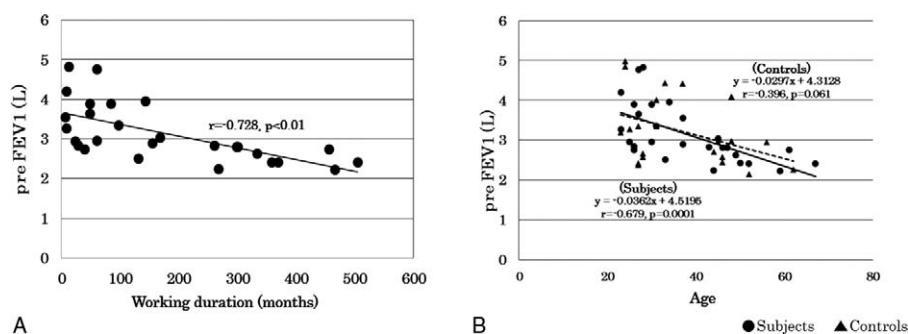


FIGURE 5. The correlation between pre-FEV1 and working duration (months) in subjects without BA history (A), and between pre-FEV1 and age in subjects and controls without BA (B). (A) The correlation between pre-FEV1 and working duration is significant ($r = -0.728, P < 0.01$). (B) The degree of the decrease of pre-work FEV1 levels by aging of the subjects was bigger than that of the controls.

Significantly increased FeNO levels after 5 days of work were found in subjects with a current or past history of BA. It can be presumed that their airway inflammation was evoked or airway irritation was enhanced by exposure to low-concentration chemicals including FA in workers that have airway hypersensitivity with potential exacerbation of their clinical condition. However, the FeNO levels of the subjects without a past history of BA significantly decreased after the study investigation period. It is difficult to explain clearly these data, but one interpretation based on past reports is oxidative stress-induced response.

Murta et al²⁴ examined the changes in inflammatory cells in bronchoalveolar lavage fluid (BALF), the activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in the blood, the activity of superoxide dismutase enzyme (SOD), and the activity of the catalase enzyme (CAT) of Fischer rats exposed to ambient air (control group: CG) or different concentrations of FA: 1% (FA1%), 5% (FA5%), and 10% (FA10%). In BALF, the exposure to a concentration of 10% promoted the increase of macrophages and lymphocytes in FA10% and lymphocytes in FA5% compared with CG, but eosinophil did not increase significantly in any concentration of FA. The activity of NADPH oxidase in the blood had been higher in FA5% and FA10% than in CG. The activity of SOD had an increase in FA5% and the activity of CAT showed an increase in FA1% compared with CG. These results suggest that airway inflammation occurs due to inhalation exposure of FA, and the main stream of its inflammation is likely to be oxidative stress rather than eosinophils. In addition, in patients with chronic obstructive pulmonary disease (COPD), the nitric oxide (NO) in the respiratory tract is consumed for producing peroxynitrite (ONOO⁻) in response to superoxide (O₂⁻), which is representative reactive oxygen species (ROS).^{25,26} It can be presumed that similar oxidative stress may be the main stream of inflammation even in the respiratory tract exposed to FA, and that, by such a reason, FeNO levels in the subjects without history of BA decreased in this study. Future studies will need to elucidate the mechanism of decrease in FeNO by low-concentration FA exposure in subjects.

In the current study, the subjects underwent spirometry before (pre) and after (post) a 5-day work; and FVC and FEV1 in the subjects showed significant decrease on chemical exposure mainly to FA, even though these exposure levels were less than OELs. In other words, the likelihood of evaluation of the short-term health effects of low-concentration FA exposure in workers by confirming FVC and FEV1 values were shown. FA, xylene, ethyl benzene, and acetone were detected by individual exposure monitoring (Table 2). Of these, FA is considered to be a group 2 occupational airway sensitizer, probably inducing allergic reactions in humans, and is known as a causative agent of occupational asthma. There is a report on a worker in a crease-resistant, trouser-product factory who handled FA on a daily basis; FEV1 was found to be decreased by 21% after normal work. After a FA inhalational exposure challenge test of 0.5 ppm for 20 minutes,

FEV1 was found to be temporarily decreased by 23% five hours later, and he was diagnosed with occupational asthma due to FA.²⁷ In addition, a previous study demonstrated airway obstruction secondary to high-concentration FA inhalational exposure.⁶ However, although the general impression is that asthma-like symptoms are common in workers who handle FA, past reports have shown that the low-concentration FA inhalation challenge test often has a low positive or negative rate.²⁸ This suggests that the airway response to low-concentration FA exposure is complicated. In the past, most of the reports are case studies or survey results in a small group of around 10 subjects. In the present study, although it is not enough subjects for detail analysis, the current consequences seem to show the reliable evidence of adverse effects on pulmonary function by exposing to low-concentration chemicals mainly including FA. In future study, it will be necessary to perform spirometry before and after research period for confirming recovery of each value in order to determine whether reductions in FVC and FEV1 are reversible. Recently, it is difficult to carry out a challenge test for an ethical problem, and it is important to continue the survey and collect more data.

Although a significant difference was not found in the pre-work FVC and FEV1 levels between the subjects and controls, there was a significant negative correlation in the subjects between pre-work FEV1 and work duration. Only work duration was found to be significantly associated with a decrease in FEV1 on multiple regression analysis, which assumed that work duration and age were contributing factors. This means that work duration influencing FEV1 decreases more than aging, and these results strongly support the effect of chronic exposure to low-concentration chemicals on lung function. Kilburn et al²⁹ performed pulmonary function tests in women who were histology technicians with chronic occupational exposure to FA, and reported that age-related decrease in FVC and FEV1 was more remarkable than in a national sample of women. The present results agree with the ones by Kilburn et al²⁹ in that age-related decrease in FEV1 was more remarkable in exposed subjects than in nonexposed controls. That leads us to presume that chronic exposure to FA is regarded as having other influences on pulmonary function in addition to aging.

This study had limitations. First, the subjects were exposed to chemicals other than FA. According to the recommendations of OEL by the JSOH, only FA is considered to be an airway sensitizer and other chemicals have not been mentioned in this regard. The findings in this current study are primarily due to FA; however, there is another report on increase in the risk of asthmatic crisis in children exposed to domestic low concentrations of VOCs containing xylene.³⁰ Therefore, our results cannot exclude effects on airways caused by chemicals other than FA. Second, other factors have been said to influence FeNO levels, in addition to asthma, smoking, and steroid use, for example, foods containing nitrites and having a current history of AR. It was not examined about nitrite-containing food intake of participant before FeNO measurement. However, it

seems that there was not a significant effect on FeNO levels through ingestion of nitrite-containing foods before the investigation because of the consistent tendency of increase in FeNO levels in asthmatic subjects and decrease in nonasthmatic subjects. In addition, regarding NIOX MINO, which was used for FeNO measurement in this investigation, it has been confirmed that NO derived from the nasal cavity is not mixed in by exhaling at a predetermined pressure. In the current study, the subjects and controls have AR accounted for 58% and 50% according to the questionnaire. Although it has been reported that FeNO levels are higher than 6 ppb on average in AR in previous papers, a significant difference was not found in the baseline and rate of change of FeNO levels when the groups with and without AR were compared in the current study. There is a possibility that the diagnosis of AR in subjects is not accurate, because these classifications were based on self-declaration. Therefore, it can be presumed that AR was not considered to have a significant influence on FeNO.

CONCLUSION

In recent years, in pathology departments of hospitals and research organizations, occupational health and safety management have greatly improved work environments. However, the current study showed that pathology workers were exposed to chemicals even at levels less than OELs. Furthermore, exposure to low levels caused adverse changes in both FeNO and spirometry indices. This suggests that even chemical exposure to concentrations less than OEL may have adverse effects on airways. In a chemical-handling work environment, it is believed that regular monitoring and evaluation of health effects secondary to chemical exposure are important. The current study suggests that FeNO, FVC, and FEV1 could be applied as indicators for the short-term evaluation of the adverse health effects of chemical exposure, and that FEV1 could be applied as an indicator for long-term evaluation.

It takes several decades until onset after exposure to carcinogen. Although FA is classified as a 2A carcinogen in the International Agency for Research on Cancer (IARC) classification, any chemical substances may have potential for carcinogens. It is noted that subclinical exposure to unconscious chemicals should be avoided and reduce exposure as much as possible. Therefore, it is important to have an indicator for assessment of health status in individual workers. It is hoped that the outcome of the present study would make a contribution to the health management of workers, who handle chemical substances.

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REFERENCES

- McGwin G, Lienert J, Kennedy JI. Formaldehyde exposure and asthma in children: a systematic review. *Environ Health Perspect*. 2010;118:313–317.
- Weisel CP. Assessing exposure to air toxics relative to asthma. *Environ Health Perspect*. 2002;110(Suppl 4):527–537.
- Persoz C, Achard S, Lelou C, Momas I, Seta N. An in vitro model to evaluate the inflammatory response after gaseous formaldehyde exposure of lung epithelial cells. *Toxicol Lett*. 2010;195:99–105.
- Alexandersson R, Hedenstierna G, Kolmodin-Hedman B. Exposure to formaldehyde: effects on pulmonary function. *Arch Environ Health*. 1982;37:279–284.
- American Academy of Allergy, Asthma and Immunology (AAAAI) Board of Directors. Idiopathic environmental intolerances. *J Allergy Clin Immunol*. 1999;103:36–40.
- Hendrick DJ, Lane DJ. Formalin asthma in hospital staff. *Br Med J*. 1975;1:607–608.
- Nordman H, Keskinen H, Tuppurainen M. Formaldehyde asthma: rare or overlooked? *J Allergy Clin Immunol*. 1985;75:91–99.
- Burge PS, Harries MG, Lam WK, O'Brien IM, Patchett PA. Occupational asthma due to formaldehyde. *Thorax*. 1985;40:255–260.
- Lemière C, Desjardins A, Cloutier Y, et al. Occupational asthma due to formaldehyde resin dust with and without reaction to formaldehyde gas. *Eur Respir J*. 1995;8:861–865.
- Kriebel D, Sama SR, Cocanour B. Reversible pulmonary responses to formaldehyde. A study of clinical anatomy students. *Am Rev Respir Dis*. 1993;148:1509–1515.
- Krzyzanowski M, Quackenboss JJ, Lebowitz MD. Chronic respiratory effects of indoor formaldehyde exposure. *Environ Res*. 1990;52:117–125.
- American Thoracic Society; European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med*. 2005;171:912–930.
- Kharitonov SA, Yates D, Robbins RA, Logan-Sinclair R, Shineboume EA, Barnes PJ. Increased nitric oxide in exhaled air of asthmatic patients. *Lancet*. 1994;343:133–135.
- Matsunaga K, Hirano T, Akamatsu K, et al. Exhaled nitric oxide cutoff values for asthma diagnosis according to rhinitis and smoking status in Japanese subjects. *Allergol Int*. 2011;60:331–337.
- Song GW, Ban GY, Nam YH, Park HS, Ye YM. Case report of occupational asthma induced by polyvinyl chloride and nickel. *J Korean Med Sci*. 2013;28:1540–1542.
- Maniscalco M, Grieco L, Galdi A, Lundberg JO, Sofia M. Increase in exhaled nitric oxide in shoe and leather workers at the end of the work-shift. *Occup Med (Lond)*. 2004;54:404–407.
- Meo SA, Alrashed AH, Almana AA, et al. Lung function and fractional exhaled nitric oxide among petroleum refinery workers. *J Occup Med Toxicol*. 2015;10:37.
- Ljubičić A, Varnai VM, Vučemić M, Matković K, Milić D, Macan J. Exhaled breath condensate pH and FeNO as biomarkers of acute and chronic exposure to hazards at swine farms. *J Occup Environ Med*. 2014;56:946–952.
- Van Amsterdam JG, Nierkens S, Vos SG, Opperhuizen A, van Loveren H, Steerenberg PA. Exhaled nitric oxide: a novel marker of adverse respiratory health effect in epidemiological studies. *Arch Environ Health*. 2000;55:418–423.
- Franklin P, Dingle P, Stick S. Raised exhaled nitric oxide in healthy children is associated with domestic formaldehyde levels. *Am J Respir Crit Care Med*. 2000;161:1757–1759.
- Chung CW, Morandi MT, Stock TH, Afshar M. Evaluation of a passive sampler for volatile organic compounds at ppb concentrations, varying temperatures, and humidities with 24-h exposures. 2. Sampler performance. *Environ Sci Technol*. 1999;33:3666–3671.
- Miller MR, Hankinson J, Brusasco V, et al. Standardization of spirometry. *Eur Respir J*. 2005;26:319–338.
- Japan Society for Occupational Health. Recommendation of occupational exposure limits (2015–2016). *J Occup Health*. 2015;57:394–417.
- Murta GL, Campos KK, Bandeira AC, et al. Oxidative effects on lung inflammatory response in rats exposed to different concentrations of formaldehyde. *Environ Pollut*. 2016;211:206–213.
- Blough NV, Zafiriou OC. Reaction of superoxide with nitric oxide to form peroxonitrite in alkaline aqueous solution. *Inorg Chem*. 1985;24:3502–3504.
- Ichinose M, Sugiura H, Yamagata S, et al. Xanthine oxidase inhibition reduces reactive nitrogen species production in COPD airways. *Eur Respir J*. 2003;22:457–461.
- Kim CW, Song JS, Ahn YS, et al. Occupational asthma due to formaldehyde. *Yonsei Med J*. 2001;42:440–445.
- Frigas E, Filley WV, Reed CE. Bronchial challenge with formaldehyde gas: lack of bronchoconstriction in 13 patients suspected of having formaldehyde-induced asthma. *Mayo Clin Proc*. 1984;59:295–299.
- Kilburn K, Warshaw R, Thornton JC. Pulmonary function in histology technicians compared with women from Michigan: effects of chronic low dose formaldehyde on a national sample of women. *Br J Ind Med*. 1989;46:468–472.
- Rumchev K, Spickett J, Bulsara M, Phillips M, Stick S. Association of domestic exposure to volatile organic compounds with asthma in young children. *Thorax*. 2004;59:746–751.