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Article

Urinary Biomarkers and Respiratory Responses to Indoor and Traffic-Related Air Pollution in Urban Children: A Prospective Cohort Study

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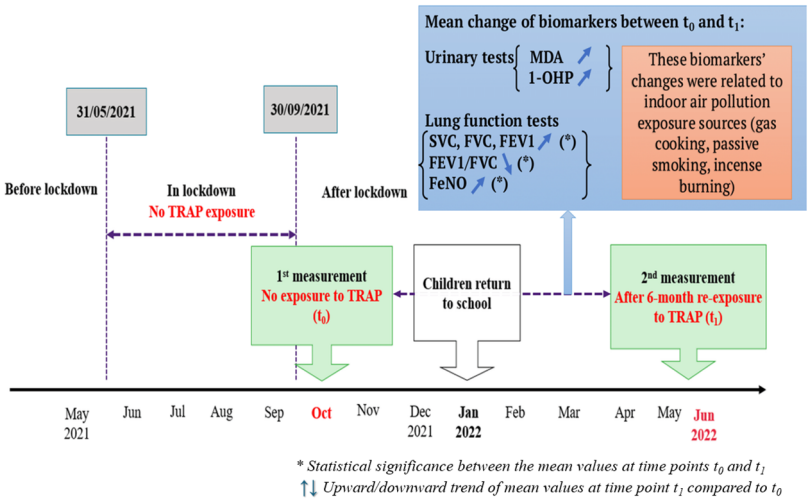
Highlights

- Study during COVID-19 lockdown offers a unique natural experiment in free traffic-related air pollution (TRAP)
- Combined biomarker and lung function tests captured children's pollution exposure

Abstract: COVID-19 lockdowns unintentionally improved air quality by reducing traffic and industrial activity. This study assessed changes in respiratory health and urinary biomarkers of air pollution exposure among children with no traffic-related air pollution (TRAP) exposure and TRAP re-exposure under COVID-19-related lockdowns. A prospective cohort study was conducted with 53 children from urban districts in Ho Chi Minh City, Vietnam. Two time measurements were taken: t0 (immediately post-lockdown, no TRAP exposure) and t1 (six months after TRAP re-exposure). The children underwent physical examinations, respiratory tests (spirometry, fractional exhaled nitric oxide (FeNO)), and urine collection for 1-hydroxypyrene (1-OHP) and malondialdehyde (MDA) analysis. Overall, thirty-five children were eligible for the analysis. FeNO and lung function parameters (SVC, FVC, FEV1, FEV1/FVC) showed significant changes after TRAP re-exposure. Following the return to normal daily activities after the COVID-19 pandemic, children exposed to passive smoking exhibited lower 1-OHP levels, which may be attributable to increased time spent outdoors. Significant increases in FeNO were observed in children exposed to passive smoke (p = 0.007), incense smoke (p = 0.003), and gas cooking (p = 0.003). The respiratory function values (SVC, FVC, and FEV1) of children underwent significant increases ($p < 0.05$) after the lockdown was eased and their exposure to indoor pollution was reduced while being re-exposed to TRAP. Urban children remained exposed to harmful indoor pollutants despite TRAP being limited during the COVID-19 lockdown. The findings call for continued efforts to reduce air pollution exposure among children and highlight the need for further investigation into passive smoking and incense smoke.

- Urban children still faced harmful indoor smoke despite reduced TRAP
- Urinary 1-OHP decreased after 6 months of TRAP re-exposure post-lockdown
- FeNO increased in children exposed to smoke from indoor air pollution sources



1. Introduction

Children are particularly vulnerable to the adverse effects of traffic-related air pollution (TRAP) [1]. They are exposed to TRAP while commuting to school, playing outside, or spending time in the schoolyard [1]. In many developing countries where public transportation is still underdeveloped, children often travel to school on motorbikes or bicycles, which increases their chance of being exposed to outdoor air pollution [2]. Moreover, children's travel time typically coincides with peak traffic hours, placing them at greater risk of exposure to high concentrations of air pollutants. Additionally, schools are often located near main traffic arteries to facilitate travel, and children's playtime in the schoolyard may expose them to significant emissions caused by traffic.

The COVID-19 pandemic, however, has significantly altered the risks related to air pollution. On March 11, 2020, the World Health Organization declared the novel coronavirus (COVID-19) outbreak as a global pandemic, leading many governments to implement lockdown measures in response to increasing outbreaks [3]. The implementation of COVID-19 lockdown measures has resulted in unprecedentedly low levels of global air pollution, attributed to a reduction in transportation. Numerous studies have established a correlation between the reduction in traffic due to COVID-19 lockdowns and improvements in air quality. For instance, a global study across 162 countries has found that $PM_{2.5}$ pollution in urban areas decreased by up to 45 percent during COVID-19-related lockdowns [4]. One question was raised as to whether the change in air pollution can positively impact human health. While ambient air quality in metropolitan areas improved significantly under lockdown measures, an increased risk of exposure to indoor pollutants requires further attention. As a result of the pandemic, people spent more time inside their houses to comply with social isolation and travel restrictions [5], leading to greater exposure to indoor air pollutant sources, which need to be taken into account.

To measure air pollution exposure, there are generally two approaches: air monitoring and biological measurement. While many past studies on air pollution exposure have typically relied on pollutant measurements from fixed-site monitoring stations, measuring exposure at an individual level is also critical to obtain a more accurate and realistic assessment of exposure levels. Biological monitoring is increasingly viewed as a desirable alternative to air sampling for characterizing environmental exposures. Recent advanced techniques using biomarkers have been suggested to identify levels of exposure to traffic-related air pollutants, particularly in epidemiological studies that assess air particle exposure at the individual level. This study used urinary 1-hydroxypyrene (1-OHP), a widely used biomarker of air pollution exposure, and malondialdehyde (MDA), a biomarker of lipid peroxidation and oxidative stress. Together, these biomarkers provide information on selected exposure pathways and potential biological responses related to air pollution exposure. However, they do not represent all air pollutants or biological mechanisms associated with traffic-related air pollution. Urinary 1-OHP and MDA have been applied in many environmental and occupational studies to assess health responses to air pollution exposure [6–11]. In addition, this study also used lung function tests, including fractional exhaled nitric oxide (FeNO) and spirometry (including the slow vital capacity (SVC), the forced expiratory volume in one second (FEV_1) and the forced vital capacity (FVC)), to evaluate the consequences of air emission-induced damage to the respiratory system.

This study aimed to assess and compare changes in urinary biomarkers (1-OHP and MDA) and respiratory indicators (FeNO, SVC, FEV_1, FVC) among children who had no TRAP exposure and TRAP re-exposure under the COVID-19-related lockdown conditions. The study also

considered indoor exposure sources as potential contributors to the higher level of exposure to air pollution.

2. Methodology

2.1. Study Population and Eligibility Criteria

A prospective cohort study was conducted among children living in the inner districts of Ho Chi Minh City. Participants were recruited through advertisements on social media, newsletters sent to schools, and communication channels between schools and parents. The inner districts are defined as the central part of Ho Chi Minh City, based on the geographical boundaries of the Vietnamese national system, where there is usually a high traffic density. This study was approved by the Board of Ethics in Biomedical Research at the University of Medicine and Pharmacy in Ho Chi Minh City (342/UMP-BOARD) at 25th June 2019.

Eligible children must be aged 12 to 14 years, have resided in the inner districts of Ho Chi Minh City for at least one year before enrollment and plan to stay in the same location until the following year. They must have available time for health examinations and sample collection and have obtained parental approval. This age range was selected because they are generally considered capable of providing reliable responses to self-based assessments and represent the standard age group recommended for a standardised International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire [12]. Children with chronic conditions that could interfere with respiratory function testing, biological sample collection, or safe participation in study procedures were excluded. Prior to data collection, all participants underwent a general health screening by a respiratory physician to confirm their suitability for study assessments. Children with a history of asthma were not excluded if they were clinically stable at the time of participation.

2.2. Study Procedure

The study location, Ho Chi Minh City, Vietnam, experienced a severe COVID-19 outbreak, resulting in strict lockdowns and social distancing measures from April 2021 to September 2021. The implemented restrictions included the prohibition of residents from leaving their homes for any reason. Additionally, transportation restrictions were imposed with enforcement by police and military forces. As a result, people spent over four months absolutely staying at home. This period was referred to as the time with no TRAP exposure. In October 2021, the city eased the lockdown, but people were only allowed to leave their homes for essential reasons such as health examinations or food shopping. Children remained at home during this time. In January 2022, social distancing measures were completely lifted, and children started returning to school, thus re-exposing themselves to TRAP. The study took place from October 2021 to June 2022 and included two exposure conditions: no exposure to TRAP (i.e., at the end of the four-month strict lockdown) and re-exposure to TRAP (i.e., six months after return to school). Two measurements were taken: t_0 (no TRAP exposure) at the initial time point after the lockdown and t_1 (re-exposure to TRAP) at the final time point after six months of re-exposure, which are summarized in the study flowchart (Figure 1).

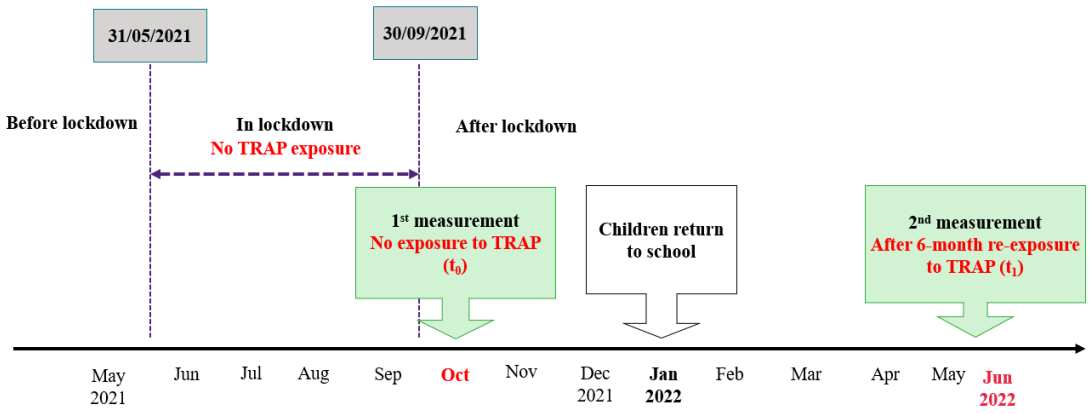


Figure 1. Study flowchart.

2.3. Study Assessments

All study assessments took place at an outpatient clinic, specialized in respiratory standards, equipped with full technical and emergency equipment, and licensed by the Ministry of Health. Each child underwent two identical assessments: once at t0 (no TRAP exposure) and again at t1 (TRAP re-exposure). The children were invited to attend the clinic to answer survey questions, undergo a physical examination, and measure lung function. The entire participation time was approximately two hours. A respiratory physician examined the child participants first to ensure their safety before conducting respiratory function tests using a bronchodilator. The tests could cause discomfort for the children while holding the breathing

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tube, so the technical staff carefully guided them on how to hold it properly to make them feel more comfortable. After completing the tests, the children remained at the clinic for at least 15 min for safety reasons.

2.4. Study Outcomes

Study outcomes of the cohort study included the presence of asthma ISAAC questionnaire, spirometry, fractional exhaled nitric oxide (FeNO), 1-hydroxypyrene (1-OHP) and malondialdehyde (MDA). Clinical assessments and Spirometry and FeNO measurements were conducted at a licensed respiratory clinic accredited by the Vietnamese Ministry of Health. Device calibration, maintenance, and quality assurance procedures were performed according to the manufacturer's specifications and national clinical regulations.

Details of these outcomes included:

- Diagnosis of asthma: The term "diagnosis of asthma" was recorded through patient self-report or diagnosis by doctors. The physician asked the child participant or caregiver if the child had ever been diagnosed with asthma in the past, and about their history of treatment and current condition. Additionally, the physician can provide a diagnosis of asthma by observing symptoms and following the GINA 2020 criteria. This process involves recognizing a particular pattern of respiratory symptoms, such as wheezing, shortness of breath (dyspnea), chest tightness, or cough, which could vary in severity and frequency, along with variable expiratory airflow limitation confirmed at least once.
- Spirometry: It was carried out using a flow-volume spirometer (Vyntus, Care Fusion) by a skilled technologist. The spirometer used in the study must adhere to the technical standards suggested by the American Thoracic Society (ATS)/European Respiratory Society (ERS) [13]. Calibration of the spirometers was performed every morning on the day of the study. All measurements in this study were made by two technicians using the same device. Participants were seated and used nose clips for the test. They were given an inhaled bronchodilator and then waited for at least 10 min before the spirometry was repeated. The software recorded the age, height, and gender of the participants, and height was measured just before the test. The results of the spirometry had to meet the ATS/ERS 2019 standard for acceptability and repeatability [13]. The primary lung function indices of interest were slow vital capacity (SVC), forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1).
- Fractional exhaled nitric oxide (FeNO): It refers to the fraction of exhaled nitric oxide concentration. A portable device (HYPAIR FeNO—Medisoft Group) was used in this study to measure FeNO, which quantifies the level of nitric oxide in parts per billion (ppb) in the air that is slowly exhaled from the lungs. The measurement technique followed guidelines and standards recommended by the American Thoracic Society/European Respiratory Society [14,15]. The device was calibrated every morning on the day of the study. All measurements in this study were taken by a technician using the same device. Child participants were advised to sit comfortably and breathe normally for about five minutes to acclimatize before inhaling close to total lung capacity (TLC). This was followed by an immediate exhalation for at least four seconds at the flow of 50 mL/s. FeNO measurements were taken before spirometry to ensure accuracy.
- MDA and 1-OHP: They were used in this study to assess internal dose and oxidative stress in children's urine samples. Analyses were conducted by environmental technologists at The University of Nature and Science in Ho Chi Minh City and followed laboratory standards. The detection limit of the 1-OHP analysis method was 0.087 ng/L. The detection limit of the MDA analysis method was 8.547 µg/L. The urinary 1-OHP and MDA levels of each individual were corrected according to urine creatinine values, which were measured using an automated method based on Jaffe's reaction.

2.5. Blinding

To prevent potential bias in outcome measurements, blinding was implemented. The primary study objectives, which were to compare outcomes between two time points, one with no TRAP exposure and one after exposure, were not disclosed to respiratory physicians or technologists. The main investigators, who were aware of the study's health outcomes, did not directly participate in the measurements of respiratory functions or urinary biomarkers.

2.6. Data Analysis

Descriptive statistics, including frequency and proportion for categorical variables, mean and standard deviation, as well as the median and interquartile range (IQR) for numeric variables, were used to summarize the characteristics of the study. Linear regression analyses were used to evaluate the correlation between two continuous variables at t_0 and t_1. The correlation coefficient and standard error (SE) were provided to represent the strength and direction of the relationship between the two variables. Models were adjusted by covariates such as sex, asthma, COVID-19 history and child's height and weight difference between the two assessments. Paired t-test was used for normally distributed data to compare the difference of continuous variables between the two time points, while non-parametric tests like the Wilcoxon signed-rank test were

used for non-normally distributed data. The mean differences and standard error (SE) were used to express the results of the comparison, with a statistical significance level set at $\alpha = 0.05$ for all tests. Stata 17.0 was used to analyze all the data.

3. Results

A total of 53 children participated in the first assessment of the study, but only 41 children returned for the second assessment after 9 months. Each child underwent assessments at two time points (referred to as t_0 and t_1), each of which included two lung function tests (FeNO and Spirometry) and urine collection for biomarker testing (MDA and 1-OHP). Out of 41 children, only 35 provided enough urine for testing and were able to conduct respiratory measurements. As a result, the final sample size for all analyses was 35 paired assessments. The characteristics of this cohort are presented in Table 1. There was an equal distribution between males and females in the study, with an age range of 12 to 14 years. The child participants had a normal birth weight, but nearly half of them were classified as overweight or obese based on their current BMI. Ten children (28.6%) were diagnosed with asthma, and three of them were diagnosed at the second assessment.

Table 2 illustrates the level of exposure to various sources of outdoor and indoor air pollution. In the first assessment (t_0), our hypothesis was that, due to the lockdown, children were not exposed to outdoor air pollution for about four months. The data revealed that most children either did not go out or went out less than once a month due to the restrictions (91.4%). Their outdoor activities were limited to short trips by motorcycle or on foot around their immediate surroundings. After the lockdown was lifted, the children were re-exposed to traffic-related air pollution due to their daily commute to school and recovered transport activities. On average, children spent approximately one hour per day travelling outside, with the majority of them using motorcycles, a common mode of transportation in the city (74.3%).

Table 1. Characteristics of the study group (n = 35).

Variables		Value (Percent)
Sex		
	Male	18 (51.4)
	Female	17 (48.6)
Age		
	12	6 (17.1)
	13	28 (80.0)
	14	1 (2.9)
Weight at birth (kg)		
	Mean (sd)	3.2 (0.29)
	Median (IQR)	3.2 (3.05–3.4)
Weight at t_0 (kg)		
	Mean (sd)	47.7 (11.5)
	Median (IQR)	46.2 (41–55.8)
Height at t_0 (cm)		
	Mean (sd)	150.5 (6.7)
	Median (IQR)	149(147–156)
BMI for age at t_0 (WHO 2007)		
	Thinness	2 (5.7)
	Normal	17 (48.6)
	Overweight	8 (22.9)
	Obesity	8 (22.9)
Weight at t_1 (kg)		
	Mean (sd)	51.3 (11.3)
	Median (IQR)	51 (44–57.7)
Height at t_1 (cm)		
	Mean (sd)	153.8 (7.2)
	Median (IQR)	154 (150–158)
BMI for age at t_1 (WHO 2007)		
	Thinness	1 (2.9)
	Normal	17 (48.6)
	Overweight	11 (31.4)
	Obesity	6 (17.1)
Diagnosed with asthma		
	Yes	10 (28.6)
	No	25 (71.4)
COVID-19 infection in six recent months		
	Yes, at the 1st assessment)	3 (8.6)
	Yes, at the 2nd assessment)	12 (34.3)

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Table 2. Exposure to outdoor and indoor air pollution.

Variables		Value (Percent)
Exposure to outdoor air pollution		
1st assessment–t_0		
Time spent outside during lockdown		
	Never	10 (28.6)
	Less than 1/months	22 (62.8)
	More than 1/months	3 (8.6)
Mean to travel during lockdown		
	Motorcycle	18 (72.0)
	Walking	4 (16.0)
	Car/Taxi	3 (12.0)
2nd assessment–t_1		
Time spent outside after lockdown (minutes)		
	Mean (sd)	63.0 ± 54.0
	Median (IQR)	45 (30–70)
Mean to travel after lockdown		
	Motorcycle	26 (74.3)
	Bicycle or e-bicycle	3 (8.6)
	Walking	3 (8.6)
	Bus	3 (8.6)
Exposure to indoor air pollution		
Children smoking		
	Yes	0
	No	35 (100)
Family member smoking		
	Yes	16 (45.7)
	No	19 (54.3)
Cooking stove		
	Gas stove	25 (71.4)
	Induction cooker/Infrared cooker	14 (40.0)
	Opened stove using coal/wood	2 (5.7)
Incense burning		
	Yes	23 (65.7)
	No	12 (34.3)

Concerning indoor exposure, our study population was exposed to three main sources. Firstly, all the children were non-smokers, but some were exposed to smoke from their family members. Around half of the study population lived with at least one family member who smoked. Additionally, nearly 71% of children lived in homes that used gas stoves, while only 5% of children resided in homes using open stoves that burn coal or wood. Furthermore, more than 65% of children reported that their families burned incense daily.

Table 3 indicates that re-exposure to TRAP resulted in a general upward trend in the urinary biomarker of MDA (with a mean change of 124 μg/g creatinine), while the level of 1-OHP slightly decreased (with a mean change of −1.16 μg/g creatinine). However, no significant differences were observed between the two time points of assessment (t_0 and t_1). This non-significant trend should be interpreted cautiously and cannot be considered conclusive evidence of increased oxidative stress associated with re-exposure. Nitric oxide concentration in exhaled air (FeNO) is a valuable biomarker for detecting and monitoring airway inflammation. As shown in Table 3, there was a significant change between t_0 and t_1 in FeNO levels at the flow of 50 mL/s, rising by 12.52 ppb. Moreover, the results indicate complex changes in lung function in children after re-exposure to TRAP from t_0 to t_1. Lung function measured on the second occasion had significantly increased for SVC, FEF_1, and FVC with a crude rise of 0.24L, 0.10L, and 0.21L, respectively, and a 3% decrease in FEV_1/FVC. After adjusting for sex, asthma, COVID-19 history and child's height and weight difference between the two assessments, SVC, FEF_1, and FVC increased by 0.19L, 0.01L, and 0.15L, respectively and FEV_1/FVC reduced by 4%.

The analysis also considered indoor sources of air pollution, adding to TRAP exposure at t_1, as presented in Tables 4 and 5. The results showed no significant difference in the change in MDA levels between two time points among both groups of children exposed and not exposed to smoke from indoor air pollution sources, such as gas cooking, passive smoking, and incense burning. Although the concentration of 1-OHP generally did not

differ significantly between children exposed or not exposed to smoke from cooking and incense, a significant difference was found in the child population exposed to smoke from family members. Children who were exposed to passive smoking at home had a decreased concentration of 1-OHP at t_1. The data also revealed a significant increase in FeNO values at 50 mL/s in children exposed to smoke from indoor air pollution sources, including gas cooking, passive smoking, and incense burning, at t_1. When considering the indoor sources of air pollution exposure that contribute to lung function, children who were exposed to gas cooking, passive smoking, and incense burning at home experienced a significant change from t_0 to t_1 in respiratory function values of SVC, FEV_1, and FVC. However, levels of FEV_1/FVC significantly decreased after TRAP exposure in both children who used gas stoves and those who did not, as well as in children who were not exposed to passive smoking.

Table 3. Association of indicators of urine tests and lung function tests between two time point assessments.

Variables	Mean (SE)			Coefficient (SE)		*p*-Value
	t_0	t_1	Change (t_1–t_0)	Crude Difference	Adjusted Difference #	
MDA_µg/g_creatinine	159.74 (16.45)	284.45 (91.28)	124.71 (93.85)	−0.38 (0.96)	−0.74 (1.06)	0.49
1OHP_µg/g_creatinine	2.55 (0.63)	1.39 (0.45)	−1.16 (0.79)	−0.03 (0.12)	−0.09 (0.14)	0.52
FENO_50 (n = 33), ppb	15.33 (2.79)	27.85 (5.20)	12.52 (3.26)	1.55 (0.19)	1.47 (0.21)	<0.001 *
SVC_pre, L	2.58 (0.08)	2.82 (0.09)	0.24 (0.03)	0.98 (0.07)	0.93 (0.07)	<0.001*
SVC_pre, %	100.2 (2.37)	102.69 (2.20)	2.49 (1.47)	0.74 (0.10)	0.64 (0.11)	<0.001 *
FEV1_pre, L	2.43(0.07)	2.53 (0.08)	0.10 (0.03)	0.96 (0.07)	0.94 (0.07)	<0.001 *
FEV1_pre, %	108.11 (2.39)	105.43 (2.33)	−2.69 (1.32)	0.82 (0.09)	0.81 (0.10)	<0.001 *
FVC_pre, L	2.60 (0.08)	2.80 (0.09)	0.21 (0.03)	0.99 (0.07)	0.94 (0.08)	<0.001 *
FVC_pre, %	100.91 (2.33)	102.03 (2.28)	1.11 (1.48)	0.78 (0.10)	0.72 (0.11)	<0.001 *
FEV1/FVC_pre,	0.94 (0.01)	0.91 (0.01)	−0.03 (0.01)	0.72 (0.14)	0.66 (0.16)	<0.001 *

Abbreviation: SE: Standard error; * Statistically significant; # Adjusted for sex, asthma, COVID-19 history, and child's height and weight difference between the two assessments.

Table 4. Comparison of the mean change of biomarkers' values between two assessments by indoor air pollution exposure sources.

Difference of Biomarkers' Values between Two Assessments (t_1–t_0)						
	MDA_µg/g_Creatinine [s]		1OHP_µg/g_Creatinine [t]		FENO_50 (n = 33) [s]	
Exposure Sources	**Mean Change (SE)**	***p*-Value**	**Mean Change (SE)**	***p*-Value**	**Mean Change (SE)**	***p*-Value**
Gas cooking						
Non-exposure (n = 10)	−20.04 (46.66)	0.109	−0.09 (0.26)	0.73	14.67 (5.42)	0.18
Exposure (n = 25)	182.61 (129.05)	1.0	−1.59 (1.09)	0.16	11.71 (4.05)	0.003 *
Passive smoking						
Non-exposure (n = 19)	224.41 (169.58)	1.0	0.30 (0.81)	0.715	12.65 (3.82)	0.049
Exposure (n = 16)	6.31 (31.17)	0.45	−2.90 (1.33)	0.043 *	12.38 (5.50)	0.007 *
Incense burning						
Non-exposure (n = 12)	0.41 (45.71)	0.388	−2.72 (1.64)	0.123	0.41 (45.71)	0.15
Exposure (n = 23)	189.56 (140.04)	1.0	−0.35 (0.82)	0.670	189.56 (140.04)	0.003 *

Abbreviation: SE: Standard error; [t]: Paired *t*-test, [s]: Wilcoxon signed-rank test, *: Statistically significant.

Table 5. Comparison of the mean change of lung function parameters between two assessments by indoor air pollution exposure sources.

Difference of Biomarkers' Values between Two Assessments (t_1–t_0)								
	SVC_pre [s]		FEV1_pre [t]		FVC_pre [s]		FEV1/FVC_pre [s]	
Exposure Sources	**Mean Change (SE)**	***p*-Value**	**Mean Change (SE)**	***p*-Value**	**Mean Change (SE)**	***p*-Value**	**Mean Change (SE)**	***p*-Value**
Gas cooking								
Non-exposure (n = 10)	0.36 (0.06)	0.002 *	0.12 (0.05)	0.005 *	0.29 (0.06)	0.02 *	−0.06 (0.01)	0.004 *
Exposure (n = 25)	0.20 (0.04)	<0.001 *	0.09 (0.04)	0.02 *	0.17 (0.04)	0.02 *	−0.03 (0.01)	0.08 *
Passive smoking								
Non-exposure (n = 19)	0.22 (0.05)	<0.001 *	0.08 (0.05)	0.10	0.20 (0.06)	0.02 *	−0.04 (0.01)	<0.001 *
Exposure (n = 16)	0.27 (0.04)	0.004 *	0.12 (0.03)	0.004 *	0.22 (0.04)	0.02 *	−0.03 (0.01)	0.42
Incense burning								
Non-exposure (n = 12)	0.31 (0.06)	<0.001 *	0.13 (0.05)	0.02 *	0.24 (0.06)	0.04 *	−0.03 (0.01)	0.18
Exposure (n = 23)	0.21 (0.04)	0.003 *	0.08 (0.04)	0.049 *	0.19 (0.04)	0.01 *	−0.04 (0.01)	0.007

Abbreviation: SE: Standard error; [t]: Paired t-test, [s]: Wilcoxon signed-rank test, *: Statistically significant.

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4. Discussion

The study utilized the strict and long-term lockdown in a Vietnamese city to evaluate the respiratory health of child participants during 4 months of limited exposure to traffic air pollution, referred to as t_0 (no TRAP exposure). Subsequently, a second health assessment was conducted six months after the lockdown was lifted, re-exposing children to usual levels of air pollution, referred to as t_1 (TRAP re-exposure). The study reveals three main findings: (1) urban children were exposed to high levels of air pollution emitted from both indoor and traffic sources; (2) urinary biomarkers, such as MDA and 1-OHP, demonstrated that urban children suffered from high concentrations of air pollution exposure even during the lockdown period when outdoor pollutant sources were minimized; and (3) re-exposure to TRAP in six months resulted in complex changes in children's respiratory health, as indicated by FeNO and function, partly related to reduced exposure to indoor air pollution, complicated by re-exposure to TRAP.

A recent report by Health Effects Institute has revealed that the impact of air pollution falls disproportionately on children in LMICs. The present study underscored the impact on children living in urban areas of LMICs. The study found that children in the urban area spent roughly one hour each day travelling outdoors, with the majority of them using motorcycles, a prevalent means of transportation in the city [16]. However, open vehicles like motorcycles expose children directly to hazardous ambient air without any enclosure, leading to higher inhalation of dust and pollutants [17,18]. Children living in urban areas experience regular and long-term exposure to air pollution, with peak exposure often occurring during going to school when significant amounts of pollutants are released due to traffic [19]. Moreover, compounded by traffic-generated air pollution, passive smoking is a significant issue among urban children in Vietnam [20]. This study indicated that urban children in Vietnam were frequently exposed to secondhand smoke, with a prevalence rate of 46%, which is consistent with other studies in Vietnam (43%) [20], Southeast Asia (53%) [21], and globally (40%) [21]. Furthermore, incense burning is considered a major source of indoor air pollution [22], with the amount of particulate matter generated by incense being up to 4.5 times that of cigarette smoke [23]. The burning of incense is a religious ritual that is commonly observed in the cultures of Southeast Asia, particularly in Vietnam. Even though it is favoured by the elderly population, children are often passively exposed to the smoke from burning incense. In addition, recent research indicates potential harms associated with gas stoves [24,25]. Cooking with gas stoves emits nitrogen dioxide and $PM_{2.5}$ particles when in use, and may also release methane gas even when turned off [25].

In Ho Chi Minh City, Vietnam, the mean $PM_{2.5}$ value during the COVID-19 lockdown period was 18% lower compared to those of normal condition estimations [26]. However, after the lockdown was lifted, increasing ambient air pollution was observed. Urinary MDA and 1-OHP measurements were used in this study to demonstrate the presence of metabolites and oxidative stress induced by ambient air pollution. The mean level of MDA among children who were free from TRAP due to staying completely at home for four months was 159.74 μg/g creatinine, but it nearly doubled to 284.45 μg/g creatinine after six months of re-exposure to TRAP. Nonetheless, no significant association was found between the changes in the two time point assessments. This may be due to potential confounders of urinary MDA other than ambient air pollution, such as indoor air pollution, diet, or psychological stress, which should be taken into account [6,11,27]. Conversely, the mean level of 1-OHP slightly decreased from 2.55 to 1.39 μg/g creatinine after six months since children returned to their usual life. The current results indicate that the levels of MDA and 1-OHP in Vietnamese urban children were higher compared to those of children living in two Chinese cities measured during normal school days, with values of between 113.1 and 196.8 μg/g creatinine for MDA measurements and between 1.19 and 2.38 μg/g creatinine for 1-OHP measurements [7]. These findings suggest that Vietnamese urban children may have experienced substantial exposure to air pollution despite reduced traffic-related emissions during the lockdown period. Possible explanations include continued exposure to indoor pollution sources, such as secondhand smoke, incense burning, and gas cooking, as well as differences in environmental conditions and exposure patterns compared with the study populations reported elsewhere. However, comparisons across studies should be interpreted cautiously because of variations in study design, population characteristics, and exposure assessment methods.

Several studies have shown that urinary 1-OHP concentrations are significantly correlated with passive smoking [8,9]. The levels of 1-OHP remained higher among children living with smoking parents compared to those with non-smoking parents [9]. Consistent with these findings, the present study indicates a significant difference in urinary 1-OHP concentrations between two time points among children exposed to passive smoke. Specifically, during the first study measurement, even though the children had not been exposed to air pollutants from traffic for a long time, they still had high levels of 1-OHP due to living with family members who smoked. However, during the second study measurement, when the children spent more time at school and less time at home with active smokers, their 1-OHP values significantly decreased. These findings suggest that passive smoking may represent an important contributor to children's air pollution exposure, particularly during periods when time spent indoors is increased. However, this does not imply that their exposure was primarily

derived from indoor sources, as both indoor and outdoor pollution sources are likely to contribute to children's overall exposure. Additionally, another contributing factor to the reduced exposure could be the fact that, after COVID-19 restrictions eased, masks remained mandatory in public places and schools. Children tended to wear masks when going outside, which could partly protect them from outdoor air pollution [28,29]. This study highlights the greater risk of indoor air pollution among children during COVID-19 lockdowns, as they spent almost the whole day at home [30].

The study's results indicated a significant increase in FeNO levels at the flow of 50mL/s after six months of re-exposure to TRAP, after adjusting for sex, history of asthma, history of COVID-19 infection, and the child's height and weight gain. These findings suggested an early presence of inflammation in the airways following increased air pollution exposure, which aligns with prior research of a link between ambient air pollution levels and FeNO levels as well as the use of FeNO as a sensitive biomarker for respiratory effects related to air pollution, particularly in children [31–36]. At a flow of 50mL/s, the average FeNO level at the second assessment was approximately 28 ppb, a 12 ppb increase compared to the first assessment, exceeding the normal range for children, which should be less than 20 ppb [37]. The outcome obtained in this study was greater than the results observed in other studies conducted among healthy schoolchildren [32–34,38,39]. These findings extend prior investigations into the relationship between FeNO levels and air pollution exposure by incorporating the contribution of indoor air pollution sources. Specifically, urban children exposed to indoor air pollutants at home, such as smoke from gas stoves, passive smoke, or incense burning, exhibited significantly higher FeNO concentrations after additional exposure to TRAP. Despite significant changes in spirometry parameters such as SVC, FEV1, FVC, and FEV1/FVC following six months of TRAP re-exposure, all values remained within the normal range, thus, no evidence of airway obstruction was proven. Consequently, the study concludes that prolonged exposure to TRAP has a significant impact on children's respiratory health, as indicated by both FeNO and spirometry parameters. Nonetheless, FeNO measurements may be more useful as an early marker for predicting airway effects resulting from air pollution exposure [31,40].

This study conducted immediately after the strict and prolonged lockdown period, possesses noteworthy strengths that are difficult to replicate at present. This unique setting offered an exceptional opportunity to investigate the impact of a dramatic reduction in air pollution on children's health. In addition, outcome measurements incorporated both biomarkers in urine and respiratory function, enabling the assessment of exposure and health effects at an individual level. Besides, the transition from lockdown to reopening involved both increased traffic-related air pollution exposure and reduced time spent indoors. Consequently, the independent effects of indoor and outdoor pollution sources could not be disentangled. Nevertheless, because children are typically exposed to both environments simultaneously in everyday life, the study was designed to capture the overall impact of these real-world exposure scenarios. Therefore, the observed health changes are likely to reflect the combined influence of multiple exposure sources rather than the effect of any single source. Several limitations should also be acknowledged. First, as with any longitudinal study, there was some sample attrition by the second point of assessment. Second, although the study did not include air quality data, which may limit its accuracy in representing actual exposure to polluted air, the two time periods of lockdown and lockdown easing can be used as proxies for low and high traffic-related air pollution levels. However, it is important to note that the high percentage of children exposed to indoor smoke could affect the calculation of the impact of TRAP in this study. Therefore, our analyses took into consideration the indoor factors that may affect the results. Third, the results of biomarkers could be influenced by factors such as sample characteristics or laboratory errors [41]. To minimize these potential errors, respiratory measurements were taken using the same devices, by the same technologists, and urine samples were collected at the same time in the early morning. Lastly, the relatively small sample size, particularly within some stratified analyses, may have reduced the statistical power to detect significant differences. Therefore, the findings should be interpreted with caution and require confirmation in larger studies before being generalized to broader populations. Additionally, local background reference values for urinary 1-OHP and MDA among unexposed children were unavailable, limiting direct contextual interpretation of the observed biomarker concentrations. Furthermore, detailed analytical validation parameters were not available for retrospective reporting and could therefore not be included.

The current study measured only 1-OHP and MDA due to budgetary and laboratory resource constraints. Future research should incorporate additional biomarkers of exposure and oxidative stress to better characterize the biological pathways linking air pollution exposure to respiratory health outcomes. Furthermore, data on physical activity, time-activity patterns, and other behavioural factors should be collected to improve exposure assessment and reduce the potential for residual confounding when evaluating changes in lung function among children.

5. Conclusions

This study employed a robust approach, using a longitudinal study to evaluate the respiratory conditions

(FeNO and Spirometry) and urinary biomarkers (MDA and 1-OHP) of 35 children at a point of no TRAP exposure (immediately after the COVID-19 lockdown was lifted) and again at a point of TRAP re-exposure (when the lockdown had been lifted for six months and children returned to school). It suggests that despite outdoor emission sources being limited, urban children still face hazardous air from their homes. The findings call for continued efforts to reduce air pollution exposure among children and highlight the need for further investigation into indoor air pollutants in urban environments, particularly passive smoking and incense smoke.

Author Contributions

H.H.T.C.L.: conceptualization, data curation, formal analysis, funding acquisition, project administration, writing—original draft, writing—review and editing; D.P., P.K.T., N.N.V. and T.N.D.: conceptualization, supervision, funding acquisition, writing—review and editing; H.N.T., L.L.T., Q.N.N., H.T.D.P., T.H.T.D., N.T.T.V. and T.T.T.D.: data curation, investigation, visualization; T.T.H.: data curation, resources, project administration; P.L.A.: conceptualization, supervision, funding acquisition, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Board of Ethics in Biomedical Research at the University of Medicine and Pharmacy in Ho Chi Minh City (342/UMP-BOARD) at 25th June 2019.

Informed Consent Statement

Individual children's consent was obtained during data collection after informing them that their participation was completely voluntary. To protect confidentiality and ensure the right to refuse participation, all questionnaire sheets were placed in an envelope. Children and caregivers were able to leave the answer sheets blank, and they remained sealed until they reached the researchers.

Data Availability Statement

The data are available from the corresponding author upon reasonable request.

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Conflicts of Interest

Given the role as an Associate Editor, Associate Professor Phong Thai had no involvement in the peer-review process of this paper and had no access to any information regarding its review. Full responsibility for the editorial handling of this manuscript was delegated to another editor of the journal. The other authors declare no conflicts of interest. The funder, the Vietnam National Foundation for Science and Technology Development (NAFOSTED), was involved in funding the data collection. The authors take full responsibility for the content of the published article.

Use of AI and AI-assisted Technologies

No AI tools were utilized for this paper.

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