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ORIGINAL ARTICLE

Effects of $PM_{2.5}$ and NO_2 on the 8-isoprostane and lung function indices of FVC and FEV_1 in students of Ahvaz city, Iran

Bayram Hashemzadeh^{a,b}, Esmaeil Idani^c, Gholamreza Goudarzi^{a,d}, Kambiz Ahmadi Ankali^d, Mohammad Javad Zare Sakhvidi^e, Ali Akbar Babaei^{a,d}, Hassan Hashemzadeh^f, Mehdi Vosoughi^{a,d}, Mohammad Javad Mohammadi^g, Abdolkazem Neisi^{a,d,*}

^a Department of Environmental Health Engineering, Health Faculty, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

^b Department of Environmental Health, Khoy School of Nursing, Urmia University of Medical Sciences, Urmia, Iran

^c Department of Internal Medicine, Division of Pulmonology, Imam Khomeini Hospital, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

^d Environmental Technologies Research Center (ETRC), Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

^eShahid Sadoughi University of Medical Sciences, Yazd, Iran

^f Department of Environmental Health Engineering, School of Public Health, Zanjan University of Medical Sciences, Zanjan, Iran ^g Abadan School of Medical Sciences, Abadan, Iran

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KEYWORDS

Air pollution; Lung function; 8-Isoprostane; Traffic; Ahvaz; Iran **Abstract** The aim of this study was to determine the correlation between $PM_{2.5}$ and NO_2 pollutants and oxidative stress marker (8-isoprostane) and lung function tests (FVC and FEV₁) in healthy children who were living and studying in three different areas of Ahvaz city including A₁: Naderi site with high traffic, A₂: Alavi Alley site with average traffic, and A₃: Ein 2 site with low traffic (a rural area on the suburb of Ahvaz). 30 students in the 12–13 year-old range were selected from each studied zone (1, 2 and 3 sites) during three months of year. Of each student, one sample was taken every two weeks to measure 8-isoprostane of exhaled breath condensate (EBC). Air pollution data were collected from three air quality monitoring stations. Also, the relationship between air pollution and 8-isoprostane as well as lung function tests were determined using generalized estimating equations (GEE). The mean concentration of $PM_{2.5}$ and NO_2 in A₁,

* Corresponding author at: Department of Environmental Health Engineering, Health Faculty, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

E-mail addresses: bhashemzadeh94@gmail.com (B. Hashemzadeh), akneisi@ajums.ac.ir (A. Neisi). Peer review under responsibility of King Saud University.



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 A_2 and A_3 areas were 116, 92 and 45 (µg/m³) also 77, 53 and 14 (ppb) respectively. Among all studied students, there was a significant correlation between the increase of mean concentration of PM_{2.5} and NO₂ in 1–4 before sampling day, increased 8-isoprostane concentration and decreased FEV₁, while there was no significant correlation between them and decreased FVC. In A_1 site, an increase in IQR (13 µg/m³) PM_{2.5} and IQR (6.5 ppb) NO₂ on 1–4 days before sampling was associated with 0.38 unit (95% CI: 0.11, 0.65) and 1.1 unit (95% CI: 0.85, 1.35) increase in 8-isoprostane concentration, also decreased 121 ml and 190 ml FEV₁, respectively. Results showed that the short-term exposure to traffic-related air pollution can decrease the values of lung function indices and increase the oxidative stress. It may adversely affect children's lungs.

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1. Introduction

The emissions from transport and industrial activities have detrimental negative impact on the quality of the inhaled air on both developed and developing countries and caused deaths due to pulmonary and cardiovascular disease (Bono et al., 2015; Wu et al., 2013; Kamkar et al., 2010). Among air pollutants, PM_{2.5} and NO₂ have been introduced as the most common pollutants emitted from vehicles. People who live near high-traffic areas are exposed to more contaminants than those living near low traffic areas (Liu et al., 2015). PM_{2.5} has been associated with adverse human health outcomes, due to the absorption of toxic substances on the surface of PM2.5 particles such as volatile organic compounds (VOCs) and polycyclic aromatic hydrocarbons (PAHs), (Cachon et al., 2014; Neisi et al., 2016). Moreover, the increase in exposure to outdoor concentrations led to an increase in lung inflammation in children and adults (Romieu et al., 2008). It seems that children are the most vulnerable groups to the harmful effects of air pollution. Because most of them spend more their time outside especially during warm seasons. Also, as a result of the great amount of activity in this age group, they have a higher breathing rate, so higher amount of ambient air pollutants can enter their lungs (Gauderman et al., 2000; Salvi, 2007). Numerous studies have investigated the adverse effects of traffic-related air pollution on human health, and reported that it has become a main problem in most of the world's largest cities (Bind et al., 2012; Götschi et al., 2008). To determine the effect of air pollutants on the lung health, several parameters have been defined such as lung inflammation and oxidative stress (PH, 8isoprostane and cytokinesis in breath condensate output) and pulmonary function parameters including forced expiratory flow in 1 s (FEV₁) and forced vital capacity (FVC) (Liu and Zhang, 2009). 8-Isoprostane is a byproduct of lipid peroxidation, which is attributed to the effects of free radicals that its amount can be determined at EBC. Its amount is different between healthy and patient individuals. 8-Isoprostane measurement in exhaust air from the lungs could be useful in air pollution studies (Rosa et al., 2014). 8-Isoprostane measurement can be performed in biological fluids such as blood, urine and especially in EBC (Commodore et al., 2013). EBC sampling has been considered as a simple, safe and non-invasive technique, which is used to sample from lower parts of the lungs and for the assessment of pulmonary inflammation especially in children. Also, this technique can be done in nonhospital environments such as houses (Corradi et al., 2002; Montuschi, 2007).

Lung function is a main marker in the evaluation of type and severity of respiratory problems. The pulmonary function tests are widely used as a marker effect, to assess the health effects of air pollutants. FVC and FEV₁ are generally used for spirometry data collection (Pellegrino et al., 2005).

Although many studies in field of air pollution and its health effect have been focused mostly on PM_{10} (Dastoorpoor et al., 2016; Dianat et al., 2016a; Dianat et al., 2016b; Khaniabadi et al., 2017; Marzouni et al., 2016; Naimabadi et al., 2016; Radmanesh et al., 2016; Soleimani et al., 2016), there is a scarcity of data about $PM_{2.5}$ and its impact on human health in Iran.

Few studies have examined the relationship between exposure to traffic and airway inflammation in healthy children, and most of them have focused on adults with largely exposed to pollutants in the roadside. Hence, changes in airway inflammation in healthy individuals with short-term exposure to particles and gases from vehicles are not well known.

Ahvaz metropolis is in southwest of Iran with an area of 528 square kilometers. In recent years, various industries have been launched (Naimabadi et al., 2016; Maleki et al., 2016). Also, the number of gasoline and diesel vehicles has dramatically improved and vehicle density and traffic volume is very high in some areas of this city. In this regard, in order to understand the relationship between short-exposure with $PM_{2.5}$ and NO_2 (as two important pollutants from vehicles) and respiratory health of children and students, three zones of Ahvaz city were selected that are different in terms of traffic volume. Moreover, biomarkers of oxidative stress and 8-isoprostane in exhaled breath condensate and lung function indices including FVC and FEV₁ were measured.

2. Material and methods

2.1. Study subjects

This cross-sectional study was carried out from March 2015 to May 2015, 90 male students in sixth grade elementary aged between 12 and 13 years and with an average of body weight of 18.3 kg/m² (in the range 17.5–19 kg/m²) were selected from three schools, which were different in term of traffic volume and intensity (Naderi, Alavi Coi and Eine 2 schools). The questionnaire of the international study of asthma and allergies in childhood (ISSAC-C) was used for selecting appropriate precipitants, who have lived on the same area and school in the whole of their lives (Mallol et al., 2013). The questionnaire included questions about the basic information of students, respiratory

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health, allergic status, demographic, and environmental impact. The questionnaires were filled by student's parents and students. The consent was taken from their parents and the children. Ethical considerations of this research were approved by the ethics committee of Ahvaz University of Medical Sciences.

2.2. Study sites

Ahvaz is a city in southwest of Iran with a population of about 1,120,000 people according to the 2015 census and an area of 528 km² (Naimabadi et al., 2016). During the study air temperature was varied from 21 °C to 38 °C. Three sites of Ahvaz city were selected including A₁: Naderi site with high traffic, A₂: Alavi Alley site with average traffic, and A₃: Ein 2 site with low traffic (a rural area on the outskirts of Ahvaz) (Fig. 1).

2.3. Lung function test

In order to perform lung function test, a portable spirometer (microlab) on daily basis was used. It was calibrated using a syringe (one liter) before spirometry. The test was conducted during study in the morning shift schools between 9 am and 1 pm Iranian local time. During spirometry test, all students were asked to fill the questionnaire containing various questions in relation to respiratory symptoms such as cough, sneezing, runny nose, and shortness of breath (5 day before test). During the study, 5 students were excluded, due to the lack of cooperation and unacceptable spirometry graph. It should be noted that five other precipitants were replaced. The spirometry device was fixed for the evaluation of pulmonary function. The lung function indices including FVC and FEV₁ were measured. Spirometry was performed in the standing position. In the measuring, the values of difference between the highest FVC and FEV₁ more than 150 ml were accepted. In order to predict the lung function of students, their height and weight were also measured during spirometry.

2.4. Sampling the breath condensate exhaled

We assembled a manmade device, which consisted of a glass soxhlet extractor condenser, water flow pumps, thermometers, exhaled breath collection location and electrical power supply for sampling the exhaled breath condensate according to devise previously described by Sakhvidi et al. (2015) (Fig 2). In order to blow the exhaled air within the device a flexible tube was used. Ice water (with a temperature of zero degrees Celsius) that had been dumped in a 2-liter container was guided through the plastic tube condenser and again returned into the container by turning a spiral flow inside condenser, and cooling the inner chamber of condenser. Exhaled air reached the saturation conditions through the inner part of the condenser glass that was cooled by water flow, and then its moisture remained on the walls of the condenser. Produced drops on the inner surface of the condenser were guided into a micro-tube that was placed under the condenser and was kept in the micro-tube. To collect the exhaled breath samples initially all participants were asked to hold their nose by using a clamp, and then asked to blow into the mouthpiece with a



Figure 1 Map of sampling site $(A_1, A_2 \text{ and } A_3)$.

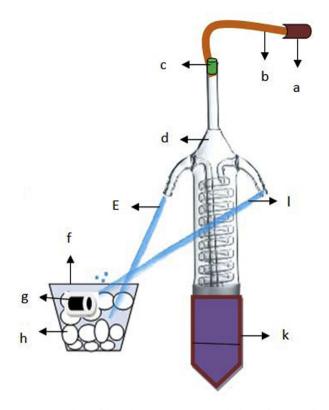


Figure 2 View of breath sampler: (a) mouthpiece, (b) polyetilen tube, (c) cap water, (d) condenser I, (e) water circulation tubes, (f) ice-water reservoir, (g) electrical pump, (h) ice, (k) sample collector.

fixed frequency for 10 min, which was separately embedded for each participant. After exposure to freezing temperatures, the exhaled air was condensed. Next, the condensed liquid was collected into micro-tubes. Also, the condenser was washed gently with normal saline, and then dried using a hair dryer. The collected samples were stored in a container of ice water. After that, they were kept in a freezer at -80 °C in a laboratory before testing.

2.5. Pollution monitoring sites

In A₁ and A₂ areas, the data of hourly average concentrations of air pollutants (PM_{2.5} and NO₂) and meteorological data were taken from the stations measuring air pollutants of AEPA for analyzing their correlation with lung function of students and 8-isoprostane. Due to the lack of measuring air pollution and lack of access to measuring devices in A3 site, the amount of PM2.5 was determined using a Grimm devices and NO2 was also measured using the diffusion method. Methods and devices in A3 were validated. In addition, in A1 and A2 sites the air pollutants were measured for five consecutive days with the same methods and devices. The obtained values were compared with the values reported by environment monitoring stations. These results showed that there was no statistically significant difference between them. The results of air pollutants collected from 7 a.m. to 6 p.m., 1-4 days prior to the sampling (lag1-4), were used for analyzing the impact of air pollution on lung function and 8-isoprostane marker. The selected sites and stations are shown in Fig. 1.

2.6. Statistical analysis

The mean and maximum amount of air pollutants on the day of spirometry testing (lag0) and 1–4 days before the test (lag1-4) were calculated and compared between three studied sites. Then, the results of the distribution of age, height, weight, 8isoprostane concentration and lung function tests including FVC and FEV₁ were compared between the three studied sites. In this study, multiple regression by using GEE statistical models was used to determine the correlation between lung function indices (FVC, FEV₁) and 8-isoprostane levels and air pollutants (0 day and 1–4 day). Statistical analyzes (GEE, multiple regression, ANOVA) were performed using SPSS software (version 22) and statistical significance for *P*values < 0.05 were considered.

2.7. 8-Isoprostane analysis

8-Isoprostane concentration was analyzed using Cayman Chemical 8-isoprostane enzyme immunoassay kit (Cayman Chemical Company, Ann Arbor, MI, USA). Marker concentrations in all samples were above the detection limit (2.7 pg/ml). In addition to the ELISA method, 10% of the samples were analyzed using GC/MS device, and then the accuracy and precision of ELISA method were also investigated (the difference between two studied methods was less than 2%) (De Prins et al., 2014).

3. Results and discussion

Table 1 shows the population characteristics in the present study. Totally 90 sixth-grade students were selected from three sites. The average and standard deviation of height, weight and age of the subjects between three sites were not significantly different.

3.1. The concentration of air pollutants

During the three-month period in this study, the mean daily concentrations of $PM_{2.5}$ and NO_2 in site 1 with a high volume and intensity of traffic were higher than in A_2 and A_3 sites (Table 2). $PM_{2.5}$ concentrations in A_1 , A_2 and A_3 sites were in the ranges 95–126 µg/m³, 83–90 and 39–51 µg/m³, respectively. Moreover, NO_2 concentration ranges in A_1 , A_2 and A_3 sites were 65–87, 43–57 and 9–18 µg/m³, respectively. Although there was no significant difference in the mean concentration of NO_2 and $PM_{2.5}$ between A_1 and A_2 sites, a significant difference was observed between the both sites and A_3 site.

3.2. Correlation between the 8-isoprostane concentrations in exhaled breath condensate and $PM_{2.5}$ and NO_2

8-Isoprostane as an oxidative stress biomarker is produced from the oxidation of phospholipids of cell membranes (Janssen, 2001). In this study, EBC was collected with the aim of sampling of exhaled breath originating from the respiratory system, which is the target organ of air pollutants. Totally, 270 condensate samples of exhaled air were taken from the participants. 8-Isoprostane concentration in three

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Table 1	Physical	characteristi	cs of schoo	I children.

	A_1	A_2	A_3	<i>P</i> -value	
Number of subjects	30	30	30	_	
Height (cm) mean \pm SD	143.7 ± 7.4	145.3 ± 8.1	144.1 ± 7.9	P = 0.79	
Weight (kg) Mean \pm SD	39.35 ± 9.2	41.3 ± 10.3	40.7 ± 9.8	P = 0.65	
Age (years) Mean ± SD	$11.9~\pm~0.8$	$12.3~\pm~0.5$	12.2 ± 0.6	P = 0.75	

Table 2Air pollutant concentration in studied areas.

	A ₁	A_2	A ₃
$\frac{\text{PM}_{2.5} \text{ (Mean } \pm \text{ SD)}}{\text{NO}_2 \text{ (Mean } \pm \text{ SD)}}$	$116 \pm 15 \ (\mu g/m^3)$ 77 ± 8 (ppb)	$92 \pm 11 \\ 53 \pm 5.5$	$\begin{array}{c} 45\ \pm\ 5\\ 14\ \pm\ 3\end{array}$

samples was lower than the detection limit (2.7 pg/ml). The mean 8-isoprostane concentration in EBC samples collected from children in A1, A2 and A3 were 49.8 (4.3-111), 41 (4.2-98) and 31 (4.5-51) pg/ml, respectively. The mean 8-isoprostane concentration in children of A₁ site was 21.5% higher than in A_2 site, and it was 60% higher than in A_3 site. Roberto Bono et al. in their study on the effect of traffic on oxidative stress indicator in Piedmont Region, North-Western Italy, showed that the oxidative stress indicator in people who lived in places of city with high traffic volume was higher than other places of the city. Also they reported that there was a significant difference in the level of oxidative stress indicator between people who lived in places with high traffic volume and other places (Bono et al., 2014). In the present study the concentration of air pollutants in A1 was higher than in other studied sites, and there was no significant difference in individual characteristics of the students. Therefore, the correlation between lung function indices (FEV1 and FVC) and PM_{2.5}, NO₂ and 8-isoprostane concentration was investigated in A_1 (Table 3).

An IQR increase $(13 \ \mu g \ m^{-3})$ in PM_{2.5} had a positive correlation with increasing 0.13 unit 8-isoprostane (95% CI: 0.04, 0.19) on the same day. Moreover, an increase in PM_{2.5} on 1–4 days before sampling was associated with 0.38 unit (95% CI: 0.11, 0.65) increase in 8-isoprostane concentration. All assessed lags (exposure to NO₂), was positively correlated with increasing 8-isoprostane concentrations, and the highest increase was attributed to the increase of the mean NO₂ concentration on 3 and 4 days (data not shown). An increase in NO₂ concentration (IQR = 6.5 ppb) was positively correlated with 0.61 unit (95% CI: 0.38, 0.79) increase in 8-isoprostane concentration on 1–4 days was positively correlated with 1.1 unit (95% CI: 0.85, 1.35) increase in 8-isoprostane concentrations.

In a similar study that was conducted by Patel et al. on the children the 8-isoprostane concentration in non-asthmatic was reported to be in the range 3.8-126 pg/ml with an average of 48.6 pg/ml. This result is consistent with our finding. However, there was no fixed correlation between PM2.5 concentration and 8-isoprostane in the mentioned study. This contradiction with our study may be due to their use of data obtained from center monitoring stations of air pollutants that have been far from the studied schools. Moreover, they reported a significant correlation between Carbon Black, NO2 concentrations and 8isoprostane. While in the mentioned study, due to the high correlation between black carbon and NO2, it was not possible to detect the effect of each of them alone, in the present study, a high correlation was also observed between 8-isoprostane and NO₂ concentrations (Patel et al., 2013). In another study on healthy young people conducted by Huang et al. it was reported that the increase in PM2.5 measured by central monitoring stations was correlated with increasing levels of 8isoprostane (Huang et al., 2016). Similar results were found in another study conducted by Antczak et al. (2012), the mean 8-isoprostane concentration was reported to be in the range 2.6-16 pg/ml (Antczak et al., 2012).

Some previous studies reported that the concentration of 8isoprostane in healthy people is lower than the limit of detection. In a study conducted by Yoda et al. (2014) in Tokyo, Japan on 21 healthy subjects showed that the 8-isoprostane concentration was less than the limit of detection. They also reported that this is due to the selection of healthy participants (Patel et al., 2013; Yoda et al., 2014). In another survey, 24 h mean and standard deviation of $PM_{2.5}$ and PM_{10} were 40.8 \pm 21 and 23.7 \pm 13.7 μ g/m³, respectively. There was no significant correlation between 8-isoprostane concentration and the mentioned pollutants (De Prins et al., 2014), while, in the present study, the mean $PM_{2.5}$ concentration was four times higher than the value in the mentioned studies. A high concentrations of 8-isoprostane has been reported for even healthy people by some previous studies.

3.3. Lung function parameters and PM_{2.5} and NO₂

For this purpose, 270 spirometry tests were conducted on the students of selected schools. The mean concentration of FVC in A_1 , A_2 and A_3 students was 2.79, 2.84 and 2.93 L, respec-

Table 3 Unit change in 8-isoprostane concentration, FVC and FEV_1 per interquartile-range (IQR^a) increases in pollutant concentration at various lags of exposure.

Pollutant	Time lag before spirometry	FVC(l)	FEV ₁ (l)	8-Isoprostane (pg/ml)
PM _{2.5}	Lag0 Lag1–4	$\begin{array}{c} -0.131(-0.19, -0.06) \\ -0.144(-0.21, -0.09) \end{array}$	$\begin{array}{c} -0.11(-0.17, \ -0.07) \\ -0.121(-0.18, \ -0.05) \end{array}$	0.13(0.04, 0.19) 0.38 (0.11, 0.65)
NO ₂	Lag0 Lag1–4	$\begin{array}{c} -0.03(-0.05, \ -0.01) \\ -0.12(-0.19, \ -0.06) \end{array}$	$\begin{array}{c} -0.04(-0.07, \ -0.01) \\ -0.19(-0.28, \ -0.08) \end{array}$	0.61 (0.38, 0.79) 1.1 (0.85, 1.35)

^a Interquartile ranges of pollutants are 13 μ g/m³ for PM_{2.5} and 6.5 ppb for NO₂.

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		Means	Lower limit	Upper limit	*P <
FVC (l)	Naderi	2.79	2.75	2.83	0.01
	Alavi	2.84	2.78	2.91	0.22
	Eine 2	2.93	2.84	3.1	
FEV ₁ (l)	Naderi	2.41	2.36	2.46	0.01
	Alavi	2.58	2.51	2.64	0.01
	Eine 2	2.64	2.55	2.73	

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omparing A_1 and A_2 , A_3 .

^b Comparing A₂ and A₃.

tively. The highest concentration of FVC was observed in A₃ students and its lowest was in A_1 site (Table 4). The FEV₁ in A₃ students was significantly higher than other sites. FEV₁ values were significantly different between the studied students of three studied sites (P < 0.01). Moreover, the concentration of NO₂ and PM_{2.5} in A₁ was higher than in A₂, and in A₂ it was higher than A₃. In a study on the people living in three regions of Kanpur, India (residential, commercial and high-traffic) that was conducted by Sharma et al., it was concluded that the lowest value of FVC and FEV1 were observed in the high-traffic region, and the highest values were attributed to the residential place, which had clean air compared to the other two sites. An IQR increase $(13 \,\mu\text{g/m}^3)$ in PM_{2.5} on the same day (lag0) was correlated with the decrease of FVC (131 ml) and FEV₁ (110 ml). Both FVC and FEV₁ were more likely to be associated with PM2.5 exposed 1-4 days before the spirometry test than in comparison to the 0-d exposure before the lung function test. Most longitudinal studies on school children have shown that the exposure to NO₂ or PM_{2.5} are attributed to the adverse effects on lung function (Gauderman et al., 2004; Rojas-Martinez et al., 2007). Also, in some investigations such as Chen et al. (2015) study, on the effects of particulate matter, it was reported that lung function was mainly related to sub-chronic exposure. They reported with $12 \,\mu g/m^3$ increase in PM_{2.5} concentration, both FVC and FEV₁ reduced by 4% (Chen et al., 2015). In the present study, the decrease in the pulmonary parameters was higher than the values in mentioned studies, which this difference may be due to the high concentration of PM_{2.5} and particle chemical characteristics in this study.

The increase of NO_2 in the sampling day (lag 0) was weak correlated with the FVC and FEV₁. These spirometry tests were negatively correlated with mean NO₂ concentration in four-day mean concentration before sampling (lag 1-4). In this regard, by increasing the mean NO₂ concentration to 6.5 ppb (IQR), the values of FVC and FEV_1 decreased by 12 and 19 mL.

The correlation between short exposure to NO₂ and reduced lung function was consistent with other previous studies. In a similar study, it was reported that short-term exposure to less than 1 ppm of NO₂ does not have any adverse effects on lungs of healthy people. However, they founded that the exposure to 0.2-0.6 ppm of NO₂ cause adverse health effects for sensitive populations (Hesterberg et al., 2009). In the present study, NO₂ concentration was lower than in the mentioned study, while spirometry tests significantly reduced. This difference may be due to the difference in the environmental conditions such as air temperature, in which the mean temperature of Ahvaz city was over 38 °C at the time of the study. This high temperature as a synergistic factor with air pollutants affects the respiratory system.

A cross-sectional study conducted by Rosenlund et al. (2006) on the effect of exposure to NO₂ on lung function changes in children between 9 and 14 years, showed that there was no significant correlation between exposure to pollutant and FVC and FEV₁ (Rosenlund et al., 2006), while, with the 6.5 ppb increase in NO₂ concentration in the present study, FVC and FEV₁ decreased by 12 and 19 mL, respectively. Some previous studies reported that the exposure to NO₂ causes a decrease in pulmonary function. For example, Chang et al. (2012) investigated the effects of air pollution on children's lungs and they reported that there is an inverse correlation between FVC and NO₂ concentration on one day before sampling. In another study, FEV1 and FVC of adolescents were significantly low after exposure to NO₂ (Rosenlund et al., 2006).

4. Conclusion

The results of this cross-sectional study showed that the pulmonary function parameters in people who lived in hightraffic areas were lower than those living in low-traffic areas, and oxidative stress indicator in high-traffic areas was higher than in low-traffic areas. According to the FVC and FEV_1 measurements, short-term exposure to PM2.5 and NO2 was significantly correlated with a decrease in pulmonary function and the increase of oxidative stress marker (8-isoprostane). In conclusion it was found that short-term changes in 8isoprostane concentration, EBC, FVC and FEV1 along with the increase of air pollutants and their exposure can be used in the pulmonary health assessment.

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