Glutathione (GSH) and superoxide dismutase (SOD) levels among junior high school students induced by indoor particulate matter 2.5 (PM$_{2.5}$) and nitrogen dioxide (NO$_2$) exposure

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Abstract

Background: Indoor air pollution has globally known as the risk factor of acute respiratory infection in young children. The exposure to indoor particulate matter 2.5 (PM$_{2.5}$) and nitrogen dioxide (NO$_2$) at house or school can be a potential risk to children’s health. This study aimed to examine the association between indoor PM$_{2.5}$ and NO$_2$ with oxidative stress markers in junior high school students.

Design and methods: This study was conducted using a cross sectional study with 75 students collected randomly from four junior high schools in Jakarta. PM$_{2.5}$ and NO$_2$ were measured in classrooms and school yards. The schools were categorized based on the exposure level of PM$_{2.5}$ and NO$_2$ in classrooms. Superoxide dismutase (SOD) and reduced glutathione (GSH) were examined from the blood sample. All students were interviewed with questionnaires to determine upper respiratory tract infection, smoking family members, mosquito repellent usage, and dietary supplement consumption.

Results: Mean concentration of indoor PM$_{2.5}$ and NO$_2$ were 0.125±0.036 mg m$^\text{-3}$ and 36.37±22.33 µg m$^\text{-3}$, respectively. The schools which located near to highway showed lower PM$_{2.5}$ and higher NO$_2$ level indicated the emission of traffic activity. Mean activity of SOD was 96.36±50.94 U mL$^{-1}$ and mean concentration of GSH was 0.62±0.09 µg mL$^{-1}$. Most of the students reported upper respiratory tract infection history, smoking family member, use mosquito repellent at home, and do not consume dietary supplement.

Conclusions: The level of oxidative stress markers and the exposure categories of classroom PM$_{2.5}$ and NO$_2$ was not significantly different, however there were significant correlation with cigarette smoke and mosquito repellent at home. Nevertheless, the exposure of indoor PM$_{2.5}$ and NO$_2$ increased the risk of the exposure to cigarette smoke and mosquito repellent at home. Further study on the air pollution at school and home is needed to affirm association towards student’s health and to design strategic control efforts.

Introduction

Indoor air pollution is one of the major health risk factors responsible for nearly 1.6 million excess deaths annually and about 3% of the global burden of disease.1 Outdoor air pollution strongly influences indoor air quality especially due to human activities such as traffic and industrial activities.2,3 Previous study reported that, based on the guideline of World Health Organization, most of the municipality of Jakarta province had poor air quality from 2011 to 2017 and was reported to be associated with respiratory infection among population in Jakarta.4,5 Traffic activity is one of the major sources of air pollutants such as particulate matters (PM$_{2.5}$) and nitrogen dioxide (NO$_2$) in Jakarta. Particulate matter with diameter of less than 2.5 µm or PM$_{2.5}$ is a major component of air pollution that can be inhaled up to systemic circulation.6,7 Meanwhile, NO$_2$ gas is mainly emitted from traffic activity that involves fuel burning and directly combines with the oxygen in the atmosphere.8,9 Vigorous development activities in Jakarta have increased air pollution source points such as highways or toll roads into adjacent school buildings, impacted to increase the exposure risk of PM$_{2.5}$ and NO$_2$ to students in school environment.2,10 School-age children are vulnerable to environmental exposure due to their immature immune and respiratory system. During school time,
this group spends most of their time six to eight hours at school.11,12 Several studies reported that high exposure of PM2.5 and NO2 in classroom were associated with respiratory problems in children such as asthma, decreased of lung function, and respiratory tract and lung inflammation.11,13-15 Several studies have reported that airborne air pollutants can induce oxidative stress resulted to increase the risk of respiratory problems.8,16 Inhaled air pollutants such as PM2.5 and NO2 initiate reactive oxygen species (ROS) production that induce inflammation response in lung resulting oxidative stress indicated by depletion of specific antioxidants or elevation of antioxidant activity such as superoxide dismutase (SOD).8,16 Superoxide dismutase (SOD) plays as an enzymatic antioxidant to catalyze the dismutation of superoxide anion radical to molecular oxygen and harmless hydrogen peroxide which is then to be scavenged by glutathione (GSH) through enzymatic reactions. GSH is the most important hydrophilic antioxidant that protects cells against free radicals. Disorder of antioxidants level has been implicated in the etiology or development of various diseases.6,17 However, study on oxidative stress effects to the inflammation of respiratory tract on school-age children in Jakarta has not well understood, particularly the production of SOD and GSH. Thus, a study on antioxidant levels of SOD and GSH was suggested to early determination on the health risk of PM2.5 and NO2 exposure towards students in Jakarta.

Design and methods

This study was conducted from December 2010 to January 2020 in Jakarta Special Region, Indonesia, using a cross-sectional design. Four junior high schools were selected randomly from four municipalities in Jakarta. Parental and student informed consents were distributed randomly to 200 first grade of junior high school students living around five kilometers from the school. Students who were willing and permitted to participate from their parents underwent examination by medical doctors to fulfill the inclusion and exclusion criteria. This research excluded students with the history of chronic respiratory disease and who were experiencing symptoms of health problems on the day of blood sampling.

Data collection

Interview was conducted to collect demographic and health information. We conducted structural interview to 75 students using standard questionnaires adapted from a set of validated questionnaires by International Study of Asthma and Allergies in childhood (ISAAC) and food frequency questionnaires. The questionnaire was used to determine history of upper respiratory tract infection, tobacco smoke exposure at home, mosquito repellent usage, and dietary supplement intake were also compared using Mann Whitney test, with p-value <0.05 considered statistically significant. The mean values of SOD and GSH between exposure categories were compared using t-test and Mann Whitney test, with p-value <0.05 considered statistically significant. The mean of SOD and GSH between exposure categories was also compared using t-test and Mann Whitney test.

Indoor air samples

We collected ambient air samples at each school according to the standardized Griess-Saltzman method by National Standardization Agency of Indonesia to measure absorbed NO2.21 In addition, we also measured the classroom’s physical conditions (i.e., temperature and humidity) and observed the classroom condition. Each school was assessed in different week due to school permis, academic activities, and availability of the instruments.

Superoxide dismutase (SOD) and glutathione (GSH) level in blood

Blood samples were collected from the students who have agreed to the term and conditions of the study. Around 5 ml blood samples were collected using sterile syringe by experienced para-medic. Blood samples were then centrifuged to separate plasma and stored into vial. Samples were then kept in refrigerator at 4°C. SOD enzyme concentration was assayed by using RanSOD Kit and read by RX-Monza. This measurement aimed to get the dismutation of the toxic superoxide anion to hydrogen peroxide and oxygen. Xanthine and Xanthine oxidase were used to generate superoxide anion radicals. It reacts with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T) to create a red fomazan dye. SOD activity was measured by inhibition degree of the reaction22.

GSH was measured using spectrophotometric method. GSH was oxidized by 5,5’-dithiobis-(2-nitrobenzoic acid) (DTNB) resulting in the formation of oxidized glutathione (GSSG) and 5-thio-2-nitrobenzoic acid (TNB). GSSG then was reduced to GSH by glutathione reductase (GR) using reducing equivalent provided by NADPH. The rate of TNB formation was proportional to the sum of GSH and GSSG presented in the sample and was determined by measuring the formation of TNB at 412 nm.23 The analysis was done in Biochemistry Laboratory, Faculty of Medicine, Universitas Indonesia.

Statistical analysis

All data in this study were analyzed using statistical analysis software package. The Skewness-Kurtosis method was used to test data normality. Students’ demographic data, nutrition status, upper respiratory tract infection history, home tobacco smoke exposure, mosquito repellent usage, and dietary supplement intake were analyzed descriptively. Schools were categorized into highly exposed and low exposed categories based on mean levels of indoor PM2.5 and NO2. To evaluate association between the exposure and oxidative stress marker, the mean values of SOD and GSH between exposure categories were compared using t-test and Mann Whitney test, with p-value <0.05 considered statistically significant. The mean of SOD and GSH between the categories of students’ demographic, nutrition status, upper respiratory tract infection history, home tobacco smoke exposure, mosquito repellent usage, and dietary supplement intake were also compared using t-test and Mann Whitney test.

Results

Subjects and location characterization

All corresponding schools in this study open in five school days per week at 6:30 am to 2:15 pm. Besides that, extracurricular activities were also performed until around 4:00 pm. Two of the four schools (school A and D) were located next to the highway, while school B was located about 100 m from the main highway, and school C was in a residential area (Table 1). All classrooms were floored without carpets and were naturally ventilated with additional fans. All classrooms were furnished with wooden desks,
Exposure of PM$_{2.5}$ and NO$_2$

The mean PM$_{2.5}$ concentrations in four schools were 0.125 mg/m$^2$ in classroom and 0.090 mg/m$^2$ in school yard (Table 3). School C (residential area) had the highest PM$_{2.5}$ concentration for both inside and outside of the classroom. All schools have higher PM$_{2.5}$ concentrations inside the classroom compared to the yard. Based on the mean PM$_{2.5}$ concentrations, school B and C were categorized as high PM$_{2.5}$/low NO$_2$.

The mean concentrations of NO$_2$ were 36.37 mg/m$^3$ and 19.24 mg/m$^3$ in classroom and school yard, respectively. School A has the highest NO$_2$ concentration (62.54 mg/m$^3$) in classroom. All schools, except for School D, showed higher NO$_2$ concentrations in classroom compared to school yard. Meanwhile, NO$_2$ concentration in School D showed no difference concentration for both inside classroom and in school yard. School D had higher NO$_2$ concentration than the other schools. Based on the mean concentrations, school A and C were highly exposed to NO$_2$ which then

### Table 1. School characteristic.

| School   | Location                     | Sampling sites                                      | Classroom shape | Classroom area (m$^2$) | Number of students/class | Environment condition |
|----------|------------------------------|-----------------------------------------------------|-----------------|------------------------|-------------------------|----------------------|
| A        | Pasar Baru, Central Jakarta  | 1st floor classroom (1) and school yard (1)        | Rectangle       | 62.01                  | 32                      | City center, business and office areas, next to main highway, near the intersection of red lights and the train station. |
| B        | Lubang Buaya, East Jakarta   | 3rd floor classroom (1) and school yard (1)        | Rectangle       | 64.8                   | 36                      | Suburban, office and business areas, 100 m from the main highway and red light intersections. |
| C        | Cipedak, South Jakarta       | 3rd floor classroom (1) and school yard (1)        | Rectangle       | 64.74                  | 36                      | Suburban, residential areas, and next to golf courses. |
| D        | Kebon Jeruk, West Jakarta    | 3rd floor classroom (1) and school yard (1)        | Rectangle       | 56.15                  | 36                      | Suburban, business and residential areas, next to main highway, and the intersection of red lights. |

### Table 2. Subject characteristic.

| Characteristic                                                                 | Description |
|-------------------------------------------------------------------------------|-------------|
| Age (year)                                                                    | 13.06±0.48* |
| Sex (n, %)                                                                    |             |
| Female                                                                       | 58          |
| Male                                                                         | 17          |
| Nutrition status (n, %)                                                      |             |
| Thin                                                                          | 3           |
| Normal                                                                       | 48          |
| Fat                                                                          | 24          |
| Upper respiratory tract infection history (last 6 months) (n, %)              |             |
| Yes                                                                           | 57          |
| No                                                                            | 18          |
| Smoking family member (n, %)                                                 |             |
| Yes                                                                           | 43          |
| No                                                                            | 32          |
| Dietary supplement consumption (at least once per week in last month) (n, %) |             |
| Yes                                                                           | 32          |
| No                                                                            | 43          |
| Mosquito repellent usage (n, %)                                              |             |
| Yes                                                                           | 42          |
| No                                                                            | 33          |
| SOD (U mL$^{-1}$)                                                            |             |
| Mean±SD                                                                       | 96.36±50.94 |
| Median                                                                       | 84.97#      |
| GSH (µg mL$^{-1}$)                                                           |             |
| Mean±SD                                                                       | 0.62±0.09*  |
| Median                                                                       | 0.63        |

*Normal distribution; #skewed distribution.
categorized as high NO2/low PM2.5, PM2.5, and NO2 measurements were carried out in the rainy season with clear or cloudy weather patterns in the morning until noon and cloudy or rainy in the afternoon. All classrooms have higher humidity levels and lower temperatures compared to school yard. PM2.5 and NO2 concentrations were also found to be higher in the classroom than in school yard.

**Oxidative stress marker**

The mean SOD activity from all samples was 96.36 U mL⁻¹ and the mean concentration of plasma GSH was 0.62 µg mL⁻¹ (Table 4). Based on our statistical analysis, there was no significant difference of GSH and SOD levels between high PM2.5 schools and high NO2 schools. However, schools with high NO2 exposure in the classroom had lower levels of GSH and higher SOD activity (Table 5). Meanwhile, mean SOD activity and GSH concentration between sex (p=0.889; 0.819), nutritional status (p=0.368; 0.612), history of upper respiratory tract infection (p=300; 0.190), and dietary supplement consumption (p=0.213; 0.617), were not statistically different. However, there was a statistically significant difference of mean SOD activity (p=0.001) and mean GSH concentration (p=0.036) between cigarette exposure and non-cigarette exposure at home. The usage of mosquito repellent resulted in a significant difference in GSH concentration (p=0.035).

**Discussion**

**Airborne particulate matter and nitrogen dioxide**

The concentration of PM2.5 in four schools was similar to a previous study conducted in schools in Depok, Indonesia and several studies in India.18,24,25 Several other studies conducted in Germany, Italy, Portugal, and Malaysia reported that PM2.5 concentrations that exceeded WHO guideline values, but still lower than our current study.11,12,26,2. According to the previous studies, high PM2.5 levels in the classroom might be caused by poor ventilation, poor classroom hygiene, occupant activities, and other outdoor sources.11,26,28 However, we did not make further observation for these factors in this study. In contrast to the studies in Germany and Malaysia, this study found that schools located in suburban settlements (far from the highway or industry) had the highest PM2.5 concentrations.26,29 Another study conducted in kindergarten in Portugal reported a similar results with this study.11,12 Higher PM2.5 concentrations in a residential area might be caused by public activities such as biomass burning and cooking, open yards or land without cement layering, and surrounding activities such as traffic and industry.30,31

The PM2.5 concentration in this study showed that rainy season did not influence the air quality in the school environment. Studies in tropical and subtropical countries reported a decreasing pattern of outdoor particulate levels which related to the increase of humidity and rainfall during rainy season. Indoor particulate level can increase during the rainy season due to student’s activity in the classroom and poor ventilation openings.11,25,26,28,30

The mean NO2 concentration in four schools did not exceed the threshold values set by the Indonesian government for one hour (400 µg m⁻³, 1 hour) and WHO (200 µg m⁻³, 1 hour). A similar study by Wispriyono et al.10 on indoor air pollution in Jakarta reported lower indoor and outdoor NO2 than our results. Other studies conducted in the United States and Brazil measured NO2 in the school environment reported there was association of NO2 in air and respiratory disorders.15,32,33 Whereas studies in India and Australia reported higher NO2 and correlated with respiratory disorders, even though they did not exceeded the WHO threshold

| Parameter | School A | School B | School C | School D | Mean ± SD | School A | School B | School C | School D | Mean ± SD |
|-----------|----------|----------|----------|----------|-----------|----------|----------|----------|----------|-----------|
| **PM2.5 (mg m⁻³)** | 0.095 | 0.128 | 0.175 | 0.103 | 0.092 | 0.109 | 0.095 | 0.065 | 0.125±0.036 | 0.090±0.018 |
| **NO2 (µg m⁻³)** | 62.54 | 24.5 | 12.37 | 46.05 | 23.72 | 4.33 | 2.87 | 46.05 | 36.37±22.33 | 19.24±20.24 |
| **Humidity (%)** | 73.5 | 71.43 | 77.03 | 76.77 | 69.53 | 56.83 | 68.77 | 72.73 | 75.24±6.85 | 68.72±7.21 |
| **Temperature (°C)** | 31.57 | 31.00 | 30.37 | 30.63 | 32.23 | 37.10 | 34.87 | 32.17 | 30.74±0.57 | 33.36±2.62 |

**Table 3. Level of pollutants in schools.**

| Antioxidant | School A (n=21) | School B (n=23) | School C (n=16) | School D (n=15) | All schools (n=75) |
|-------------|-----------------|-----------------|-----------------|-----------------|-------------------|
| **SOD (U/ml)** | Mean ± SD | Median | CI 95% | Min-Max | Mean ± SD | Median | CI 95% | Min-Max | Mean ± SD | Median | CI 95% | Min-Max |
| GSH (µg/ml) | Mean ± SD | Median | CI 95% | Min-Max | Mean ± SD | Median | CI 95% | Min-Max | Mean ± SD | Median | CI 95% | Min-Max |

*Normal distribution; #skewed distribution.
value.\textsuperscript{25,34} 

The existence of NO\textsubscript{2} in ambient air was specifically related to outdoor air pollutants sources such as traffic activities, thus related to the increase of NO\textsubscript{2} concentration in classroom and yard in school A and D. This results indicated that outdoor air pollutants affected the indoor air quality in the classroom.\textsuperscript{25,33} Beside that, the lowest NO\textsubscript{2} concentration was detected in school C (suburban settlement) indicated that buildings which located far from highway or main road received less NO\textsubscript{2} pollution. Previous studies also suggested that NO\textsubscript{2} levels was relatively lower in the rainy season and increase during the dry season.\textsuperscript{25}

### SOD and GSH levels

At the time of data collection, subjects had just enrolled in the school for six months, thus these results represent the exposure of the last six months before data collection. The median SOD activity in this study was higher than a study conducted among healthy, asthma, and down syndrome children, but was similar to a study among workers in the ready-mix concrete factory exposed to PM\textsubscript{2.5}.\textsuperscript{35,36} The production of SOD activity occurred in response to the increase of NO\textsubscript{2} concentration in classroom and yard in school A and D. This results indicated that outdoor air pollutants affected the indoor air quality in the classroom.\textsuperscript{25,33} Beside that, the lowest NO\textsubscript{2} concentration was detected in school C (suburban settlement) indicated that buildings which located far from highway or main road received less NO\textsubscript{2} pollution. Previous studies also suggested that NO\textsubscript{2} levels was relatively lower in the rainy season and increase during the dry season.\textsuperscript{25}

### Table 5. Oxidative stress marker association to various variables.

| Variable                                      | SOD (U ml\textsuperscript{-1}) | Antioxidants level | n=75 | Mean±SD/Median (IQR) | p-value | GSH (µg ml\textsuperscript{-1}) | n=5 | Mean±SD/Median (IQR) | p-value |
|-----------------------------------------------|---------------------------------|--------------------|------|----------------------|---------|---------------------------------|------|----------------------|---------|
| PM\textsubscript{2.5} and NO\textsubscript{2} exposure |
| High PM\textsubscript{2.5}/low NO\textsubscript{2} | 39 | 83.03(69.49) | 0.714 | 39 | 0.63±0.06 | 0.197 |
| Low PM\textsubscript{2.5}/high NO\textsubscript{2} | 36 | 89.54(51.42) | | 36 | 0.60±0.12 | |
| Sex                                           |                                |                    | 58   | 83.44(60.10) | 0.889   | 58 | 83.44(60.10) | 0.889   | 58 | 83.44(60.10) | 0.889   |
| Male                                          | 17 | 93.92(60.17) | 17 | 93.92(60.17) | 0.62±0.09 | 0.819 |
| Female                                        |                               |                    | 58   | 83.44(60.10) | 0.889   | 58 | 83.44(60.10) | 0.889   | 58 | 83.44(60.10) | 0.889   |
| Nutrition status (BMI age)                    |                                |                    | 27   | 83.03(53.35) | 0.368   | 27 | 83.03(53.35) | 0.368   | 27 | 83.03(53.35) | 0.368   |
| Normal                                        | 48 | 88.23(63.83) | 48 | 88.23(63.83) | 0.61±0.10 | 0.662 |
| Abnormal (thin and fat)                       |                                |                    | 27   | 83.03(53.35) | 0.368   | 27 | 83.03(53.35) | 0.368   | 27 | 83.03(53.35) | 0.368   |
| Upper respiratory tract infection history (last 6 months) |
| Yes                                           | 57 | 80.29(59.09) | 57 | 80.29(59.09) | 0.61±0.10 | 0.190 |
| No                                            | 18 | 104.25(64.57) | 18 | 104.25(64.57) | 0.65±0.09 | |
| Smoking family member                          |                                |                    | 43   | 110.71(62.96) | 0.001   | 43 | 110.71(62.96) | 0.001   | 43 | 110.71(62.96) | 0.001   |
| No                                            | 32 | 68.99(36.44) | 32 | 68.99(36.44) | 0.59±0.10 | 0.036 |
| Yes                                           | 32 | 92.93(43.94) | 32 | 92.93(43.94) | 0.60±0.10 | 0.617 |
| No                                            | 43 | 98.59(55.97) | 43 | 98.59(55.97) | 0.60±0.10 | 0.617 |
| Dietary supplement consumption (at least once per week last month) |
| Yes                                           | 32 | 89.82(53.44) | 32 | 89.82(53.44) | 0.60±0.10 | 0.005 |
| No                                            | 33 | 84.87(73.19) | 33 | 84.87(73.19) | 0.64±0.08 | 0.005 |

**Limitations**

This study did not record the number of daily cigarettes and periods of smoking among family members so the frequency of exposure could not be further explained specifically.
Conclusion

The concentration of PM2.5 and NO2 showed higher level in the schools located near to highway or main road. The relation between PM2.5 and NO2 exposure with antioxidant level showed no significant correlation. However, there was a significant difference between SOD and GSH concentration with the students who were exposed to cigarette smoke and mosquito repellent. The upper respiratory tract infection history in students suggested that students had been exposed to poor air quality in both school and house. Further study should be improved to measure other airborne pollutants which related to the increase of oxidative stress by determining other potential factors.

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Contributions: The study presented here was carried out in collaboration between all authors. All authors prepared the study design and protocols; BW, HK, JJ, SPa, interpreted and drafted the manuscript; BW, JJ, SPa, SPR, NE, FF, conducted data collection and analysis; RN, SPa, GP, edited and translated the manuscript. All authors have read and agreed to the manuscript submission.

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Data availability: The data used to support the study are available from the corresponding author upon request.

Ethical approval and consent to participate: This study has been reviewed and passed the ethical clearance from Faculty of Public Health Universitas Indonesia with the reference number of 665/UN2.F10.PPM.00.02/2019.

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