NITROGEN DIOXIDE CONCENTRATIONS IN THE AIR IN INCREASING SUPEROXIDE DISMUTASE ENZYME AND MALONDIALDEHYDE SERUM LEVELS IN BUS TERMINAL OFFICERS

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Abstract

Introduction: Free radicals in the air such as NO\textsubscript{2}, O\textsubscript{3}, PM\textsubscript{2.5} induce the production of reactive oxygen species (ROS) in the body and stimulate the production of superoxide dismutase (SOD) enzyme. Imbalanced levels of free radicals and antioxidants will trigger the production of malondialdehyde (MDA). This study aimed to analyze the correlation between NO\textsubscript{2} concentrations in the air with SOD and MDA levels in bus terminal officers. Methods: This study was an analytical observational and used a cross-sectional design. The population included terminal operational officers and desk administrative officers in the bus terminal. A number of 12 people was chosen as a sample calculated with averaging formula between case and control. The measurement of NO\textsubscript{2} concentrations in the ambient air was performed using the Griess Saltzman method. The ELISA method was utilized to analyze the levels of the SOD enzyme, and the TBARS method was used to analyze the level of MDA serum. A statistical test was conducted using a Mann-Whitney test and Independent-2 Sample T-test. Results and Discussion: The average of NO\textsubscript{2} concentrations in the ambient air showed 106.5 µg/Nm\textsuperscript{3} and in the administration room at 17.8 µg/Nm\textsuperscript{3}. There was a very significantly higher exposure to NO\textsubscript{2} in the air towards the level of SOD enzyme (p < 0.01) and to the level of MDA serum (p < 0.01). Conclusion: The high level of exposure to NO\textsubscript{2} in the ambient air will increase SOD enzyme and MDA serum production in the body. It is recommended that the officers consume more fruit and vegetables containing vitamins C and E to fulfill the daily nutritional intake for antioxidants, and for the operational officers, always use a mask.
INTRODUCTION

Vehicle density indicates high levels of air pollution due to vehicle emissions. One of the vehicle emissions is nitrogen dioxide (NO₂) that is brownish to its appearance. The importance of monitoring NO₂ levels in the ambient air must be a concern as the negative impacts of NO₂ are dangerous to human health and environmental sustainability. The NO₂ concentration monitoring in ambient air using Citra Satellite in Indonesia showed an increase up to 18.49% and in a temperature to 0.7% (1).

A terminal is a location with a high level of pollution. The Purabaya Terminal is a type A bus terminal in East Java and has 120,000 passengers daily, and around 1,000 buses are operating every day for 24 hours. With that said, the Purabaya Terminal is the busiest in East Indonesia. This terminal, located in Sidoarjo District and managed by the Surabaya City Government, becomes the entry point to Surabaya and potentially an icon or landmark in Indonesia, especially the middle and eastern parts. Based on the previous studies, the average level of NO₂ at 188.5 µg/m³ in the Departure Gate of Purabaya Terminal has exceeded the threshold limit value. The concentration of NO₂ in the ambient air is influenced not only by buses but also other the motor vehicle (2). It then becomes the basis for the researchers to determine research locations.

Nitrogen oxide (NOₓ) is one of the primary pollutants in urban areas (3). NOₓ is very reactive and plays a role in the chemistry pollutants and being a free radical as NO (oxide nitrate radical) (4). The NOₓ concentrations in the ambient air are processing from accumulation, dispersion, transformation, and elimination. Generally, the concentrations of NOₓ vary and react quickly with photochemistry in urban areas entirely in a day and remaining days (5). NOₓ acts as a precursor of several poisonous pollutants. The reaction between NOₓ and O₂ will be producing NO₂ (6).

NO₂ is a free radical exposed into the human body through the respiratory tract system. NO₂ will yield reactive oxygen species (ROS) in the cells. ROS is an oxygen compound that has one non-paired electron and thus is reactive. In a high concentration of ROS in the body, the ROS will be reactive with non-radical compounds and will trigger a chain reaction, e.g., peroxy for fats (7).

Regarding the potency of being intoxicated by free radical compounds, the body has a natural mechanism of the immune system by producing an antioxidant enzyme that functions to neutralize and accelerate the degradation of free radicals in preventing the disruption of macromolecules components. Superoxide dismutase (SOD) cell is one of the endogen antioxidants which functions to catalyze the dismutation of superoxide ion (O₂⁻) into hydrogen peroxide (H₂O₂) and oxygen molecules. The imbalance between antioxidants and free radicals will cause oxidative stress (4,8). The antioxidant enzyme activity increases when oxidative stress occurs (9).

The United States Environmental Protection Agency (USEPA) sets the threshold limits value of NO₂ concentrations in the ambient air. It is at 53 ppb in one year and 100 ppb in one-hour concentration exposed. Indonesia sets the threshold limits value of NO₂ at 203.77 ppb on average of an hour concentration; 76.41 ppb in 24-hour concentration; and 50.94 ppb in one-year concentration exposed (5). The environmental quality standards by The Government Regulation No 41 Years 1999 for the NO₂ in Indonesia is at 400 ug/Nm³ in one-hour concentration; 400 ug/Nm³ in 24-hour concentration; and 100 400 ug/Nm³ in one-year concentration.

Due to NO₂ concentrations, reactive oxygen compounds may harm the body molecules such as protein, carbohydrate, and DNA. Due to the possible damage to the biological system, the oxygen compounds with radical properties are considered factors for aging and chronic diseases (8) and disrupt the body’s immune system (10). An antioxidant can also oxidate toxic chemicals and help prevent oxidative harm to protein, lipids, and DNA (9). The management of free radicals in maintaining redox homeostasis is important to keep the organism’s physiological health (11).

Antioxidant responses can be in the form of enzymatic responses (SOD, GPx, and catalase) and non-enzymatic ones (glutathione, vitamins C and E, carotenoids, lipoic acid, transferrin, and polyphenols) (7). The mechanism of oxidative cell damage occurs in the following steps. The number of free radicals declines due to the collaboration of three main antioxidant enzymes: SOD, catalase, and GPx. The hydroxyl radicals as part of hydroperoxide may cause oxidative cell damage: DNA damage, carboxylation of protein, and peroxy lipids, including lipids from the mitochondria cells (12-13). Reactive product responses may cause DNA damage due to other modified molecules induced by the ROS, e.g., lipids. The peroxy lipids can be used as a biomarker of oxidative stress and function to mark potentials in assessing the severity of diseases vulnerable to inflammation cancer (8).

Oxidative stress occurs if the ROS is not neutralized enough by an antioxidant. The value of superoxide dismutase (SOD) is an indicator of antioxidant status, while the value of malondialdehyde (MDA) is an
indicator of peroxide lipids. The ratio of MDA can be used as an indicator of oxidative stress, which is measure from the serum. If the value of MDA inclines, oxidative stress will be high as well (14). A biomarker of oxidative stress is relevant in evaluating a disease status and antioxidant effects to improve health (8,15).

Bus terminal officers on the morning shift are the group who is the most highly exposed to air pollution. Therefore, it is required to conduct biomonitoring on personal activities and the workplace environment. Continuous oxidative damage will affect the cell, tissue, and body organ system damage resulting in degenerative diseases and deaths. This study aimed to analyze the effect and differences in NO\textsubscript{2} concentrations in the ambient air on the level of superoxide dismutase (SOD) enzyme and malondialdehyde (MDA) serum in bus terminal officers.

**METHODS**

This study was observational analytic and used a cross-sectional design. The research population was terminal operational officers as the exposed group and desk administrative officers as the non-exposed group who shifted in the morning for eight working hours. The samples were selected through random sampling. The inclusion criteria were male officers who worked for more than two years and did not suffer any degenerative diseases. The sample size was determined by using the average formula. It obtained 12 male operational officers and desk administrative staff, respectively. The average value was obtained from the average value of biochemical change due to accumulation in free radicals and was referred to previous studies where the control MDA level in village police was about 3.34±0.81 and the case MDA level in urban police was about 4.28±0.77 (16). The values were calculated from the adjusted standard deviation for α two-way test (Z\textsubscript{a}=1.96), the adjusted standard deviation for β two-way test (Z\textsubscript{β} =0.84), the standard deviation of the control group (σ=0.81) with a formula

\[ n = \frac{2 \sigma^2 (Z_a + Z_b)^2}{(\mu_1 - \mu_2)^2} \]

The measurement of NO\textsubscript{2} in the air was carried out using the Griess Saltzman and a spectrophotometer at 550 nm of wavelength. It was conducted in three locations, i.e., the administration room (control), the arrival and departure gates, which each location would be taken three times in the morning, noon, and afternoon. The measurement of MDA level was performed using the Thiobarbituric Acid Reactive Substance (TBARS) and a spectrophotometer with a λ-value of 532 nm. The measurement of the SOD enzyme was done with the Enzyme-Linked Immunosorbent Assay (ELISA) and Human Superoxide Dismutase (SOD) ELISA Kit E0918Hu. A statistical test was performed using Independent-Sample 2 T-test and Mann-Whitney Test.

**RESULTS**

The results of the NO\textsubscript{2} measurement at the Surabaya Bus Terminal in Table 1 showed the average NO\textsubscript{2} concentration was at 106.5 µg/Nm\textsuperscript{3}. The SOD enzyme and MDA serum levels had significantly different between terminal operational officers and desk administrative staff. Figures 2 and 3 showed the correlation between NO\textsubscript{2} concentrations on the increase of SOD and MDA levels. Furthermore, it would be inducing the production of ROS and causing oxidative stress, and finally yielding MDA serum.

| Sampling Locations          | Measurement Time | Ambient Air (Outdoor) | Administration Room (Indoor) |
|----------------------------|------------------|-----------------------|-------------------------------|
|                            |                  | NO\textsubscript{2}  | Temperature (°C) | RH (%) | NO\textsubscript{2}  | Temperature (°C) | RH (%) |
| Arrival gate                | 07.30            | 53.3                  | 35.8             | 48     | 17.8                  | 29.0              | 68     |
|                            | 12.00            | 51.3                  | 33.5             | 62     | 19.0                  | 29.8              | 68     |
|                            | 16.00            | 104.8                 | 32.0             | 62     | 16.7                  | 29.0              | 65     |
| Departure gate for local trips | 07.30        | 66.8                  | 36.4             | 48     | 17.8                  | 29.0              | 68     |
|                            | 12.00            | 232.2                 | 33.2             | 61     | 19.0                  | 29.8              | 68     |
|                            | 16.00            | 78.1                  | 30.3             | 64     | 16.7                  | 29.0              | 65     |
| Departure gate for interlocal trips | 07.30     | 41.3                  | 33.4             | 50     | 17.8                  | 29.0              | 67     |
|                            | 12.00            | 252.9                 | 33.0             | 61     | 17.8                  | 29.0              | 65     |
|                            | 16.00            | 77.8                  | 30.0             | 65     | 17.8                  | 29.0              | 67     |
| Mean ±SD                   | 106.5±79.5       | 33.0±2.1              | 57.8±7.0         | 17.8±1.1 | 29.4±0.4             | 67.0±1.7          |
The results showed that the outdoor NO₂ concentrations were extremely high compared to indoor. The air pollution has resulted from the fuel burning from the gasoline and diesel vehicle around the terminal. There was high traffic of buses departing at noon and buses arriving in the afternoon. Besides, temperature and humidity greatly influence improving NO₂ concentrations in the air (6). The temperature around the working area of the terminal operational officers was about 33°C, which resulted in the average concentrations of NO₂ at 106.5%. It indicated that the high NO₂ concentrations would be proportionally comparable to temperature and humidity due to photochemistry effects (6). The chemical reaction between peroxy radical with NO would produce NO₂, and it stays in the atmosphere for a half time (5,17).

Our findings showed that the NO₂ level administration room tended to be lower, and there was less possibility for the pollutant to enter the room. The room was designed with an exhausted system and completed with air conditioning to control the temperature and humidity. Besides, the door was opened and closed regularly. The bus operational officers who worked in the morning were more likely exposed to NO₂ than those who shifted in the evening and desk administrative officers. Furthermore, the SOD enzyme levels significantly differed between the exposed and the non-exposed groups (ρ < 0.01). The exposed group was higher SOD enzyme levels with the mean of 0.52 U/ml than the non-exposed group with the mean of 0.15 U/ml at the NO₂ exposure level of 17.8 ppm. Our result inlined with the study in Sidoarjo showed a difference SOD enzyme level in the exposed group with the mean of 17.92 U/ml and non-exposed group with the mean of 2 U/ml in the NO₂ exposure level of 0.1454 ppm (17).

Due to the exposure to NO₂, the higher chemicals level in the forms of gas and particles may lead the air pollution to cause oxidative stress and inflammation (18-19). During this condition, the body’s response to the free radical pollution will produce ROS and RNS, which harm the proteins, lipids, and DNA. There is a balance between ROS production endogen antioxidant levels in the cells (20). In a normal condition, the concentration of ROS/RNS is minimized through the antioxidant activity in either the cells or lung epithelial fluid (21). The ROS impede the respiratory system. This fact is fascinating to understanding the global epidemic, e.g., obesity, since there is a possibility of inadequate antioxidant nutrition intake or toxic and xenobiotic, producing ROS in the adipocyte mitochondria (22-24).

The metabolism process occurs either in the erythrocyte or other body cells and produces some
strong oxidants. The results in the oxygen metabolism are the outcome of the reduction process of one electron. Reactive oxygen species (ROS) consist of superoxide \( \text{O}_2^- \), hydroxyl (\( \text{OH} \)), hydrogen peroxide (\( \text{H}_2\text{O}_2 \)), and peroxyl radical (\( \text{RCOO}^- \)). A large amount of ROS will be continuously produced in the cells through the metabolic pathway. The production in the normal biological process occurs due to many stimulations such as exposure to radiation, highly partial oxygen pressure (pO\textsubscript{2}), exposure to certain chemicals; thus, either an infection or inflammation may occur. The ROS in a stable state can survive in milliseconds \( (10^{-6}-10^{-12}) \) before reacting with other molecules (13,25). Both superoxide and NO are reactive and easily interact with the series of other ROS and RNS (23). In the cells, many redox signaling pathways include effects or molecules (e.g., kinase and transcription factor) that are negatively regulated by a protein sensor with thiolate ions in a normal condition (24).

The Independent Sample Test showed a significant difference in the MDA serum level in the exposed and non-exposed groups \( (p \leq 0.05) \). The MDA level in the exposed group was higher at the mean of 9.44 nmol/ml, while in the non-exposed group at the mean of 5.60 nmol/ml. Another study showed the discovery of exposure to NO\textsubscript{2} could increase the MDA serum level (26). The officers exposed more to NO\textsubscript{2} will have ROS and finally high levels of MDA serum. Besides, the high levels of MDA serum in the officers may be caused by smoking habits, which exposes them to cigarette chemicals, obesity, disease infection, and lack of nutrition (27).

The ROS are usually produced inside the mitochondria membrane through the electron transport chain in the mitochondria. In the mitochondria, the electron transport from the Krebs cycle to \( \text{FADH}_2 \) or NADH is regulated by four complexes to catalyze the reduction of oxygen to be water as caused by the four single electrons as a result of transport reaction. The electrons can reduce molecules that decline oxygen or other electron acceptors and also produce free radicals. The electron transport in the mitochondria reduces 95% \( \text{O}_2 \) by reducing tetravalent compounds to be \( \text{H}_2\text{O} \) without the intermediation of free radicals. The reduction of 5% remaining oxygen through the univalent pathway may produce free radicals such as superoxide, which becomes dismutase transforming to hydrogen peroxide due to superoxide dismutase (SOD), which can regenerate the ROS (28).

Naturally, the human body can avert free radicals with antioxidant defense from the body such as GPx (glutathione peroxidase), catalase, and the activity of superoxide dismutase (SOD) (29). SOD is the first defense that needs to be passed if the peroxide lipids are excessive in the body. The activity of SOD depends on the mineral metals such as Mangan (Mn), Zinc (Zn), and Copper (Cu) to work optimally (30).

Conversely, the antioxidant enzymes such as SOD, CAT, and glutathione peroxidase (GPx) catch ROS and thus become cell protectors from the deadly effect of ROS and maintain cellular homeostasis. The superoxide dismutase activity catalyzes dismutase of \( \text{O}_2^- \) to be \( \text{H}_2\text{O}_2 \), which mediates the decomposition of \( \text{H}_2\text{O}_2 \) to be \( \text{H}_2\text{O} \) and oxygen. The GPx helps in the conversion of \( \text{H}_2\text{O}_2 \) to \( \text{H}_2\text{O} \). In a normal condition, it is a balance between the production of oxidants and their elimination by antioxidants to prevent free radicals (31-32).

One of the decomposers of ROS is SOD (33). The SOD transforms the mechanism of \( \text{O}_2 \) free radicals into \( \text{H}_2\text{O}_2 \), which the CAT then decomposes to be water and oxygen to prevent hydroxyl free radicals. Moreover, GSH-Px changes the peroxide and hydroxyl radicals to be non-toxic compounds to be glutathione disulfide through reduced glutathione oxidation (GSH). Other antioxidants include glutathione-S-transferase and glucose-6-phosphate dehydrogenase (10,34).

The immune system mechanism will be well-integrated by a natural antioxidant from food and enzymatic oxidant from the body to dampen free radicals and impede ROS production leading to oxidative stress (31,35-36). It also blocks free radicals from the remaining oxidant, changes toxic free radicals to be less toxic, and ceases the production of toxic secondary metabolite and inflammation mediators. Further, it propagates the secondary oxidation chain, repairs broken molecules and initiation, and improves endogenous antioxidant as a system defense. All the defense mechanisms work hand in hand to protect the body from oxidative stress (35).

The insufficient SOD can lead to the production of peroxynitrite and other oxidative species, while enough SOD can chase superoxide and allow NO to stay active as a signaling molecule (33,37). The incapacity of the SOD enzyme in dampening the ROS will incline the MDA level as the final product of the decomposed arachidonic acid and peroxidation of polyunsaturated fatty acids (PUFAs), which is present in the cell walls. The increase of free radicals causes excessive MDA production, commonly known as oxidative stress and antioxidant biomarker (20).

The comparison results between the terminal operational officers and desk administrative officers showed that the capacity of the SOD enzyme could not dampen the production of ROS in the body. As a result, oxidative stress created can be observed from the high
level of MDA serum. It showed that the increase in SOD levels was proportional to the MDA serum levels in the terminal operational officers. The supplementation of SOD showed a slight tendency to decline the MDA levels in the plasma insignificantly. It may occur as the MDA quickly evaporates. Thus, if it is not immediately evaluated after the sampling process, the actual MDA levels will change (38). The relationship between the oxidative damage levels causes irreversible damage in the cellular macromolecules at the beginning of disease infections such as atherosclerosis, ischemic heart disease, liver disease, diabetes, and carcinogenic diseases (39).

Therefore, it is recommended that people consume natural dietary products consisting of high antioxidants to protect them from toxic agents and related diseases (37). Non-enzymatic antioxidants from food containing vitamins will interact with RONS and cease the chain reaction of free radicals such as bilirubin, tocopherol (vitamin E), and beta carotene (10,22).

**CONCLUSION**

$\text{NO}_2$ influenced the incline in SOD enzyme and MDA serum levels in terminal operational officers. Higher exposure to $\text{NO}_2$ could increase the SOD enzyme and MDA serum. It is recommended that the terminal management check the bus’s emission, monitor the ambient air quality regularly, use personal protective equipment, e.g., respiratory mask or N95 for those who primarily work outside. Furthermore, it can provide some natural antioxidants such as fruit which contains many vitamins C and E.

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