Glutathione and malondialdehyde levels in gas station attendants of Semarang city

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Abstract. Gas station is one of the environments with the highest oxidant content such as toluene, benzene, xylene or polycyclic aromatic hydrocarbons (PAHs), forming as free radicals. Glutathione (GSH) as the antioxidant plays a role in capturing free radicals in order to prevent the cell from damage. The objective of this study was to determine the GSH and MDA levels in gas station attendants of Semarang City. This study was an analytic observational and cross-sectional study conducted from April to August 2017. The blood samples were obtained from 34 gas station attendants and 22 volunteers who live in the countryside as the control group. Blood samples were analyzed by using ELISA kit to determine the GSH levels biochemically. Measurement of MDA levels were conducted using the Thiobarbituric Acid Reactive Substance (TBARSC). The results showed that the average GSH level in gas station attendants was 0.587 µg/mL and in controls was 0.208 µg/mL. While the average MDA level in gas station attendants was 2.55 nmol/mL and in controls was 0.827 nmol/mL. Statistical test result of GSH levels showed the significant value $p = 0.000$, and for MDA levels $p=0.001$. There is a relationship between working in gas station and GSH and MDA levels.

1. Introduction
Gas station officers are vulnerable to exposure by exhaust gases from motorized vehicles. Motor vehicle exhaust gas contains of compounds that can increase oxidants in the body such as toluene, benzene, xylene and polycyclic aromatic hydrocarbons (PAH). These compounds will be metabolized by the body and produce free radicals. Free radical is an atom or molecule that has one or more unpaired electrons [1]. Free radicals is not harmful for the body if it is within the normal limits, but will cause problems if the levels exceed the normal limits [2]. Free radicals enhancement and antioxidants level reduction in the body cause oxidative stress.

Oxidative stress have been implicated in a variety of pathological conditions such as diabetes mellitus, cancer, aging, liver damage, atherosclerosis etc [3]. Measurement of lipid peroxidation is used as an indicator of cells oxidative stress. Increased lipid peroxidation due to disruption of the balance of oxidants and antioxidants [4]. Lipid peroxidation is unstable and breaks down to produce a reactive carbonyl compound. Malondialdehyde(MDA) is a key product of lipid peroxidation due to oxidative stress [5]. The antioxidants act as sacrificial substrates scavenging oxidant pollutants from the airways and thereby preventing oxidation of macromolecules such as lipids, proteins and carbohydrates. Antioxidants such as glutathione (GSH) present in epithelial lining fluid (ELF) may protect the airways from oxidant injury induced by exposure to air pollutants [6]. The exposure to petroleum fumes is a risk...
factor and is associated with oxidative stress which raises the need for public awareness about the health hazards in order to enable petrol attendants to take necessary precautionary measures [7].

Gas station officers are vulnerable to exposed by a heavy Pb which causes free radicals in it body. Pollutants Exposure can increase the free radicals. Free radicals can be resisted by antioxidants. Due to those findings this research will be done to realized the antioxidant levels and lipid peroxidation of MDA. The amount of antioxidant GSH level in gas station officers that is expected to be resisted free radicals. The high amount of antioxidants GSH and the MDA low, shows that exposure to pollutants in gas station officers has not caused a oxidative stress. Oxidative stress is characterized by high free radicals and low antioxidants levels. This research purposed is to identify glutathionine (GSH) and Malondialdehyde (MDA) levels at gas station officers in the Semarang City. The Information about GSH and MDA levels at gas station officers can be used as a knowledge to gas station officers in particular, in order to do that taking precautions against free radical on him. The significance of the study for science is for futher research on free radicals at gas station officers.

2. Methods
The study comprised of atotal of 24 subjects, consisting of 7 petrol attendants and 24 healthy control matched with respecto age, sex and with chemical exposure at work in Gas station operators in Semarang City, who were eligible and willing to participate in this study and signed an informed consent. Only those individuals who had not been on antioxidant supplements or had conditions (such asdiabetes, asthma, hypertension, malaria) withunderlying inflammatory or immune responses and the use of drugs which interfere with oxidative metabolism were recruited. Five (5) ml of blood sample were collected from the ante-cubital vein of subjects for analysis. Blood plasma of the samples were separated by a centrifuge in speed of 400 rpm and stored in a freezer before the examination. Lipid peroxidization was estimated spectrophotometrically by the thiobarbituric acid reactive substance (TBARS) method as described by Varshney and Kale (1990) and measurement of MDA levels was conducted using the Thiobarbituric Acid Reactive Substance (TBARSC) [8]. Malondialdehyde (MDA) were measured using blood plasma malondialdehyde (MDA) was quantified using S=1.56×10/M/cm [8]. Measurement of MDA solution was performed using a spectrophotometer with a wavelength at 532 nm. Glutathione level was estimated by the methodof Beutler et al.[9]. Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 17.0 [10]. Comparison between Petrol attendants and control was performed using Student’s t-test for unpaired data. The statistical significance was set at p<0.05.

3. Result and Discussion
A statistic analysis was conducted to see the average, highest and lowest values of MDA and GSH in blood of gas station operators and control (healthy volunteer) in Semarang City. The measurement results of MDA and GSH levels are presented in Table 1.

Table 1. Results of measurement of MDA and GSH levels in blood of gas station operators healthy volunteer (Control) in Semarang.

| Indicator       | Gas station Operator (n=24) | Kontrol (n=24) | p-value |
|-----------------|----------------------------|----------------|---------|
| GSH (ug/mL)     | 0.587 ± 0.23               | 0.208 ± 0.27   | 0.000   |
| MDA (nmol/mL)   | 2.55 ± 0.16                | 0.87 ± 0.20    | 0.001   |

Notes: MDA: malondialdehyde; GSH: glutathione; SOD: superoxide dismutase; CAT: catalase

The comparison between the levels of MDA and GSH in blood of gas station operators and control were analyzed using independent t-test. Average the levels of MDA in gas station operators level of 2.55 nmol/mL and control 0.827 nmol/mL. Whereas the average of GSH level has a gas operation 0.587 ug/mL and control 0.28 ug/mL. Statistical analysis showed that there were significant differences
between MDA levels in gas station officers and controls (p = 0.001; p<0.05). Likewise, GHS levels in gas station officers were significantly different from control (p = 0.000; p <0.05).

The highest MDA levels is on the gas station operators in Jl. Kemerdekaan Sukun, 3.24 nmol / ml. This is because the operators work longer than any other operator that is for 28 years. This data was obtained from the interviews with the operators. Mean while the lowest level of MDA was for the gas station operators in Jl. Kemerdekaan Gombel which at 1.87 nmol / ml. MDA level sex amination results in seven filling stations is relatively high for normal MDA levels below 1.04 ± 0.43 mol / L . The results showed that increasing levels of MDA followed by increasing levels of GSH in the blood. Other studies suggest that MDA enhancement was caused by benzene exposure [11-12]. Glutathion was significantly decreased with the increase of the period of petroleum station workers. GSH is an important antioxidant produced by liver cells and the major soluble antioxidant in cell compartment. The significant decrease in GSH concentration might be attributed to its use in various protective roles against oxidants [13].

Free radicals are compounds containing unpaired electrons in the outer most shell, so it will highly reactive attracting the electrons from the molecules around it to complete the lack of electrons in it. As a result of reactivity, the molecule that loses an electron turns into a new radical and eventually cause cell damage, impaired cell function, and even cell death. Important molecules in the body that are easy damaged by free radicals is deoxyribonucleic acid (DNA), fat, and protein[14].

As a result of an increase in free radicals causing increased use of endogenous antioxidants, so that the levels of endogenous antioxidants in the body decline. The imbalance between free radicals and antioxidants in oxidation-reduction reactions cause oxidative stress [15].

Oxidative stress is the situation coming from the imbalance between free radicals and antioxidants in the body (the number of free radicals more than the antioxidants) as a result of excessive lipid peroxidation events. The results of lipid peroxidation is MDA thus increase lipid peroxidation and will increase the levels of MDA. Therefore, MDA isused as a biomarker of oxidative stress [2].

Endogenous antioxidants can help the body with stand from oxidative stress, but when the number of free radicals more than the amount of endogenous antioxidant produced, it will require exogenous antioxidants, antioxidants that come from outside the body. Natural exogenous antioxidants can be derived from fruits. Through a different mechanism in counteracting free radicals, antioxidants can help the work of exogenous endogenous antioxidantagainst free radicals.

The exposure to gasoline fumes may be harmful to the normal body physiology by increasing serum lipid peroxidation, corticosterone and aldosterone levels while reducing the activities of antioxidant enzymes such as CAT and BuChE. It is therefore imperative for individuals, especially people whose daily activities predispose them to gasoline fume to be well enlightened and equipped against its inhalation [16]. Benzene levels were estimated to pose a significant risk with HQ$_{50}$ > 1 and HQ$_{90}$ > 1 for workers exposed to benzene as base estimates for petroleum refinery workers [17]. The exposure to petroleum fumes is a risk factor and is associated with oxidative stress which raises the need for public awareness about the health hazards in order to enable petrol attendants to take necessary precautionary measures [18].

4. Conclusion
The conclusion of this study is that relationship between working in gas station and GSH and MDA levels. The GSH levels of gas station attendants tend to be lower compared to the control group. Meanwhile, the blood lipid peroxidation levels were higher in gas station attendants compared to the control group.

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