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Conference Paper

Superoxide Dismutase Levels of Operator Gas Stations in Semarang, Central Java, Indonesia

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Abstract

Air pollutants exposure on the gas station generally comes from motor vehicle emissions, such as toluene, benzene, xylene or polycyclic aromatic hydrocarbons (PAH) compounds. These compounds are metabolized by the liver to form free radicals, thus are harmful to health. Gas station operators have a substantial risk of air pollutants exposure released by motor vehicle emissions. The objective of this research is to find out the levels of superoxide dismutase (SOD) in gas station operator on Semarang, Indonesia. This research was analytic observational cross-sectional study conducted from April to August 2015. The number of samples obtained in this research was 21 gas station operators and 10 volunteers who live in the countryside. Blood SOD levels biochemically determined using Randox kit SOD. The results showed that the average of SOD level in gas station operators was 0.76 $u \cdot mL^{-1}$ and volunteers was 0.87 $u \cdot mL^{-1}$. Statistical test results of SOD levels showed the significant value p = 0.860. The SOD levels of gas stations operators are lower than the SOD levels of volunteers who live in the countryside. Gas stations operator does not significantly influence SOD levels.

Keywords: gas station operators, pollutants, superoxide dismutase.

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Nomenclature

FFS	Fuel filling station
PAH	Polycyclic aromatic hydrocarbon
SOD	Superoxide dismutase
TCA	Trichloroacetic acid
DTNB	Ellman's Reagent (5, 5-dithio-bis-(2-nitrobenzoic acid)

1. Introduction

Fuel Filling Station (FFS) is the place where oil and natural gas product especially fuel marketing and distributing happens, where the main processes are receiving, saving/stockpiling and distributing fuel to consumers [1, 2]. The environment of FFS is

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one of the environments with high exogenous which can increase oxidant in the body such as toluene, benzene, xylene, or hydrocarbon aromatic polycyclic (PAH) [3, 4].

Oxidant compounds will be metabolized by the body and produce free radical, such as hydroxyl radical and anion superoxide. Free radical oxides macromolecule in the body such as fat, protein and nucleate acid, causes cell damage. High and long exposure by oxidant can cause free radical accumulation, cell damage and health disorder [5–7].

The body needs antioxidant to prevent free radical. Antioxidant is gained from outside the body (food) or produced by the body itself (endogen) [8, 9]. One of the antioxidant is SOD. Superoxide dismutase (SOD) is endogen antioxidant enzyme that catches free radical in order to prevent cell damage.

FFS employees or operators are one of the worker populations who have risk of high benzene exposure, especially through inhalation with continually exposure time. Several researches showed that oxidant exposure from FFS increased free radical and decreased status of antioxidant in the body. The research conducted by Ariyani [3] in Jakarta showed that those FFS employees were more susceptible to have DNA damage caused by free radical. Besides that, the research conducted in Iraq and Nigeria showed that there was the increasing of free radical and decreasing antioxidant status on FFS employees [9–11].

Looking at the data about oxidant exposure and free radical in FFS environment, a research which measures SOD content to FFS employees in Semarang city has not been conducted yet, Therefore, it is necessary to conduct this kind of research in Semarang. The aim of this research is to find out SOD content in FFS employees' blood in Semarang city.

2. Material and Methods

This research was observational analytic research with cross section approach. Three FFS were chosen randomly and they were FFS in Kaligawe, Pengapon, and Gunungpati, Semarang, Indonesia. The sample of this research was the entire FFS operators who fulfilled inclusion and exclusion criteria. Inclusion criteria were male or female FFS operators who were 20 yr to 50 yr with work period more than 5 yr. Exclusion criteria were FFS operators who did not want to involve in this research. The subjects of this research were 21 FFS operators and 10 controls. The subjects of the research were chosen after they willingly participated in this research and they were explained about the benefits and uncomfortableness involving in this research. The willingness involved in this research was written down through informed consent. Sampling technique used by signing research subject in workplace. The sample was taken during break time. The blood samples were taken from Vena medianacubiti on the subjects' left hands

Characteristic	Frequency (F)
Age	
(20 to 30) old	16
(31 to 40) old	10
(41 to50) old	5
Length of work	
(1 to 3) yr	1
(4 to 6) yr	20
>10 yr	10

Table 1: Characteristic of subject.

for 2 mL. The blood was then centrifuged with 2 000 rpm speed (1 rpm = 1/60 Hz) for 3 min to get the plasma. Then, the serums were stored in a −20 °C refrigerator, before SOD examination was done. SOD content was measured by mixing 50 μL serum with 1.78 mL phosphate buffer 0.1 M pH 8 and 0.2 mL TCA 5%. The mixture was centrifuged in 1500 g for 5 min [g = Relative Centrifugal Force (RCF)], temperature 4 °C. The supernatant then added by 0.01 mL DNTB and left for 1 h. That mixture examined by spectrophotometry on 550 nm wave length to determine serum's SOD content. SOD content examination in serum was done in Molecular Biochemical and Biology Laboratory Biology Department, Semarang State University. The data was processed and analyzed by using SPSS 16. Statistical analysis used in this research was Mann Whitney U test.

3. Result and Discussion

The research was conducted after getting the ethics permission from Bioethics Unit Semarang State University by the number 94/kepk/unnes/2015. The average age of the subjects in this research were 25 yr and had worked for more than 3 yr as FFS operators. The longest service period of FFS operator was 11 yr. The characteristics of the subjects were presented on Table 1.

SOD content was measured based on the speed cytochrome c obstructing by anion superoxide produced by xanthine or xanthine oxidase. Xanthine oxidation produces uric acid and anion superoxide, which then reduces the cytochrome c. Cytochrome c reduction was observed based on the increasing of absorption on 550 nm wave length.

SOD content analysis using spectrophotometry shows that average SOD content in stocktickerFFS operators' bloods was 0.267 $\mu g \cdot mL^{-1}$ and the average of SOD control was 0.305 $\mu g \cdot mL^{-1}$. After doing the normal distribution data test of SOD content by using Kolmogorov smirnov, the significant value obtained was 0.004 ($\leq$ 0.05). It shows that SOD content data was not normally distributed. Therefore, nonparametric Mann

Subject	Levels of SOD ($\mu g \cdot mL^{-1}$)	p
FFS	0.267	0.860
Control	0.305	

TABLE 2: Levels of Superoxide Dismutase.

Whitney U test was done to see the influence of working at stocktickerFFS toward SOD content. The SOD content of research subjects can be seen on Table 2.

Dismutase superoxide enzyme is one of the endogen antioxidant enzymes which can protect cell from oxidative damage. This enzyme decomposes anion superoxide free radical (O_2) which is very reactive to be hydrogen peroxide (H_2O_2) which is less reactive. Then, H_2O_2 compound which is free radical, can be decomposed into water and oxygen by catalase enzyme or glutathione peroxidase [12, 13]

SOD enzyme holds important role as endogen antioxidant. Based on the mechanism, this enzyme is categorized as primary antioxidant which decreases new free radical forming by delinks the chain reaction and changes it into more stable product. SOD enzyme protects body cells and prevents inflammation process caused by free radical. Actually, this enzyme has been in the body, but it needs the help of mineral nutrition substances such as manganese (Mn), zinc (Zn), and copper (Cu) in order to work. Dismutase superoxide works to avoid free radical to prevent or reduce cell damage [5, 6, 8] .

The result of Table 1 above shows that SOD content of FFS operators was lower than SOD content of control. It was in line with the existing theory that FFS environment contains more oxidants such as benzene, toluene, and xylene [3–5]. The longer employees work in FFS, the more exposure and accumulation of benzene, toluene, and xylene compound so that it tends to decrease antioxidant in the body [7, 9].

The result of statistical test of SOD content (Table 2) shows the significant value at 0.860 ($p > 0.05$). It means that work period didn't significantly affect the SOD content. The low SOD content in this research was probably because of the number of endogen antioxidant (GSH, Glutathione peroxide, catalase) which were involved in preventing free radical in the body and young age factor [14]. But, from this result it can be seen that SOD content of FFS operators were lower than control. It means that SOD content in FFS operators were used to avoid free radical as a result of FFS oxidant exposure. This result was still in line with the theory that oxidant exposure could cause the decrease of endogen antioxidant [5, 6, 15, 16].

The decrease of SOD content was not statistically significant. It was probably caused by the number of other antioxidants (glutathione peroxide, SOD and catalase) which muffled free radical [5, 8, 15]. The decrease of FFS operators SOD content in this research was also corresponded to the research conducted by Ariyani in Jakarta, which stated that cancer risk of FFS employees increased because of DNA damage as the result of free radical exposure [3]. Al-Helaly and Al-Fartosy's studies in Iraq also had the same result. They stated that free radical content and MDA in FFS employees' bloods were higher than control [10, 11]. Mahmood's research also stated that there was a decrease of antioxidant content (dismutase superoxide and catalase) on FFS employees [7]. In addition, Odewabi's research at Nigeria stated that free radical exposure on FFS employees increased MDA content and reduced antioxidant content in blood significantly than control [9].

4. Conclusion

Even though the SOD content of gas stations operators were lower than the SOD content of volunteers who live in the countryside, the stastistical test showed that they are not significantly different. It means that working as a gas stations operator does not significantly affect the SOD content.

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