Cigarette Smoke Exposure and Oxidative Stress in Junior High School Children

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Abstract

Cigarette consumption annually increases. The number of smokers worldwide has reached 1.2 billion; 800 million are from developing countries. While smokers are at risk for smoking-related diseases, non-smokers exposed to smoking are also at risk. Cigarette smoke may lead to oxidative stress via active or passive exposure. The malondialdehyde (MDA) level in spot urine samples is a relevant biomarker representing oxidative stress. This study aimed to analyze urinary MDA levels in junior high school students in relation to cigarette smoke exposure. The population included all grade VIII students. Urinary MDA levels were evaluated using spectrophotometric measurement of thiobarbituric acid reactive substances in urine normalized to creatinine level, and fine particulate matter was measured. Other variables including smoking status (active or passive) and supplement consumption were assessed via questionnaires. The mean urinary MDA level of grade VIII was 32.26 μmol/g creatinine with a variation of 21.99 μmol/g creatinine. There was no significant difference in urinary MDA levels among students with active smoke exposure (active smokers). Likewise, there was no significant difference in urine MDA levels among students with passive smoke exposure (passive smokers). However, students who consumed supplements had significantly lower MDA levels than those who did not. Future studies are needed to explore other oxidative stress biomarkers that might play important roles in exposure to cigarette. Moreover, other biomarkers are needed to assess cigarette smoke exposure and antioxidant consumptions.

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Introduction

Cigarette consumption continues to show a yearly increase. The number of smokers across the world has now reached 1.2 billion, of which 800 million are in developing countries.[1] The World Health Organization attributes nearly six million deaths a year to tobacco use. This figure is expected to rise to more than eight million deaths a year by the year 2030.[2,3] Statistics show that 84% of smokers live in developing countries.[3] Data from the Ministry of Health of the Republic of Indonesia show an increase in the prevalence of smokers from 27% in 1995 to 36.3% in 2013, representing an increase from one to two smokers among every three Indonesians over a period of 20 years.[4,5] Increased consumption of cigarettes in the community will lead to an increase in smoking-related diseases and deaths. The findings of the Indonesia Global Youth Tobacco Survey (GYTS) 2014 raised alarm regarding high prevalence of tobacco use among youth aged 13–15 years in Indonesia.[2]

While smokers are at risk for smoking-related disease, non-smokers exposed to smoking are at risk as well. Secondhand smoke (SHS) causes disease and premature death in non-smokers, including children. SHS is the combination of smoke from the burning end of a cigarette and the smoke exhaled by the smoker.[3] It is a mixture of thousands of components in mainstream smoke exhaled by the smoker and sidestream smoke expelled from the end of a lit tobacco product, several of which are toxic and carcinogenic.[6] Overall, almost three out of five students (57.3%) have been reported to be exposed to smoke in their homes and 60.1% were exposed to SHS in enclosed public places.[2] Passive smokers are people exposed to cigarette smoke, although they do not smoke, they are as negatively affected by cigarette smoke as active smokers.[7]

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In Indonesia, 36.2% of boys and 4.3% of girls (comprising 20.3% of all students) currently use tobacco in smoked and/or smokeless form. Among current tobacco users, 18.3% consume cigarettes. Overall, 35.6% smoke one stick per day, whereas more than half of girls (58.3%) smoke less than one cigarette per day. The age at initiation into cigarette smoking among 43.2% of those who have ever smoked a cigarette is 12–13 years.[2] Tobacco smoke contains large numbers of gas and tar phase radicals and other oxidants. It has been estimated that a single puff of a cigarette contains as much as 10^{15} gas phase radicals and 10^{14} tar phase radicals potentially capable of modifying endogenous macromole-cules including lipids.[8-10] Oxidative stress is thought to be produced by cigarette smoking whether via active or passive exposure. An assessment of smoking-related health hazards has involved the use of several oxidative stress biomarkers.[11]

Malondialdehyde (MDA) is one of the most well-known secondary products of lipid peroxidation, and it can be used as a marker of cell membrane injury.[12] MDA concentrations in spot urine samples are considered a relevant biomarker representing oxidative stress, considering the duration between exposure and biological effects. In humans, MDA is known to be an end-product of free radical reactions and elevated MDA levels are found in several diseases including cardiovascular disease and cancer.[13] Lipid peroxidation, which is an indicator used to determine oxidative stress, and MDA level, which is a product of this reactive chain, are significantly higher in smokers. A similarly high level of MDA in passive smokers indicates that smoking also affects non-smokers negatively.[7] MDA, NO metabolites, and urinary cotinine levels were found to be significantly higher in SHS cases compared with those in a control group (P<0.0001), while total antioxidant levels were significantly lower in SHS cases than those in the control group (P<0.0001).[11] Salivary MDA levels in smokers were found to be significantly higher than those in a control group and a group of passive smokers (P<0.05). Compared with a control group, the MDA levels in passive smokers and active smokers were higher; when passive and active smokers were compared with each other, the MDA levels in active smokers were higher.[7] The present study was focused on MDA level as an oxidative stress biomarker due to cigarette exposure in schoolchildren aged 13–15 years. Such studies are important in understanding possible health risks of active and passive cigarette smoke exposure.

Materials and methods

This study used a cross-sectional study design in which the independent variables were smoking status (active or passive), the covariates were fine particulate matter ($PM_{2.5}$) and supplement consumption and urinary MDA level was the dependent variable. The population of this study comprised all students of grade VIII in a Junior High School in Bandung, West Java, Indonesia. A total of 68 students who had attended the school for at least 1 year were included as the sample using a stratified random sampling method. The primary study data were obtained via measurement of $PM_{2.5}$ using the particulate monitor HAZ-DUST Epam 5000, urine MDA level using spectrophotometric detection of thiobarbituric acid reactive substances (T-Bars) and measurement of urine creatinine. In biological monitoring using urine as a sample, creatinine level has been used for normalization.[14,15] Data regarding behavioral characteristics were obtained by means of interviews using questionnaires. Data analysis began with a univariate descriptive analysis to characterize the behavior of the respondents. Bivariate analysis was performed to identify the relationship between smoking status (active or passive), $PM_{2.5}$ concentration and supplement consumption and urinary malondialdehyde level. This analysis was conducted by using correlation analysis and independent t-test with 95 % confidence level (P<0.05). Students who were current or previous smokers were considered active smokers, whereas students who were currently or previously exposed to smokers at home were considered passive smokers. Consumption of supplements was assessed based on habitual consumption of vitamin C or E by the respondents. Ethical approval and required permissions were obtained from the Ethics Committee at the Faculty of Public Health, Universitas Indonesia, Indonesia (Ref. No. 212/UN2.F10/ PPM.00.02/ 2017). Informed consent was obtained from parents of all the children included in the study.

Results

This study was conducted between May and June 2017 on a sample comprising 68 grade VIII students (39 females and 29 males).

MDA levels

The mean urinary MDA level of the students was 32.26 μ mol/g creatinine with a variation of 21.99 μ mol/g creatinine. The mean log transformed MDA level was 1.44 μ mol/g creatinine (Table 1).

Variable	Mean	Standard Deviation
Urinary MDA level	32.26	21.99
Urinary MDA level $_{Log10}$	1.44	0.38

Table 1. Distribution of urinary MDA level (μmol/g creatinine).

The relationship of behavioral characteristics with MDA levels

The result of the analysis showed that smoking status in either active or passive smokers did not have a significant relationship with urine MDA level, with p-values of 0.822 and 0.252 in active and passive smokers, respectively. Meanwhile, there was a significant relationship between consumption of supplements and MDA levels *(P*=0.001). The results of the analysis are shown in Table 2.

The relationship of $PM_{2.5}$ with MDA levels

The mean $PM_{2.5}$ was 34.05 μ g/m^3 and mean $PM_{2.5}$ Log 10 (geometric) was 1.53 μ g/m^3. Analysis of the relationship between $PM_{2.5}$ and urinary MDA yielded, a result showing r = −0.070 with a p-value of 0.573. Statistical analysis showed no significant relationship between $PM_{2.5}$ and urinary MDA level (Table 3).

Variable	Urinary MDA Level (μmol/g creatinine)		
	n	R	P-*value*
$PM_{2.5}$ log 10	68	−0.070	0.573

Table 3. Relationship of $PM_{2.5}$ with Urinary Malondialdehyde (MDA) Levels.

Variable	Urinary MDA Level (μmol/g creatinine)				
	n	Mean (SD)	Mean diff	P-value	95% CI
Active Smokers					
Smoker	5	38.416 (30.962)	2.324	0.822	−18.228–22.877
Non-smoker	63	36.096 (21.464)			
Passive Smokers					
Yes	57	37.612 (22.801)	8.343	0.525	−6.085–22.771
Non-smoker	11	29.2691 (16.333)			
Supplement Consumption					
No	47	41.913 (22,246)	18.295	0.001	7.586–29.005
Yes	21	23.617 (15.484)			

Table 2. Relationship of the Characteristics Respondents with Malondialdehyde (MDA) Level.

Discussion

The mean urinary MDA level in the study respondents was 32.26 ± 21.99 (SD) μmol/g creatinine (mean $MDA_{log\ 10}$ 1.44 ± 0.38 (SD) μ mol/g creatinine). These results are lower than those obtained by Kim et al.[13] who compared urinary MDA levels between kindergarten children and young adults, showing a mean MDA 3.6 ± 1.9 μ mol/g creatinine in children and 4.0 ± 1.6 μ mol/g creatinine in young adults.[13] However, MDA levels in junior high school students are still in agreement with those found in another study conducted by Bae et al in two cities in China and two cities in South Korea on school children, obtaining urinary MDA levels of 1.74: 1.00, and 1.17: 0.90 (μ mol / g creatinine).[16] MDA levels do not have a default value, and differing results in MDA level measurements are due to differences in measurement methods, tools and materials, reagents, and derivatization methods. Moreover, risk factors in individuals also affect MDA levels. Inconsistent results in the literature suggest that harsh derivatization conditions that are often used, result in post-sampling formation of MDA and related derivatives and that more effort should be made to ensure a consistent level of quality in the analysis of MDA in general.[10] All the

methods used to assess lipid peroxidation andoxidative stress in humans should be used with caution because of their lack of accuracy, validity, or both. It is recommended that more than one technique be used to provide a better estimate of oxidative stress.[17]

The result of this study showed no significant difference in urinary MDA level among students with active smoke exposure (active smoker), with a mean difference of 2.324 µmol/g creatinine between current smokers and non-smokers who previously smoked (*P*=0.822). Likewise, no significant difference in urine MDA levels was found among students with passive smoke exposure (passive smokers), with a mean difference of 8.343 µmol/g creatinine between those currently exposed to smokers and those previously exposed to smoke (*P*=0.252)

This study had several limitations. First, it was of a cross-sectional design, and we were therefore unable to demonstrate a causal relationship between cigarette smoke and MDA. However, the strength of this study was the use of biomarkers to evaluate oxidative stress, which is not commonly reported in Indonesia. Second, we assessed cigarette smoke exposure using questionnaires alone and not using biomarkers such as cotinine. This allows for a recall bias against smoking habits and the impact of cigarette smoke on the respondents. We were unable to obtain information on the duration of cigarette smoke exposure due to the simplicity of the questions asked. Chronic diseases caused by exposure to cigarette smoke and oxidative stress involved continuous and long-term exposure to risk factors. Therefore, the duration and total amount of exposure are important variables.[18]

A study conducted by Kim et al. (2009) found that $PM_{2.5}$ was related to MDA levels in individuals; a 0.3% rise in log MDA value was associated with an increase of 1 µg/m^3 $PM_{2.5}$ in adults exposed to $PM_{2.5}$. However, such a relationship was not found in children even after controlling for age, sex, body mass index, passive smoking, and measurement sessions.[13] In the present study, the respondents represented early teens between 13 and 15 years of age. In the study conducted by Kim et al,[13] the elderly subjects were more susceptible to oxidative stress due to ambient exposure to $PM_{2.5}$ than young children were. Other factors that must be considered, include concomitant personal exposure to other pollutants, e.g., ozone, nitrous oxides, polycyclic aromatic hydrocarbons, heavy metals, and volatile organic chemicals.[13] Air pollution causes an increase in airborne reactive oxygen species (ROS) which directly leads to oxidative stress in the lungs. In addition, air pollution indirectly results in increased exposure of respiratory tract cells to ROS through different mechanisms,[19] suggesting that $PM_{2.5}$ is first deposited in the airway and then interacts with lipid to form MDA and other peroxide products.

Antioxidants are compounds that are able to combat and neutralize free radicals and repair oxidative damage in biological molecules. Antioxidants can be obtained from natural or synthetic sources. Natural antioxidants are antioxidants derived from plants that are often consumed, and such compounds have been isolated. Plants contain antioxidants in the form of vitamin C, vitamin E, polyphenols, carotene, bioflavonoids, catechins, and resveratrol. Synthetic antioxidants are those added to food ingredients to control quality and prevent changes in chemical properties of the food due to oxidation processes, especially during storage; examples include butylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT).[20] The antioxidant supplements evaluated in this study represent synthetic antioxidants (vitamin C and vitamin E supplements). The result showed that 21 students consumed such supplements and 47 did not. There was a significant difference in urinary MDA levels based on the supplement consumption (*P*=0.001). These results were in accordance with the possibility that cellular damage caused by ROS in normal physiological conditions is prevented by antioxidants, both enzymatic (catalase, superoxide dismutase, glutathione peroxidase) and non-enzymatic (vitamin E, vitamin C, glutathione).[21]

Conclusions

This study found no statistically significant relationship between smoking (active or passive) and oxidative stress or environmental factors ($PM_{2.5}$). However, the result indicate that habitual supplement consumption may affect levels of MDA; students who consumed supplements had significantly lower levels of MDA than those

who did not. Further studies, are needed to investigate other oxidative stress biomarkers that play important roles in exposure to cigarette smoke. In addition, other biomarkers to assess cigarette smoke exposure and antioxidant consumption are also needed.

Declaration of Interest

The authors report no conflict of interest.

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