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Oxidative Stress Biomarkers and 25 Hydroxy Vitamin D in Online Motorcycle Taxi Drivers in Jakarta, Indonesia

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ABSTRACT

Online motorcycle taxi drivers are frequently exposed to oxidative stress originating from air pollutants. Vitamin 25(OH)D is one of the potent antioxidants in the body. Studies on the relationship between vitamin 25(OH)D concentration and oxidative stress markers in online motorcycle taxi drivers are still rare. Objective of this study was to determine the differences between oxidative stress biomarkers levels in online motorcycle taxi drivers and control subjects, and the relationship of 25(OH)D concentration and oxidative stress biomarkers in both groups. This cross-sectional study involving 70 online motorcycle taxi drivers and 70 subjects as a control, aged 20-50 years. The study was conducted from September 2022 to March 2023. Subjects meeting inclusion criteria were asked to fill in a questionnaire, then underwent examination of vital signs and anthropometric data, after which 10 mL of their venous blood was drawn for examination of 25(OH)D, 8-OHdG, and MDA. Spearman's correlation and T-test was used with $p < 0.05$ indicating significant difference. Mean age of online drivers was 36.4±7.9 years, whereas mean age of control subjects was 38.5±6.7 years, There was significant differences between 8-OHdG and MDA concentration at online drivers and control subjects. There was no significant correlation of 25(OH)D concentration with MDA and 8-OHdG concentrations in both groups.

1. Introduction

The occupation of online motorcycle taxi driver carries the risk of exposure to chemical and physical agents. Some of the diseases that can be predicted to result from this occupational exposure are among others cardiovascular diseases, musculoskeletal and gastrointestinal disorders, as well as an increase in malignant diseases [1], [2]. The causes associated with these risks are among others traffic density, traffic congestion, irregular work times, and low physical activity during work. In addition, exposure to chemical agents such as dust, heavy metals, volatile organic compounds (VOCs), and polycyclic aromatic hydrocarbons (PAHs) are important factors in the medical management of these online motorcycle taxi workers.1 Airborne pollutants that frequently cause health problems in online motorcycle taxi drivers, such as pollutants that originate from both organic and inorganic gases, including nitric oxide (NO) produced by the burning of fuels, sulfur oxide (SO), and particularly sulfur dioxide (SO_2) that is produced by the burning of diesel fuel and hydrocarbons. These pollutants contain toxic substances such as aromatic polycyclic hydrocarbons (e.g. benzopyrenes, nitropyrenes), heavy metals, dioxins, and furans [1]. All of these components are absorbed by dust particles. Gas and chemical particles that are adsorbed by dust particles have negative effects on human health, such as disorders of the circulatory, nervous, and endocrine systems, and are probably carcinogenic. Chemicals in contaminated air enter the human body through inhalation or are absorbed through the skin. These particles may cause disorders in the human body, both directly and indirectly after undergoing biotransformation into reactive metabolites, such that they can cause activation of inflammatory mediators (cytokines, heat shock proteins) and may induce oxidative stress.3 Oxidative stress is a condition of imbalance between pro- and antioxidants that results in molecular and cellular damage. Markers for determining the oxidative stress levels in the body may be in the form of markers of DNA damage (8-hydroxy-2-deoxyguanosine/8-OHdG=8-oxo-2'-deoxyguanosine/8-oxodG) and lipid peroxidation (malondialdehyde/MDA) [3], [4].

Vitamin D is a secosteroid hormone that has important functions in various systems in the human body, apart from the previously known musculoskeletal system. Vitamin D is also known to play an important role in the inflammatory system, oxidative stress, and the functioning of the mitochondrial respiratory system, including the aging process in humans [5]. Hypovitaminosis D reportedly also causes an increased incidence and severity of diseases that are associated with increased age as a result of the presence of oxidative stress, such as obesity, insulin resistance, diabetes mellitus, hypertension, memory disorders, autoimmune diseases, osteoporosis, and diseases associated with inflammation. An adequate 25-hydroxyvitamin D [25(OH)D] concentration reduces oxidative stress and increases mitochondrial and endocrine functions, thereby reducing the risk of various abnormalities associated with oxidative stress. 25(OH)D is also said to be a potent antioxidant that facilitates balanced mitochondrial activity, and prevents oxidative stress associated with oxidation of proteins, lipid peroxidation, and DNA damage [5], [6]. The relationship between 25(OH)D concentration and oxidative stress is still a matter of debate. The study of [6] found a significant relationship between a deficient 25(OH)D concentration and an increase in the release of reactive oxygen species (ROS) and DNA damage. The study of [7] on taxi drivers shows that 25(OH)D concentration is not associated with a protective effect on cardiometabolic risk. Deficiency of 25(OH)D is frequently encountered in countries that are located far from the equator, but the incidence of the deficiency is also high in areas that are situated 1000 km from the equator, in countries such as Sri Lanka, India, the Far East, Middle East, Central America, and Persia. This is caused by a combination of climate, ethnicity, habits, and skin color [5]. The prevalence of 25(OH)D deficiency in Indonesia is also high. A study on 25(OH)D in Jakarta reported that 68.8% of the subjects had a 25(OH)D concentration of ≤20 ng/mL and 31.2 % had a 25(OH)D concentration of >20 ng/mL [8]. Although online motorcycle taxi drivers are always exposed to sunlight when at work, the efficiency of the exposure in vitamin D synthesis is not known with certainty. The aim of this study was to determine the relationship of 25(OH)D concentration and age with MDA and 8OHdG.

2. METHODS

This study of cross-sectional design involving 70 online motorcycle taxi drivers and 70 subjects as a control, was conducted from September 2022 until March 2023. The inclusion criteria in this study for the online drivers were males and females who had worked as online motorcycle taxi drivers for at least 1 year continuously; and for control subjects were male and females who worked at office and not riding motorcycle to the office; were 20 – 50 years old, did not smoke, and were willing to participate in this study after receiving explanations, by signing informed consent. The exclusion criterion was currently consuming vitamin D, vitamin C, or vitamin E.

2.1 Data collection

The study subjects were obtained through the use of googleforms that contained a questionnaire on demographic data, length of time as online motorcycle taxi driver (for online drives), smoking habit, and habit of taking supplements. The googleforms were spread through the Whatsapp community group of online motorcycle taxi drivers for Jakarta. The control groups also asked to fullfill the googleforms that contained the same questionare except question about driving motocycle. The subjects meeting the inclusion criteria were asked to be present at a predetermined time and examination were conducted on systolic blood pressure, diastolic blood pressure, pulse rate, body weight, height, abdominal circumference, followed by drawing of venous blood samples.

2.2 Laboratory examinations

A 10 mL venous blood sample was drawn from each subject, made into serum, and frozen at a temperature of minus 200C for use in the simultaneous determination of 25(OH)D, MDA, and 8-OHdG concentrations, after the total number of subjects was reached. The determination of 25(OH)D was performed by direct competitive chemiluminescent immunoassay (CLIA) using Diasorin Liaison reagents. The determination of MDA used reagents from Abbexa (catalog number abx-257171) by enzyme linked immunosorbent assay (ELISA), while 8-OHdG was determined by enzyme immunoassay using the reagents in the Bioxytech 8-OHdG EIA kit (catalog number 21026).

2.3 Statistical analysis

To evaluate the differences between MDA and 8-OHdG at both groups, the t test was used, with $p<0.05$ as level of significance; and to evaluate any relationships of 25(OH)D concentration with the parameters of oxidative stress, in both groups, the Spearman correlation test was used, with $p<0.05$ as level of significance.

2.4 Ethical Clearance

This study obtained ethical clearance from the Research Ethics Committee of the Faculty of Medicine, Universitas Trisakti under No. 158/KER/FK/VIII/2022.

3. RESULTS

Table 1 shows the characteristic of study subjects, it is apparent that the majority of the subjects at both groups were of male gender, with no regular physical activity or sports. Table 2 shows the characteristics that are associated with biochemical data. Table 3 shows the correlation of 25(OH)D with MDA and 8-OHdG concentrations.

Table 1. Characteristics of study subjects

Variable	X±SD / n (%) Online driver (n=70)	X±SD / n (%) Control (n=70)
Age (years)	36.4±7.9	38.5±6.7
Gender		
Male	59 (84.3)	55 (78.6)
Female	11 (15.7)	15 (21.4)

Weight (Kg)	69.36±17.9	70.54±16.7
Height (Cm)	163.13±7.9	160.12±8.2
Body Mass Index (BMI) (Kg/m2)	26.06±6.42	25.9±5.51
Systolic blood pressure (mmHg)	128.9±17.8	126.5±16.8
Diastolic blood pressure (mmHg)	87.8±12.4	85.7±11.8
Regular physical activity or sports		
Yes	17 (24.3)	15 (21.4)
No	42 (60)	45 (64.3)
Never	11 (15.7)	10 (14.3)
Duration of physical activity or sports		
<30 minutes	45 (64.3)	30 (42.8)
30-60 minutes	18 (25.7)	31 (44.3)
> 1 hour	7 (10)	10 (12.8)
Habit of sunning		
Yes	65 (92.9)	12 (17.1)
No	5 (7.1)	58 (82.9)
Duration of work as online driver (years)	4.6±1.8	-
< 5 years	55(78.6)	-
≥ 5 years	15(21.4)	-

Table 2. Biochemical characteristics

Parameter	X±SD / n (%) Online driver (n=70)	X±SD / n (%) Control (n=70)	p value
25 (OH)D----ng/mL	19.78±7.52	19.12±7.5	
<20	38(54.3)	39 (55.7)	
20-30	26 (37.1)	24(34.3)	0.679
>30	6 (8.6)	7(10)	
8-OHdG-----ng/mL	1.98±0.5	0.15±0.02	0.000*
MDA--------ng/mL	311.83±26.94	57.21±12.36	0.000*

*p<0.05 significantly difference in T-test

25(OH) D: 25-hydroxyvitamin D; 8-OHdG: 8-hydroxy-2-deoxyguanosine; MDA; malondialdehyde

Table 3. Correlation of vitamin D and age with MDA and 8-oxoDG in online motocycle driver

Parameter		Online driver		Control	
		r	*p*	r	*p*
25 (OH)D	MDA	-0.056	0.648	0.235	0.453
	8-OHdG	0.000	0.997	0.432	0.213

*p <0.05 significant difference in Spearman correlation test; 8-OHdG: 8-hydroxy-2-deoxyguanosine; MDA: malondialdehyde; 25 (OH)D : 25-hydroxyvitamin D

4. DISCUSSION

Reactive oxygen species (ROS) are substances that are always produced in a living organism, because of biochemical and metabolic reactions as well as exposure to physical, chemical, and biological agents. Large increases in ROS may cause oxidative stress and result in DNA damage. The frequently used biomarker for ROS-mediated DNA lesions is 8OhdG [9]. The 8OHdG concentration in this study had significantly higher than in the control subjects (Tabel 2). This 8OHdG concentration is categorized as high when compared with other studies on healthy individuals. In the study conducted in China, it was reported that the 8OHdG concentration in healthy individuals was 0.19 ng/mL [10]. The serum 8OHdG concentration was indeed low, i.e. less than 10 pg/mL or 0.010 ng/mL. Mean serum 8OHdG concentration in healthy individuals was reportedly around 0-70 pg/mL, with mean of 25.5±13.8 pg/mL or 0.0255±0.0138 ng/mL [9]. The study subjects in this study were online motorcycle taxi drivers that were daily exposed to pollutants that may cause oxidative stress. When compared with the value of 0.15 ng/mL, the mean 8OHdG concentration of 1.98 ng/mL was 13 -fold higher than the mean in control subjects. This shows that the online motorcycle taxi drivers undergo high levels of oxidative stress. Air pollution comes from various sources of emission, both natural and even anthropogenic emissions. Anthropogenic emissions are the current globally dominant emissions, because of the rapid advances of the industrial world. The industrial process with the largest contribution to air pollution is the burning process, such as the burning of fossil fuel and biomass to produce energy. Other combustion processes that are sources of pollutants are the results of combustion during transportation by land, air, and water, and also from industrial and biomass burning. In addition, there are the beneficial controlled as well as uncontrolled burning processes, such as forest and savannah fires, and burning of agricultural and urban waste. Other sources that contribute to air pollution are dust and building construction activities [11], [12]. The most frequent causes of air pollution that causes health problems are among others particulate matter (PM) including $PM_{2.5}$ (particles with aerodynamic diameter of ≤ 2.5 µm), PM_{10} (particles with aerodynamic diameter of ≤ 10 µm), ozone (O_3), nitrogen dioxide (NO_2), sulfur dioxide (SO_2), and carbon monoxide (CO). In fact, air pollution is produced by many more pollutant elements [11]. More than 90% of residents live in areas with air that is unhealthy to breathe, resulting in 4.2 billion deaths globally each year (data for 2016). Of all mortality from air pollution, around 38% causes ischemic heart disease, 20% causes stroke, and 43% causes chronic obstructive pulmonary disease (COPD) [11]. The mean length of time the subjects in this study worked as online motorcycle taxi driver was 4.6±1.8 years, and the majority worked < 5 years (78.8%) (Table 1). The increase in 8OHdG concentration in this study was not associated with length of employment as online motorcycle taxi driver, with r=0.022; p=0.856 (data not shown). This may have been due to other factors influencing the increase in 8OHdG concentration, among others the severity of the air pollution in the Jakarta region. High carbon dioxide emissions lead to aggravation of the air pollution. Urban areas are the areas that are the most impacted by air pollution. The transportation sector is the largest contributor to air pollution (80%), which is followed by emissions from the industry, forest fires, and domestic activities. The large number of vehicles with the addition of inadequate road infrastructure, result in traffic congestion that produces a high level of pollutants, which has a negative impact on public health. In Jakarta the index of the quality monitoring system from 2001-2015 showed a 3-fold poorer annual mean than World Health Organization standards (20µg/m^3) [13]. The air pollution problem in Indonesia is extremely severe and results in a morbidity of 50%. The diseases caused by vehicular emissions and air pollution, are acute respiratory infections, bronchial asthma, bronchitis, eye and skin irritation, lung cancer, and cardiovascular diseases [13]. The meta-analysis that was conducted by [14] showed that the association between 8OHdG and air pollution obtained variable results, depending on the research methods used and the pollutants studied. Other factors influencing 8OHdG concentration are among others age, gender, physical exercise, alcohol consumption, smoking, body weight, and nutrition [15].

Malondialdehyde is formed in vivo through the process of polyunsaturated fatty acid peroxidation. Malondialdehyde interacts with proteins while MDA itself is atherogenic in nature [16], [17]. Malondialdehyde is said to be a reliable indicator of oxidative injury in cells and tissues [18]. The MDA concentration in this study showed a significant higher concentration than in control subjects with p<0.000 (Table 2). The conversion from nmol/mL to ng/ml for MDA was divided with 0.013887 [20]. A double blind randomized controlled trial in menopausal women showed that baseline MDA was 0.81±0.23 nmol/mL or 58.32±16.56 ng/mL [21]. The results of this study showed that online motorcycle taxi drivers experience high oxidative stress. The results of a meta-analysis conducted by [14] showed that short-term exposure to $PM_{2.5}$ radicals was associated with increased MDA, showing that air pollution contributes to increased oxidative stress. The increased MDA was reported in the serum of taxi drivers, bus drivers, and technical service workers that were exposed to polluted air [1]. A study in Praha on bus drivers showed that the lipid peroxidation biomarker F2 isoprostane is correlated with age and low density lipoprotein cholesterol [16]. Lipids are important components that maintain the structure and function of the control cells. The main lipid target of attack by reactive oxygen species (ROS) such as oxygen free radicals and lipid oxidation products are associated with pathological conditions. Oxidation in organic compounds by free radicals is historically called autoxidation. Lipids and other biological components such as proteins, carbohydrates, and nucleic acids, undergo an oxidation reaction called peroxidation. The peroxidation process is involved in a number of diseases in humans, such as atherosclerosis, cancer, diabetes, and Alzheimer's and Parkinson's diseases. Lipid peroxidation is involved in various pathological conditions such as inflammation and cancer. In addition, lipid peroxidation also acts as regulator of non-apoptotic cell death [21], [22].

In this study, the 25(OH)D concentration in online motorcycle drivers was around 19.78±7.52 ng/mL and 19.12±7.5 ng/mL in control subjects (Table 2), showing that the concentration was in the deficiency range. At present the definition of 25(OH)D deficiency that is frequently used is a concentration of <20 ng/mL. The concentration of 20-29 ng/mL is categorized as insufficient and ≥ 30 ng/mL as sufficient [24- 26]. The majority of subjects in this study had 25(OH)D deficiency in both groups. The study results on 160 subjects aged 55-65 years in South Jakarta showed that 68.8% of subjects had 25(OH)D deficiency [8]. The study of [27] showed that South Asian countries had a prevalence of 25(OH)D deficiency that was considered to be high, among others Pakistan 78%, Bangladesh 67%, India 67%, Nepal 57%, and Sri Lanka 48%. The factors that influence the development of 25(OH) deficiency are among others the degree, duration, and intensity of exposure to sunlight. In addition to these, other influencing factors are age, skin color, food intake, cultural characteristics, local ethnic grouping, and religious clothing practice [27]. Because of the demands of their work as online motorcycle taxi drivers, they have to use helmets, jackets, long pants, and gloves, such that although they were daily always exposed to sunlight, the sunlight was prevented from entering the skin. In addition in Indonesia, the foods that are sources of vitamin D, such as cow's milk, butter, shrimps, egg yolk, salmon, and sardines, are seldom consumed by the community. Foods that contain vitamin D tend to be expensive, such that many Indonesians have difficulty in obtaining foods that contain vitamin D [8].

Vitamin D is said to have beneficial effects on the organ systems in the human body, such as controlling systemic inflammation, cellular oxidative stress, mitochondrial respiratory functions, and retarding the aging process [5]. The majority of the subjects in this study were in the categories of 25(OH)D deficiency and insufficiency, showing that the concentration of 25(OH)D, which functions as controller of oxidative stress, was also reduced. The 8OHdG and MDA concentrations of the subjects of this study were at a high level, indicating an increase in oxidative stress. 8OHdG is one of the oxidative biomarkers of DNA damage, whereas MDA is a biomarker of lipid peroxidation [9], [16]. This study shows that the correlation of 25(OH)D concentration with 8OHdG and MDA was not significant (Table 3). From these results it is apparent that although the 25(OH)D results were low, and the 8OHdG and MDA results were high in online motocycle drivers, these parameters were not correlated. This may have been due to the presence of another mechanism underlying the role of 25(OH)D as controller of oxidative stress. The 25(OH)D concentration is normally needed to protect against excessive oxidative stress, but increased risk of diseases and reduced longevity may occur even though the vitamin D concentration is adequate, which is due to the fact that the mechanism to protect the cells against oxidative stress does not come only from this process. Vitamin D metabolism and function is influenced by many factors, such as physical activity, life style, medications, environmental pollutants, and epigenetic factors, that as a whole also modify the balance between energy intake and expenditure through mitochondrial control [5]. The results of the study of [28] showed the role of vitamin D as anti-inflammatory and oxidative stress in human retinal cells invitro. Exposure to vitamin

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D can neutralize the production of 8OHdG under oxidative induced conditions.

5. CONCLUSION

There was significant differences between 8OHdG and MDA concentration in online motocycle drivers and control with p<0.000. There was no significant correlation of 25(OH)D concentration with 8OHdG and MDA concentrations, at r=0.000;p=0.997 and r=-0.056; p=0.648 in online motocycle drivers; Further studies are necessary with randomized controlled trials on vitamin D supplementation to online motorcycle taxi drivers.

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AUTHORS' CONTRIBUTIONS

Conceptualization: Pr, M. Data curation: Pr, M, Alv, LTM. Formal analysis: Pr, M. Methodology: Pr, LTM. Writing of original draft: Pr. Writing of review and editing: M, Alv, and LTM.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this study.

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