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COMPARISON OF VITAMIN D AND EXHALED CARBON MONOXIDE LEVELS BETWEEN SMOKERS AND NONSMOKERS AMONG INDONESIAN ADULT MALES

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ABSTRACT

The relationship between smoking and lung damage is part of the inflammatory process, increased oxidative stress, and protease. Many of these processes are modulated by Vitamin D. Current data shows that Vitamin D deficiency is associated with respiratory disorders. This study aims to compare the Vitamin D values of male smokers and nonsmokers in Indonesia and their exhaled (Carbon Monoxide) CO values. A cross-sectional study was conducted on healthy male subjects at X office Jakarta in August 2017. A sample of 60 people consisting of 30 smokers and 30 nonsmokers was selected via consecutive sampling. Interviews were conducted to fill in the baseline data questionnaire, Fagerstrom questionnaire, sun exposure score, and nutritional intake. Exhaled CO measurement was done using a portable CO measuring device, and Vitamin D level was examined from blood drawn. This study found that most participants (90%) had Vitamin D deficiency. The mean value of Vitamin D of the smoker group was lower than the nonsmoker group (15.21 ± 3.15 ng/ml vs. 16.9 ± 2.9 ng/ml, p = 0.029). The Mean exhaled CO level was higher in smokers than nonsmokers (17.3 ± 12.54 ppm vs. 5.4 ± 2.51 ppm, p = 0.000). Most participants had Vitamin D deficiency. The value of Vitamin D in smokers was lower than that of nonsmokers. Exhaled CO level of smokers was higher than nonsmokers.

1. Introduction

Tobacco smoking is an addictive potential and has influenced human behavior in the past four centuries. Smoking habit develops so fast that they become the biggest threat to world health now and in the future. Some reports suggest that the prevalence of tobacco use has declined in men in several high-income countries but is still increasing in young adults and women. In 2013, the current proportion of smokers in

Indonesia was 29.3% [1].

Several studies have been conducted to assess the effect of smoking on the value of Vitamin D. Most cells and tissues in the body express Vitamin D receptor (VDR), which stimulates the core transcription of various housekeeping genes for cellular function; hence Vitamin D deficiency can precipitate, accelerate, and worsen the disease [2]. Recent data indicates that Vitamin D deficiency is associated with respiratory disorders. Vitamin D can affect pulmonary cell biology systems, such as the respiratory and immune systems [3]. An in vitro study states that smoking can interfere with the local anti-inflammatory effects of Vitamin D [4].

Among the health-altering substances found in cigarettes, there is carbon monoxide (CO). Exhaled carbon monoxide is used as a marker of environmental cigarette smoke exposure. This substance is found in mainstream and side-stream cigarette smoke and is absorbed in the alveoli. Carbon monoxide will enter the bloodstream and bind to hemoglobin to form carboxyhemoglobin (COHb) and inhibit the release of oxygen from hemoglobin, thus causing hypoxemia. If the tissue does not receive enough supply of oxygen continuously, the tissue will become hypoxic, malfunction, and finally, cell death occurs [5]. This data is necessary to compare the Vitamin D level between adult male smokers and nonsmokers in Indonesia and their exhaled CO levels as objective supporting data on the impact of smoking in Indonesia.

2. METHODS

This study used a cross-sectional design to compare Vitamin D and CO levels between adult male smokers and nonsmokers among workers at X office Jakarta. The research was conducted from August 24th to 31st, 2017. The study received ethical approval from the Faculty of Medicine Ethics Committee, University of Indonesia, number 728 / UN2.F1 / ETIK / 2017.

The subject of this study is divided into two groups, male smokers who did not change their smoking habits (suddenly increased/decreased the number of cigarettes smoked per day) in the past month with mild and moderate Brinkman index and men who are not smokers. The inclusion criteria are adults male with normal BMI and at risk of being overweight and agree to fill and sign the consent form and questionnaire following the research procedure explanation. Exclusion criteria are the presence of fever, cough, runny nose, shortness of breath, or other respiratory symptoms within two weeks before the examination, a history of pulmonary disease, male smokers who use electronic cigarettes or shisha, and those who could not perform the maneuver for exhaled CO examination.

Participants who fulfilled the inclusion criteria were recruited via consecutive sampling. The data was collected through interviews and questionnaires, including demographic data, a smoking habits questionnaire, a fagerstorm questionnaire for nicotine dependence, and Vitamin D intake using a food frequency questionnaire (FFQ). Furthermore, the patient's nutritional status was assessed using anthropometric measurements for calculating body mass index by using average measurements of body weight and height that were taken twice each. We also performed weekly sun exposure assessments with scoring system counts for the length of exposure and body skin area exposed.

Vitamin D level was measured by direct competitive chemiluminescence immunoassay (CLIA) method with a DiaSorin Liaison device. The total Vitamin D measurement uses a quantitative measurement of total Vitamin D 25-OH concentration. In the first incubation, Vitamin D 25-OH will be separated from its binding protein and consecutively bound to specific antibodies in a solid phase for 10 minutes. The tracer was added (Vitamin D bound to isoluminol derivatives) and incubated for 10 minutes. After that, the

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unbound material will be removed through the washing cycle. Then, a starter reagent is added to initiate a chemiluminescent reaction. The photon signal will then be measured by photomultiplier as relative light units (RLU) and is inversely proportional to the concentration of Vitamin D 25-OH in the sample.

Subsequently, the measurement of expiratory air CO levels in this study was carried out using the portable piCO+ Smokerlyzer® device from Bedfont Scientific Limited via the following steps:

1. The respondent is asked to breathe, inhale, and hold his breath for 15 seconds.
2. After 15 seconds of holding their breath, the respondent immediately exhales completely on the measuring device.
3. Within a few seconds, the measuring instrument will show the level of expiratory air CO on the screen. CO levels are measured using part-per-million (ppm) units.

Statistical analysis was performed using the Statistical Package for Social Science (SPSS) software version 20 (IBM Corp, Armonk, NY, USA). P value <0.05 was referred to as statistically significant.

3. RESULTS

We included 60 subjects that fulfilled the criteria of inclusion and exclusion. The subjects were divided into two groups, 30 subjects with smoking habits and 30 subjects with non-smoking habits. The characteristics of the subjects are described in Table 1.

Table 1. Subjects' Characteristics

Characteristics	(n)	%
Age (years)*	26 (20-34)	
Age		
< 30 years old	49	81.7
≥ 30 years old	11	18.3
BMI		
Normal	31	51.7
At risk of overweight	29	48.3
Intake of Vitamin D-containing food		
≤ 2.6 mcg	32	53.3
> 2.6 mcg	28	46.7
Sun exposure score		
= 0	48	80
> 0	12	20

*The numerical group data were present as median(minimum-maximum)

Entire smoker subjects smoke 1-19 cigarettes per day. The subject consumes two types of cigarettes: white and clove. Among the subjects, 60% use white cigarettes, 30% use clove cigarettes, and only 10% use mixed cigarettes. We use the Brinkmann index to classify smoking habits. We found that 86.7% of subjects were mild smokers, and 13.3% were moderate smokers. Most of the subjects (21 subjects,70%) had very low Fagerstorm Scores. The result of the Fagerstorm Score describes in figure 1.

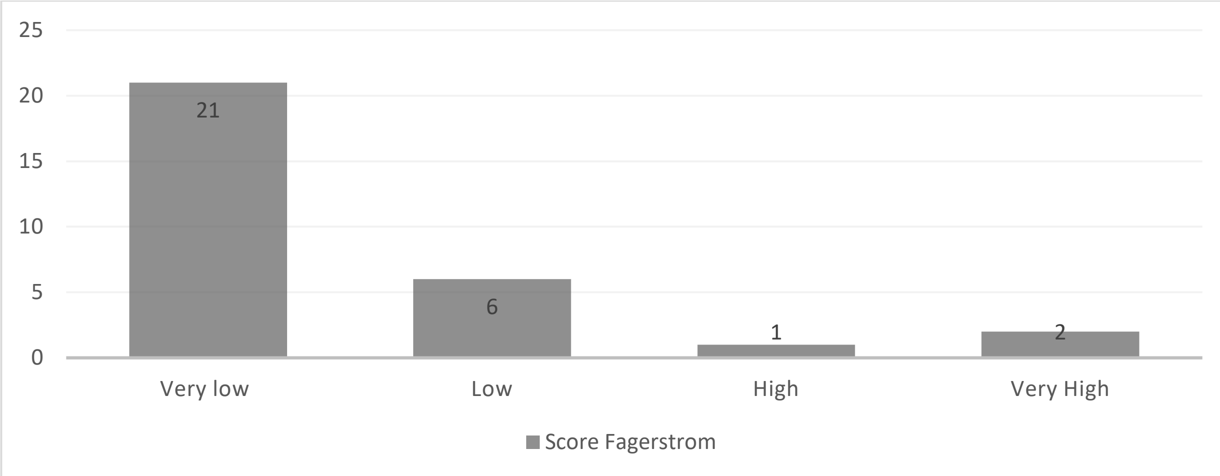

Figure 1. Fagerstorm score

All participants' mean Vitamin D levels were 16.06 ± 3.14. The mean value of Vitamin D in the nonsmoker's group was 16.9 ± 2.9 ng/ml and a median of 16.7 ng/ml with a minimum and maximum range of 12-25.3 ng/ml. The mean value of Vitamin D in the smoker's group was 15.21 ± 3.15 ng/ml with a median of 15.8 ng/ml, a minimum range, and a maximum of 8.6-22 ng/ml (table 2). Data transformation was carried out by log and unpaired T-test analysis, resulting in a statistically significant difference between Vitamin D's value in smokers and nonsmokers (p = 0.029).

Table 2. Comparison between Vitamin D level of smokers and nonsmokers

	Smokers (mean ± SD)	Nonsmokers (mean ± SD)	P value
Vitamin D	15.21 ± 3.15	16.9 ± 2.9	**0.029***

* statistical test of unpaired T-test from statistical value transformed using the log function

We also compared exhaled CO levels in smokers and nonsmokers. Exhaled CO level for all participants did not have a normal distribution, with a mean of 11.35 ± 10.79 ppm. The Mean exhaled CO level in the nonsmokers was 5.4 ± 2.51 ppm. Exhaled CO level in the smoker's group had a mean of 17.3 ± 12.54 ppm with a median of 14.5 ppm. A comparison of exhaled CO levels in smokers and nonsmokers was statistically significant (p = 0.000) shown in table 3.

Table 3. Comparison of exhaled CO levels between smokers and nonsmokers

	Smokers Median (min-max)	Nonsmokers Median (min-max)	P value
Exhaled CO (ppm)	14.5 (3-49)	5 (3-14)	**0.000***

*Mann-Whitney test

4. DISCUSSION

This research was conducted using a cross-sectional method. The study duration was one week to collect the number of subjects according to the inclusion criteria. The subjects examined were healthy employees. Vitamin D examination was not included in a routine health examination; hence for all participants, this was their first examination. Some of the variables assessed in this study, such as gender, workplace, and work environment, were limited by the initial design to display homogeneous research subjects. Gender, age,

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BMI, Vitamin D intake, and sun exposure are confounding factors affecting Vitamin D levels. Basic health research in 2013 stated that smoking prevalence was higher in men than women [1].

This study found an age range of 20-34 years old divided into two groups: 20-29 years old and ≥30 years old. This study is different from the study of [6] who divided the age group into 20-29 years old, 30-39 years old, and 40-50 years old, with a total number of 181 participants. This study did not include age groups of 30-39 years old and 40-50 years old as the age range was limited to obtain participants of similar characteristics. In this study, a similar age group was obtained so that age as a confounder in Vitamin D assessment could be neglected.

In this study, the WHO BMI criteria used was the one for the Asia Pacific region, with a standard value of 18.5 - 22.9 kg/m^2. This study did not include obesity status in the inclusion criteria because there is an association between obesity and 25(OH)D deficiency. Obesity affects the bioavailability of Vitamin D. Cholecalciferol derived from biosynthesis in the skin and intake will enter the blood circulation and bind to DBP, which then will be transported to the body tissues, especially the liver, to be processed further into an inactive form of 25D(OH)D and a small portion to be stored in adipose tissue. In obesity, there is an increase in adipose tissue hence an increased ability to store cholecalciferol, consequently, a decrease in the level of cholecalciferol transported to the liver, which ultimately causes a decrease in 25(OH)D production [7].

This study found the mean value of Vitamin D intake was 3.6 ± 2.6 mcg with a median of 2.6 mcg/day. The minimum value of Vitamin D intake was 0.3 mcg, and the maximum value of 12.2 mcg. A study by [8] found that the median Vitamin D intake in the study subjects was 8.5 mcg/day with a range of 0.2–327.6 mcg/day. [8] study compared the intake value with Indonesian Recommended Dietary Allowance (RDA) 2004, which recommends a value of 5 mcg/day as a daily intake for adults so that more Vitamin D intake (≥ 90% RDA) was obtained in 75% of the study subjects. This study used RDA in 2013, which recommends a daily Vitamin D intake of 15 mcg, so based on this recommendation, all respondents were declared to have insufficient Vitamin D intake. Vitamin D intake was lacking in all samples, thus eliminating intake as a confounder in Vitamin D assessment in both groups of participants.

Subjects assessed in this study were employees who worked indoors and were not exposed to the sun. A total of 48 participants had a zero-sun exposure score, and twelve participants had an exposure score > 0. There was no variation in either clothing or activities among respondents. There was no variation in the subjects' jobs. Subjects often wear clothes that cover the upper and lower limbs, following the regulations regarding the official dress code. This study assessed sun exposure with a questionnaire that only counts for the length of exposure and area of limbs exposed to sunlight for one week, while the questionnaire could not account for other factors. Subjects with sun exposure score > 0 had exposure scores on holidays only, namely Saturday and Sunday, and some periods during walks between spaces in the same working environment but different buildings, although there were no significant differences between exposure scores group with a score of 0 and > 0.

Assessment of sun exposure is often associated with 25(OH)D levels. Various evidence suggests Vitamin D deficiency often occurs even in tropical areas with high sun exposure, such as in Asian countries. Asian countries developing into industrialized countries causes most urban citizens to have more indoor jobs, hence not being exposed to sunlight [4].

The high prevalence of Vitamin D deficiency in young adults aged 18-29 can be explained by the low consumption of Vitamin D and the tendency to avoid sun exposure [9]. The same thing was also found in this study. Subjects in our study were indoor employees who had similar characteristics as the study subjects of [9] which were students who spent part of their time in classrooms. [9] research concluded that subjects aged 18-29 had the same risk as those older for Vitamin D deficiency. Vitamin D supplementation is recommended in subjects with a tendency to avoid sun exposure.

Currently, there are two methods of assessing sun exposure: the personal observation questionnaire and measurement with UV dosimetry. These methods have not become the gold standard in assessing sun exposure. Measurement with UV dosimetry provides objective quantitative data, but ambient UV level is influenced by various factors such as latitude, season, and daily time so this method cannot be used as a gold standard. Both methods cannot determine the UVB photon levels of sunlight reaching the skin, which are the main determinants of Vitamin D synthesis in the skin [10].

This study measures 25 (OH) D as a Vitamin D level parameter because this is the recommended form in assessing individual Vitamin D status. 54 participants (90%) in this study had Vitamin D deficiency. This study used a cutoff point of ≥30 ng/ml to indicate Vitamin D sufficiency and <20 ng/mL to indicate Vitamin D deficiency, similar to a study by [6] which found a 50.3% prevalence of Vitamin D deficiency with stated cutoff. There was no participant with sufficient Vitamin D or ≥30 ng/ml; thus, for statistical analysis purposes, the group is divided into two: <20 ng/ml and ≥20 ng/ml. Nimitphong reported that the prevalence of Vitamin D deficiency is >70% in South Asia and varies between 6-70% in Southeast Asia. This conclusion is contrary to the assumption that Vitamin D deficiency does not occur in countries with sufficient sunlight, such as Asia [11].

This study found a significant difference between the value of Vitamin D of smokers and nonsmokers through unpaired T-test analysis on data that had been log-transformed. In smokers, the mean value of Vitamin D was lower than that of nonsmokers. Kassi's research found that smoking status was the only significant variable in determining the difference in Vitamin D values in the group tested. There is an increase in the likelihood of Vitamin D deficiency among smokers. In logistic regression analysis in Kassi's study, it was found that young people (20-29 years old) have a 58% chance of Vitamin D deficiency compared to nonsmokers of the same age [6].

In this study, among all participants, there were no participants who had sufficient Vitamin D (≥ 30 ng/ml). [9] research stated that young adults aged 17-35 do not drink adequate amounts of milk and avoid sun exposure because of the assumption that skin cancer would occur due to exposure to sunlight. This condition increases the risk of Vitamin D insufficiency. Another concern is that there is some source suggesting Vitamin D receptor polymorphisms as the cause of Vitamin D deficiency among healthy adult subjects.

One of the essential examinations in the smoking cessation program is measuring CO levels. Currently, portable CO monitor is widely used in clinical practice making it easier to assess CO level. Clinicians can explain the effect of exhaled CO levels in assessing the impact of smoking on patients in the smoking cessation program. The measurement of exhaled CO using the smokerlyzer piCO device provides immediate results and is a non-invasive smoking status assessment. A study by [12] stated that measurement of expiratory CO levels is a fast, non-invasive, easy, and practical test to assess a patient's smoking status and is proportional to measurements of COHb levels in the blood [12].

This study found that the average exhaled CO level in smokers was greater than nonsmokers. The difference between CO levels in smokers and nonsmokers was statistically significant ($p = 0.000$). This result is similar to a study by Inayatillah in terms of exhaled CO level in the smoker group that is higher than the nonsmoker group. In the study by [13] median CO levels in smokers were 22 (4.48) ppm, while in the nonsmoker group, the average CO level was 5.83 ± 1.82 ($p = 0.000$). Indonesian Association of Pulmonologists stated that the cutoff of <4 ppm is the criteria for nonsmokers while ≥ 4 ppm for smokers [5].

5. RESEARCH LIMITATIONS

This study did not examine kidney and liver function to assess the influence of these two organs in the process of Vitamin D synthesis and did not check the patient's cholesterol level. Cholesterol is a precursor for Vitamin D synthesis. Researchers were allowed to view the patient's medical record data through written permission that the researcher had submitted, but the age range of the subjects was 20-34 years old; therefore, no data on liver function, kidney function, and cholesterol were found on the patient's medical record. Examination of liver and kidney function was only performed on employees >35 years old. History of liver and kidney disorders was asked during anamnesis. There was no history of liver and kidney disorders among the participants; hence liver and kidney function was assumed to be normal. Vitamin D has various forms in the body. All forms of Vitamin D can be examined using the High-Performance Liquid Chromatography (HPLC) method. This method can measure the levels of Vitamin D3, 25 (OH) D3, and 25 (OH) D2 in a single sample, but there is no laboratory in the research location that is capable of conducting this examination; furthermore, this method is considerably expensive and requires laboratory technical expertise.

6. Conclusion

All study participants were male employees who worked indoors and had a diploma background; the average age was 25.6 ± 3.7 years old, had a nutritional status of normal BMI, and was at risk of being overweight, lack of daily Vitamin D intake and most were not exposed to sunlight. There is no significant difference between the characteristics of smokers and nonsmokers group. The mean Vitamin D level in the nonsmoker group was higher compared smokers group (16.9 ± 2.9 ng/ml vs. 15.21 ± 3.15 ng /ml), and there was a significant difference in the value of Vitamin D between the two groups ($p = 0.029$). The mean Vitamin D level in participants with normal BMI was higher than those with the risk of overweight BMI (16.98 ± 3.42 vs. 15.072 ± 2.51). The Mean exhaled CO of the smoker group was higher than in the nonsmoker group (17.3 ± 12.54 vs. 5.24 ± 2.51), and a significant difference was found between the two groups ($p = 0.000$).

7. Recommendation

Further research must be done to compare Vitamin D levels in smokers and nonsmokers.

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