Correlation between Nicotine Dependence to Exhaled Breath Carbon Monoxide Levels and TNF-α Serum Levels in Adolescents

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Abstract

Background: Nicotine addiction occurs rapidly in adolescents; 25% of adolescents who smoke show signs of addiction within 1 month. **Objective:** To analyze correlation between nicotine dependence to exhaled breath carbon monoxide levels and TNF-α serum levels. **Methods:** This study used cross sectional method conducted on October 2020. A Total subject 35 adolescents smoker, selected based on consecutive sampling. The level of exhaled breath CO of all participants who accepted to participate in the study was measured by piCO+ Smokerlyzer Bedfront. Nicotine dependency was measured by a six-item version of the Fagerstrom Tolerance Questionnaire. The serum levels of TNF-α were assessed by ELISA. **Results:** Average nicotine dependence were 0.37 ± 1.03, exhaled breath CO levels were 4.31 ± 2.91 ppm and TNF-α serum levels were 4.90 ± 1.64 pg/mL. There was not statistically significant correlation between nicotine dependence to exhaled breath carbon monoxide levels and TNF-α serum levels ($p = 0.439$ and $p = 0.595$, rescpectively). **Conclusion:** There was not statistically significant correlation between nicotine dependence to exhaled breath carbon monoxide levels and TNF-α serum levels.

Keywords: *nicotine dependence, exhaled breath carbon monoxide levels, TNF-α serum levels*

Introduction

The use of cigarettes by adolescents is still a major public health problem around the world. There are 1.2 billion smokers worldwide with around 600 million smokers in Southeast Asian countries, and more than 50% are young smokers. Many countries have made policies and programs that are quite effective in reducing smoking-related deaths, namely through education about the harmful effects of smoking, control of smoking at a young age also includes control of passive smoking, providing information on smoking cessation programs and increasing cigarette taxes and prices[1, 2].

Indonesia is the fifth largest producer of tobacco leaves and ranks fifth in the world in terms of cigarette use, after China, the United States, Russia and Japan. Based on the National Baseline Health Research, the prevalence of smoking among Indonesians aged 15 years and over increased from 34.2% in 2007 to 34.7% in 2010 and 36.3% in 2013. Smoker status among students is 20.3%, one of the five students were smokers (36.2% male and 4.4% female). In Indonesia, the age of onset of smoking is 8.9% at the age of 7 years or younger, 10.9% at the age of 8-9 years, 25.6% at the age of 10-11 years, 43.2% at the age of 12-13 years and 11.4% at the age of 14-15 years[3].

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Most of the smokers started smoking habits in adolescence before the age of 15 years(4). Nicotine dependence occurs rapidly in adolescents, 25% of adolescent smokers show signs of addiction within 1 month(5). The severity of nicotine dependence is important in the evaluation of smoking habits in smoking cessation programs. The Fagerstrom Test for Nicotine Dependence (FTND) and the Brinkman index (assessing the degree of smoking) are the most commonly used quantitative measures of nicotine dependence(6).

Cigarette smoke consists of gaseous components and particles that contain more than 4000 compounds, which are toxic and carcinogenic. Each puff of cigarette smoke contains about 1015 free radicals(7). When cigarette smoke is inhaled, carbon monoxide (CO) is absorbed through the lungs into the bloodstream and binds to hemoglobin to form carboxy-hemoglobin (COHb), whose levels in the blood can be measured as a marker of cigarette smoke absorption. Increased CO levels lead to tissue hypoxia, shortness of breath, fatigue and reduced exercise tolerance(8-10). Exhaled breath CO levels correlate with blood COHb concentrations so that the exhaled breath CO measurement method is preferred because it is not invasive and the examination procedure is easy. CO measured as blood COHb or in expired air has a sensitivity and specificity of about 90%(8, 11, 12).

Smoking can affect cellular and humoral immune responses and is associated with the release of proinflammatory mediators such as tumor necrosis factor (TNF-α), interleukin (IL) -1, IL-6, IL-8 and granulocyte macrophage colony stimulating factor (GM-CSF). TNF-α is a strong proinflammatory and one of the cytokines produced by alveolar macrophages, has pleiotropic properties as well as a major mediator of inflammation(7, 13). TNF-α can increase the production of metalloproteinases (MMPs) which are involved in airway inflammation and lung damage(13).

Exhaled breath CO can be used as a tool for assessing smoking status and evaluating the degree of nicotine dependence. Previous studies have shown a significant positive correlation between the amount of daily cigarette consumption and the CO levels in exhaled breath. Other studies have also shown a positive correlation between exhaled breath CO levels and FTND scores(14). Serum TNF-α levels were significantly higher in the smokers group than in the nonsmokers group, and there was a positive correlation between serum TNF-α levels and the number of daily cigarettes(13). This finding is important because the measurement of exhaled breath CO levels and serum TNF-α levels can be used as an easy tool in early detection of nicotine dependence so that it can form the basis of policy interventions in smoking cessation programs prior to clinical symptoms of respiratory disorders in adolescents. Based on this background, the researchers felt the need to conduct research to find a relationship between the degree of nicotine dependence on exhaled breath CO levels and serum TNF-α levels in adolescents.

Method

The participants of this study were students at SMK PGRI 4 Surabaya. The inclusion criteria for participants included men aged 16-19 years, smokers, WHO category normal body mass index (BMI) (18.5 - 24.9), and being cooperative when the examination procedure was carried out. Exclusion criteria included patients having both intra pulmonary and extra pulmonary diseases such as bronchiectasis, upper and lower respiratory tract infections, heart disease and allergic rhinitis.

This study used a cross sectional study design in the period August - October 2020. The number of participants in this study was 35 participants with consecutive sampling. All participants were examined for the degree of nicotine dependence on exhaled breath CO and serum TNF-α levels. The degree of nicotine dependence was measured using the Fagerstrom Test for Dependence (FTND) questionnaire where the assessments were categorized as follows: 0-3 (mild dependence), 4-6 (moderate dependence), and 7-10 (high dependence). The exhaled breath CO levels was measured using a portable CO measuring instrument Pico smokerlyzer, where the CO level of the expansion air was an indicator of the blood COHb level. The exhaled breath CO levels were categorized into 5 using the following cut of points: 0-4 ppm (non-smoker), 5-6 ppm (light or casual smoker), 7-10 ppm (smoker), 11-15 ppm (frequent smoker), and 16-26 ppm (addicted smoker). Meanwhile, serum TNF-α levels were checked using the ELISA method.

The data obtained were analyzed according to the objectives and scale of the variables studied. Statistical analysis using computer software. The analysis in this study was divided into 2, namely descriptive and bivariate analysis. Descriptive analysis of this research includes presenting the results descriptively using distribution tables, mean, standard deviation, maximum value and minimum value. Bivariate analysis of this study to analyze the relationship between the degree of dependence of nicotine on CO levels in exhaled breath was analyzed using the Spearman correlation test. The relationship between the degree of nicotine dependence on serum TNF-α levels was analyzed using the Spearman correlation test. Statistical analysis was declared significant if $p < 0.05$.

Result

Participant Characteristics

Most of the participants were 16 years old as many as 25 participants (71.4%) and most of the participants onset smoking at the age <15 years as many as 24 participants (68.8%). Most of the participants were regular smokers as many as 19 participants (54.3%) and most of the participants also used kretek cigarettes as many as 23 participants (65.7%). The majority of participants did shallow suction (94.3%) where most of the last participants smoked for >5 hours as many as 27 participants (77.1%). The majority of the participants received Fagerstrom scores in the range 0-3 (94.3%) and the mean Fagerstrom score was 0.37 ± 1.03 (table 1).

The mean duration of smoking for the participants was 2.95 ± 1.58 years, with the shortest duration being 1 year and the longest being 7 years. The average of exhaled breath CO level was 4.31 ± 2.91 ppm with a minimum value of 1 ppm and the highest value of 13 ppm. Meanwhile, the mean serum TNF-α level was 4.90 ± 1.64 pg/ml with a range of 2.49 – 8.72 pg/ml.

Correlation of Smoking Onset and Duration of Smoking to Nicotine Dependence

Based on the correlation analysis between smoking onset and nicotine dependence, the value of $r = -0.115$ and $p = 0.511$ was obtained. Meanwhile, the correlation analysis between smoking duration and nicotine dependence found $r = 0.123$ and $p = 0.480$. Based on the above analysis, it can be concluded that there is no significant relationship between smoking onset and nicotine dependence and smoking duration with nicotine dependence.

Correlation of Smoking Status, Last Smoking Time and Types of Cigarettes to Exhaled breath CO Levels

Based on the analysis, there were significant results between smoking status and exhaled breath CO levels ($r = 0.528$; $p = 0.004$) and the last time smoking with exhaled breath CO levels ($r = 0.556$; $p = 0.001$). Different results were obtained in the correlation analysis between types of cigarettes and exhaled breath CO levels where there was no significant correlation between the two ($r = 0.318$; $p = 0.269$; table 2).

Correlation of Degree of Dependence of Nicotine on Exhaled breath CO Levels

Most of the participants had a degree of nicotine dependence of 0 and had exhaled breath CO of 0-4, of which 21 participants (87.5%). Based on the analysis, it was found that there was no significant correlation between the degree of nicotine dependence and exhaled breath CO levels ($r = 0.135$; $p = 0.439$; table 3).

Correlation between Smoking Status and Last Time Smoking on Serum TNF-α Levels

The mean TNF-α value of the participants in the regular smoker group was 4.77 ± 1.42 pg/mL and in the Occasional smoker group it was 5.07 ± 1.92 pg/mL ($p = 0.595$). Meanwhile, the mean TNF-α value of the participants in the group who last smoked <5 hours was 4.81 ± 1.40 pg/mL and the last group smoked ≥5 hours was 4.94 ± 1.74 pg/mL ($p = 0.848$; table 4).

Correlation between Degree of Nicotine Dependence on Serum TNF-α Levels

Based on the analysis, the results obtained were $r = 0.019$ and $p = 0.913$, so it can be concluded that there was no significant correlation between the degree of nicotine dependence on serum TNF-α levels.

Table 1. Characteristics of participants

Characteristics	n (%)
Age	
16 years old	25 (71.4)
17 years old	10 (28.6)
Onset of smoking	
<15 years	24 (68.8)
≥15 years	11 (31.4)
Smoking status	
Reguler smoker	19 (54.3)
Occasional smoker	16 (45.7)
Types of cigarettes	
Mild	12 (34.3)
Kretek	23 (65.7)
Suction pattern	
Shallow suction	33 (94.3)
Deep suction	2 (5.7)
Last time smoking	
<5 hours	8 (22.9)
≥5 hours	27 (77.1)
Skor Fagerstrom	
0-3	33 (94.3)
4-6	2 (5.7)
7-10	0 (0.0)

Table 2. Correlation between Smoking Status, Last Smoking Time and Type of Cigarette on CO Levels in Expiratory Air

Variables	Expiratory Air CO Levels				r	p
	0-4	5-6	7-10	11-15		
Smoking status Reguler smoker Occasional smoker	8 (42.1) 16 (100.0)	4 (21.1) 0 (0.0)	6 (31.6) 0 (0.0)	1 (5.3) 0 (0.0)	0.528	0.004
Last time smoking <5 hours ≥5 hours	2 (25.0) 16 (59.3)	1 (12.5) 4 (14.8)	5 (62.5) 6 (22.2)	0 (0.0) 1 (3.7)	0.556	0.001
Types of cigarettes Mild Kretek	6 (46.2) 18 (78.3)	2 (15.4) 4 (11.4)	3 (23.1) 6 (17.1)	2 (15.4) 1 (2.9)	0.318	0.269

Table 3. Correlation of degree of nicotine dependence with expansion air CO levels

Expiratory Air CO Levels	Degree of Nicotine Dependence				r	p
	0	1	2	4		
0-4 5-6 7-10 11-15	21 (87.5) 4 (100.0) 4 (66.7) 1 (100.0)	1 (4.2) 0 (0.0) 0 (0.0) 0 (0.0)	2 (8.3) 0 (0.0) 0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0) 2 (33.3) 0 (0.0)	0.135	0.439

Table 4. Differences in serum TNF-α based on smoking status and time of last smoking

Variables	n	mean ± SD	p
Smoking status Reguler smoker Occasional smoker	 19 16	 4.77 ± 1.42 5.07 ± 1.92	0.595
Last time smoking <5 hours ≥5 hours	 8 27	 4.81 ± 1.40 4.94 ± 1.74	0.848

Discussion

Most smokers initiate smoking habits in adolescence before the age of 15 years and nicotine dependence occurs rapidly in adolescents, 25% of adolescent smokers show signs of addiction within 1 month[4, 5]. Smoking behavior in adolescents can be influenced by several factors, including adolescent development and psychological satisfaction. There are 4 stages in smoking behavior, the first is the preparatory stage, where a person gets a pleasant picture of smoking by hearing, seeing or reading results and arouses an interest in smoking. Second, the initiation stage, which is the stage where a person will continue or not the smoking behavior. Third, the stage of becoming a smoker, which is when a person has consumed 4 cigarettes per day, has a tendency to become a smoker and fourth, the maintenance of smoking stage, which is smoking has become a part of self-regulation and smoking is done to get a pleasant physiological effect[4].

When a person inhales cigarette smoke, nicotine within 7 seconds can diffuse easily into brain tissue. Nicotine stimulates the dopamine pathway in the mesolimbic system of the brain. Nicotine binds to nAChRs, then causes the release of dopamine in the nucleus accumbens and subsequently releases other neurotransmitters such as norepinephrine, acetylcholine, glutamate, serotonin, beta endorphins and GABA which produce various physiological effects including behavioral arousal and nerve activation[15].

Nicotine is an example of a substance that can cause addiction in its users. Addiction can take the form of physical or psychological dependence[16]. Physical dependence is indicated by two factors, namely the factor of tolerance and abstinence or withdrawal symptoms. Tolerance is the decrease in the efficacy of the addict after repeated use. If a person has experienced tolerance to a type of drug, the amount (dose) of addiction needed is getting bigger and bigger to give the same effect or effect. If an addict wants to stop using it, he will experience withdrawal symptoms (withdrawal syndrome or withdrawal) or abstinence[15].

Measurement of the degree of nicotine dependence in this study using the FTND questionnaire which aims to diagnose the degree of physical dependence among smokers. One of the drawbacks of FTND is that it does not differentiate well among smokers with very low dependence[16]. When exposed to nicotine, dopamine in the brain increases, thereby amplifying brain stimulation and activating reward pathways. This reward system causes the desire to reuse and triggers physical dependence on nicotine more quickly and intensely so that smokers will consume more and more cigarettes per day and smoke deeper cigarettes to get the desired sensation[17].

Cigarette smoke consists of gaseous components and particles. CO is a gaseous component produced from incomplete combustion of cigarettes and can be absorbed quickly immediately after inhalation. Then the CO enters the bloodstream and binds to hemoglobin to form COHb[7, 8, 18]. Exhaled breath CO levels correlate with blood COHb concentrations so that the exhaled breath CO measurement method is preferred because it is not invasive and the examination procedure is easy. The value of exhaled breath CO levels is one of the clinical value tools to determine the degree of nicotine dependence[19].

There was no significant correlation between the degree of nicotine dependence on exhaled breath CO levels. These results contradict previous studies which also showed a positive correlation between the degree of nicotine dependence on exhaled breath CO levels. The duration of smoking will further increase dependence on nicotine in which the relationship between the two variables has also been shown to be significant[20].

The absence of a correlation between the degree of dependence of nicotine on CO levels in exhaled breath in this study could be caused by one or both of these parameters. There are several factors that affect the measurement of exhaled breath CO levels, including the type of cigarette, the suction pattern, the number of cigarettes per day, the last time smoking, the measurement time and the environment in which they live[21]. In this study, we found a significant correlation between smoking status and the last time smoking on exhaled breath CO levels. However, it is not sufficient to support a correlation between the degree of nicotine dependence on exhaled breath CO levels. This can be due to the fact that the majority of types of cigarette suction in this study are shallow. The concentration of nicotine in the blood depends on the type of smoker's suction, the deeper the suction, the higher the concentration of nicotine in the blood. This then explains that the higher the concentration of nicotine in the blood, the nAChRs of the α4β2 subtype will increase through an upregulation mechanism which will facilitate nicotine dependence[5].

Excessive inhalation of cigarette smoke per day to meet tolerance needs and prevent withdrawal symptoms, can activate epithelial and immune cells in the airway, then will induce the secretion of proinflammatory factors that will lead to the recruitment of other immune cells, such as neutrophils, macrophages, T cells and dendritic cells[22]. TNF-α is one of the proinflammation cytokines produced by alveolar macrophages. Inflammation is responsible for airflow obstruction because inflammation causes bronchoconstriction by releasing inflammatory mediators that can act directly on bronchial smooth muscle[23].

There was no significant correlation between the degree of nicotine dependence on serum TNF-α levels. This can be caused by the majority of students with a mild degree of dependence and shallow types of cigarette suction. These two factors lead to the lack of tolerance and efforts to prevent withdrawal symptoms so that there is no increase in the release of inflammatory mediators, namely TNF-α in serum due to excessive cigarette smoke inhalation[15, 22].

Conclusion

The mean degree of adolescent nicotine dependence was 0.37 ± 1.03. The mean CO content of exhaled breath in adolescents was 4.31 ± 2.91 ppm. The mean serum TNF-α level of adolescents was 4.90 ± 1.64 pg/mL. There is no correlation between the degree of dependence of nicotine on exhaled breath CO levels. There was no correlation between the degree of nicotine dependence on serum TNF-α levels.

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