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Research Paper

Factors Affecting the Serum Cotinine Level of Male Smokers in Malang, Indonesia

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ABSTRACT

Background: In tobacco, nicotine is the primary toxic agent that causes health problems. As a primary metabolite of nicotine, cotinine is frequently used as an accurate measure of exposure to tobacco smoke. This study aims to find the factors affecting the serum cotinine level of male smokers in Malang, Indonesia.

Methods: This cross-sectional study was conducted on 183 men who smoke regularly in Malang, Indonesia. They were selected by a purposive sampling method. Nicotine dependency and cotinine level were measured using the Fagerstrom test for nicotine dependence and the human cotinine ELISA Kit, respectively. Multiple linear regression analysis was performed to determine the effect of age, smoking duration, cigarette type, and the nicotine dependence on the continue level. ANOVA and independent t-test were also used for testing the study hypothesis. The significance level was set at 0.05.

Results: Based on the ANOVA results, the factors of age, smoking duration, type of cigarette, and nicotine dependence together had a significant effect on the cotinine level ($P<0.001$). Based on the independent t-test results, only the age factor had a significant effect on the cotinine level ($P<0.001$).

Conclusion: Older male smokers may have higher cotinine level. Other factors including duration of smoking, type of cigarette, and nicotine dependence have no significant effect on cotinine level of male smokers.

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1. Introduction

Cardiovascular diseases are considered as a significant cause of morbidity and mortality worldwide. According to data from the World Health Organization (WHO) [1], cardiovascular diseases account for most death cases (About 17.9 million) each year worldwide, followed by diabetes (1.5 million), respiratory disease (4.1 million), and cancer (9.3 million). Risk factors of cardiovascular diseases include family history, hypertension, diabetes, smoking, and low-density lipoprotein (LDL) level >130 mg/dL [2]. Smoking is the only preventable cause of premature death. Death from tobacco smoking is mainly due to the development of respiratory and cardiovascular diseases [3]. Smoking is responsible for 80% of lung cancer deaths worldwide [4]. Currently, China (26.5 million), India (19.8 million), and Indonesia (9.91 million) are the top three countries with high smoking rate. Globally, near 155 million people aged 15-24 smoked tobacco in 2019 [5]. According to the WHO, 6 million people die from tobacco smoking each year worldwide. The number is expected to reach 7 million by 2020 and over 8 million by 2030. It is estimated that, by the end of the 21st century, tobacco smoking will kill one billion people [6]. The dangers of smoking are related to the existence of toxic chemicals in tobacco. Tobacco contain more than 7,000 chemicals, most of which are poisonous when the tobacco is burned [7]. In tobacco, nicotine is the primary toxic agent, causing health problems. Nicotine is an alkaloid derived from many tobacco plants, where it acts as a pesticide [8]. A typical cigarette contains 1-1.5 mg of nicotine absorbed by the smoker. When the smoke is inhaled, nicotine enters the lung, blood steam, and brain in only 15-20 seconds [9]. In the liver, 70-80% of absorbed nicotine is quickly converted to cotinine. As a primary metabolite of nicotine, cotinine is frequently used as a quantifiable measure of exposure to tobacco smoke [10], since it has a longer half-life than nicotine and has larger quantities in bodily fluids than nicotine [11]. The level of nicotine exposure in the human body over three to four days may be measured by the cotinine level [12]. The development of health problems and nicotine dependence after tobacco smoking is most likely due to nicotine metabolism [13]. Until now, few studies have investigated the factors that affect cotinine levels in the body. Previous studies have shown that the use of different cigarette types affect cotinine level in urine [14, 15]. The age factor also seems to affect cotinine plasma level due to high nicotine intake and aging-related functional decline [16]. This study aims to examine the factors affecting cotinine level among active male smokers. It is hypothesized that age, duration of smoking, cigarette type, and nicotine dependency level affect the cotinine level.

2. Materials and Methods

Study design and participants

This is a single-group study with a cross-sectional design that was conducted in the laboratory of a clinic at Saiful Anwar Hospital, Malang, Indonesia during February-June 2022. The minimum sample size was determined 111 using G*Power software, version 3.1 by considering a test power (1-β error probability) of 95%, an effect size (d) of 0.3, and an error probability (α) of 0.05. Participants were 183 active male smokers who were selected using a purposive sampling method and based on the inclusion criteria: Age 20-40 years, declaring consent, and not receiving any medication therapy during the study. The exclusion criteria were: No history of cardiovascular disease, diabetes mellitus, or dyslipidemia.

Measures

The nicotine dependency level is estimated using The Fagerstrom test for nicotine dependence (FTND) test recommended by the American Association of Respiratory Care [17]. A score >8 show high dependency, a score of 5-7 indicates moderate dependency, a score of 3-4 shows low-to-moderate dependency, and a score of 1-2 indicates low dependency. Then, each subject underwent venipuncture to collect 2.5 mL of peripheral blood. The blood was then placed into a tube containing ethylene diamine tetra acetic (EDTA). The plasma was collected after centrifuging the blood for 15 minutes at a speed of 2000-3000 rpm at a temperature of 2-8°C to measure the cotinine level. The amount of cotinine was then measured in the samples using the ELISA Kit (Human Cotinine, Bioassay Technology Laboratory, E2043Hu).

Statistical analysis

We used multiple linear regression analysis using WarpPLS software, version 7 to predict the effect of age, duration of smoking, type of cigarette, and nicotine dependency on cotinine level. Before carrying out this analysis, the normality of data distribution, multicollinearity, heteroscedasticity, and autocorrelation assumptions were examined. Furthermore, simultaneous and partial hypothesis testing was carried out using ANOVA and independent t-test. The significance level was set at 0.05.

3. Results

Table 1 presents the characteristics of respondents in terms of demographic factors, smoking behaviour, and cotinine level.

Based on Table 1, most of participants (n=79, 43.1%) were at the age group of 26-35 years. Most of them were smoking for 6-10 years (n=60, 32.8%). The most common type of smoked cigarette was from Mild brand (n=123, 67.2%). Most of them had low nicotine dependency (n=88, 48%). Most of them (n=135, 73.8%) had no underlying diseases. Furthermore, most of them (52%) are moderate smokers (11-20 cigarettes/day). In this study, cotinine level of active smokers was 32.84±24.97 ng/mL.

The Kolmogorov-Smirnov test was used to check if data distribution was normal. This test is used when there are more than 30 pieces of data. The P was 0.055, indicating that the distribution was normal. The multicollinearity test was used to check if there was a connection between the variables used in the regression model. The result is determined by using the variance inflation factor (VIF) and the tolerance value. A tolerance value is <0.1 or VIF >10 indicates that multicollinearity exists. Table 2 shows the findings of multicollinearity test. As can be seen, there were no signs of multicollinearity in the study variables.

The heteroscedasticity test was conducted to determine whether there is a variance inequality between different residuals in the regression model. The lack of heteroscedasticity is a sign of a robust regression model. The heteroscedasticity test results are shown in Table 3. The assumption of homoscedasticity was met since all variables had a P>0.05, indicating no signs of heteroscedasticity.

The Durbin-Watson (DW) test was used to assess whether residuals in one observation are correlated with other data in the regression model. Lack of autocorrelation show a good regression model. if the Durbin-Watson (DW) statistic (D) falls within the D_U<DW<4-D_U range, there is no autocorrelation. Table 4 shows the results of the autocorrelation test. As can be seen, the D statistic is 2.069. The D_U (upper bound D) is 1.791 and P=0.05. Therefore, 4-D_U has a value of 2.208. Thus, no autocorrelation exists because the D value is between 1.791 and 2.208 (1.791<2.06<2.208).

The degree to which the model explains the dependent variable's variation is shown by the coefficient of determination (adjusted R^2). In our study, the R^2 value was 0.152 (Table 5). It indicates that the four independent variables (age, smoking duration, type of cigarette, and nicotine dependency) can explain 15.2% of the variation in cotinine level. The remaining 84.8% is explained by other factors. Multiple linear regression analysis was performed to predict nicotine levels based on age, smoking duration, cigarette type, and nicotine dependency (Table 6).

The linear regression equation (Equation 1) that can be compiled based on Table 6 is as follows

1. $Y\ (cotinine\ level)=-11.728+0.950X_1+0.399X_2+3.095X_3+1.885X_4$

Where, X_1=Age, X_2=Smoking duration, X_3=Cigarette type, and X_4=Nicotine dependence.

The equation indicates that: A) Cotinine level is -11,728 ng/mL, if there is no change in age, smoking duration, cigarette type, and nicotine dependency; B) One year increase in the smoker's age can increase cotinine level by 0.950 ng/mL; C) One year increase in the duration of smoking can increase the cotinine level by 0.399 ng/mL; D) Kretek cigarettes with higher nicotine level compared to mild cigarettes can increase cotinine level by 3,095 ng/mL; F) One level increase in nicotine dependency can increase the cotinine level by 1.885 ng/mL.

ANOVA was used to determine the effect of four independent variables on the dependent variable (cotinine level). The results are shown in Table 7. Since P<0.05 (<0.001), at least one of the independent variables could affect the cotinine level. T-test was carried out to determine the effect of each independent variable on the cotinine level (Table 8). The results showed that only age factor had a significant effect on the cotinine level (P<0.001). Smoking duration, cigarette type, and nicotine dependency had no significant effect (P>0.05).

4. Discussion

Second-hand smoke is a significant public health hazard worldwide [18]. Burning tobacco generates many mutagens including 70 carcinogens. There are two types of mutagens and carcinogens, one type damage DNA directly and other type is procarcinogens that require metabolic activation to damage DNA [19]. A study conducted by Shrestha et al. showed that tobacco use is a risk factor of cardiovascular diseases, which

Table 1. Characteristics of participants (n=183)

Variables		No. (%)/Mean±SD
		Respondents
Age	≤20	12(6.6)
	21-25	48(26.2)
	26-35	79(43.1)
	36-40	20(11)
	≥40	24(13.1)
Smoking duration (y)	≤1	12(6.6)
	2-5	51(28)
	6-10	60(32.8)
	11-20	52(28.4)
	21-30	4(2.1)
	≥30	4(2.1)
Cigarette type	Mild	123(67.2)
	Kretek	48(26.2)
	E-cigarette	12(6.6)
Nicotine dependency	Low	88(48)
	Low to moderate	47(25.7)
	Moderate	20(11)
	High	28(15.3)
History of underlying diseases	None	134(73.2)
	Hypertension	12(6.6)
	Bronchitis	8(4.4)
	Asthma	12(6.6)
	Cardiovascular disease	8(4.4)
	Gastritis	5(2.7)
	Dyslipidemia	4(2.2)
Cigarette consumption per day	≤10 (Low)	72(39.3)
	11-20 (Moderate)	95(52)
	≥20 (Heavy)	16(8.7)
Serum cotinine level (ng/mL)		32.84±24.97

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Table 2. Results of multicollinearity test

Model		Collinearity Statistics	
		Tolerance	VIF
Constant	Age	0.467	2.142
	Smoking duration	0.379	2.636
	Cigarette type	0.944	1.059
	Nicotine dependence	0.690	1.449

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is positively correlated with the cotinine level [20]. Smoking tobacco can also cause severe degeneration in the alveolar structures of epithelial tissue in the lung. Pathophysiological and biochemical findings confirm that the reduction of antioxidant enzymes during tobacco smoking can lead to the formation of supra-oxide radicals and cause damage [21].

Cotinine, as nicotine's primary metabolite, has been widely used as a measure of nicotine exposure [22]. Because of its extended half-life, cotinine has long been used as a biomarker for tobacco smoke exposure and can be detected in urine, saliva, or blood [23]. When exposed to tobacco smoke, nicotine attaches to particles in the smoke inhaled by active smokers and only in the vapour phase [24]. Nicotine remains in the body for about 2-3 hours, while cotinine remains for about 12-20 hours [25]. A small percentage of cotinine eliminated from the urine (approximately 10%) is degraded to trans-3'-hydroxycotinine and different products [26]. Cotinine is an inactive metabolite used for evaluating second-hand smoke and indicators of adherence to smoking cessation [27, 28]. However, current evidence states that cotinine activities imply that cotinine can mediate some psychoactive effects of nicotine [29]. High level of cotinine can affect oxidative stress and vascular inflammation [30].

Table 3. Results of heteroscedasticity test

Model	T	P
Constant	2.436	0.016
Age	-1.662	0.098
Smoking duration	0.752	0.453
Cigarette type	0.478	0.633
Nicotine dependence	1.489	0.138

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Table 4. Results of autocorrelation test

Model	R	R^2	Adjusted R^2	Std. Error of the Estimate	Durbin-Watson
1	0.491	0.242	0.224	0.162	2.069

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Table 5. Regression model summary

Model	R	R^2	Adjusted R^2	Std. Error of the Estimate
1	0.413	0.171	0.152	13.33997

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Table 6. Coefficients of the regression model (dependent variable: Cotinine level)

Model	Unstandardized Coefficients	
	B	Std. Error
Constant	-11.728	5.315
Age	0.95	0.176
Smoking duration	0.399	0.227
Cigarette type	3.095	1.669
Nicotine dependency	1.885	1.085

Table 7. ANOVA results

Model	Sum of Squares	Df	Mean Square	F	p
Regression	6513.710	4	1628.427		
Residual	31675.976	178	177.955	9.151	<0.001
Total	38189.685	182			

Table 8. Partial test results (t-test)

Variables	B	t	SE	P
Age	0.95	-2.207	0.176	<0.001
Smoking duration	0.399	5.399	0.227	0.081
Cigarette type	3.095	-1.756	1.669	0.065
Nicotine dependence	1.885	1.855	1.085	0.084

Our study shows that, in overall, cotinine level is affected by age, cigarette type, smoking duration, and nicotine dependency. However, in the partial test, it was found that only the age variable significantly affected cotinine level. Older age has an effect on high cotinine level. This may be related to the length of time they are exposed to cigarette smoke; older people tend to have more outdoor activities where there is high risk of exposure to cigarette smoke. Another reason is that older people have more prolonged smoking and more nicotine intake compared to younger people. Prior studies have reported that the cotinine level can increase with the increase in the number of cigarettes smoked daily [31, 32]. Increased cotinine level in older people may also due to decreased cotinine metabolic rate caused by reduced hepatic extraction of cotinine from the bloodstream [16].

In this study, it was found that the type of cigarette (mild, kretek, e-cigarette) had no significant effect on the cotinine level. A previous study also found no difference in nicotine or cotinine level between those smoking e-cigarettes and conventional cigarettes [14]. However, e-cigarette smokers have lower urinary cotinine level than conventional cigarette smokers, because e-cigarettes vary in nicotine level, from liquids that do not contain nicotine to those containing >20 mg/mL nicotine [14, 32, 33]. In our study, the effect of other factors such as the weight of smokers were not examined. BMI may also affect nicotine metabolism. The blood of a person with higher BMI may contain lower nicotine, because they have more blood or because their body fat absorb nicotine [34, 35].

5. Conclusion

Older smokers may have higher cotinine level than younger smokers. Further research is needed regarding the underlying mechanism. Other factors including duration of smoking, type of cigarette, and nicotine dependence separately have no significant effect on cotinine level. However, they together with age significantly affect cotinine level in male smokers.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Research Ethics Committee of Saiful Anwar Hospital (Code: 400/097/K.3./302/2021).

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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