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RESEARCH ARTICLE

Variation of Nicotine Metabolism in Adult Men in the Jakarta Clinical Laboratory

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Abstract

Objective: Nicotine, a mayor addictive constituent of tobacco plays a critical role in smoking addiction. Nicotine is primarily metabolized to cotinine. The objective of the research was to determine the variation of Nicotine metabolism in adult men in the Jakarta Clinical Laboratory. Methods: The research was done to 27 men aged 19-45 years old, which consisted of 6 smokers and 21 nonsmokers. Nicotine and cotinine of the plasma concentration 2 hours after chewing one piece of nicotine gum were determined by Liquid Chromatography-Mass Spectrometry (LC-MS) method. Results: The cotinine/nicotine ratio of the plasma concentration was calculated as an index of nicotine metabolism. Scatter plot cotinine/nicotine ratio of showed distribution heterogeneous (not homogeneous).Conclusion: It could be concluded that variation nicotine metabolism could be categorized to light nicotine metabolism (<0, 22), medium (0, 22 - 4, 19), and heavy (> 4, 19).

Keyword: *Nicotine metabolism, Cotinine/nicotine ratio.*

Introduction

Smoking has been known to cause health problems. This health disorder can be caused by nicotine from mainstream smoke and side-stream smoke from cigarettes smoked by smokers, so it is not only dangerous for smokers (active smokers) but also for people who are in cigarette smoke or passive smoking [1].Exposure to cigarette smoke contains millions of chemicals and free radicals that are toxic, both in the gas phase and in the tar phase. Nicotine as the main component of tobacco which is addictive is part of tar phase particles [2, 3]. After someone breathes cigarette smoke, within 10-19 seconds nicotine will reach the brain. But the presence of nicotine does not last long, this is because nicotine is in the form of its metabolites [4]. Nicotine metabolism occurs in the liver through the oxidation process by CYP2A6 cytochrome into cotinine [5, 7].The most nicotine metabolites are cotinine.

Then cotinine will be metabolized again to trans-3'-hydroxycotinine.

Nicotine is converted in the form of cotinine to approximately 70-80% in the blood [8].The amount of cotinine in various biological fluids is often used to estimate nicotine exposure in tobacco users [9].Nicotine can be metabolized quickly or slowly which will affect the degree of dependence on cigarettes [10].To determine nicotine and cotinine levels in plasma or urine it is important to study the pharmacokinetics of nicotine and cotinine. The half-life of nicotine in the body is 1-4 hours, while cotinine has a half-life of 12-22 hours. Nicotine and cotinine levels in biological samples can be analyzed using the liquid chromatography-tandem mass spectrometry method. The cotinine/nicotine ratio is determined as an index of nicotine metabolism [11-14].

The current study reports determining the variation of nicotine metabolism in adult men in the Jakarta Clinical Laboratory.

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Materials and Methods

Materials

The tools used in this study include: Greiner Bio-one® syringe, Liquid Chromatography - Luna® HILIC Mass Spectrometry, 5-100 µL micropipette, Pasteur pipette, tube rack, Sensi® glove, centrifugation, tube screw-caped, Turbo Vap® LV Caliper dryer, vial Chromatography, Vortex mixer.

Sample

In this study, the sample used was blood plasma from 27 research subjects who met the following criteria: Inclusion criteria (Men aged 19-45 years, willing to take part in research). Exclusion criteria (Suffering from Diabetes Mellitus, fasting glucose disorders, Liver disease, Heart disease, Kidney disease or having a history of the disease, Obese with Body Mass Index (BMI)> 25, Being on treatment therapy, and consume alcohol).

Chemicals

The ingredients used in this study were nicotine gum (Nicorette®), 70% alcohol, K3EDTA + 1.8 mg / 1 mL, acetonitrile, methanol. If not stated otherwise, all materials were analytical grades.

Research Methods

Collection and Storage of Samples

Blood samples were taken from 27 respondents. Respondents, who would be taken blood, previously fasted from food and drink, except water for 12 hours. Then the blood was taken before and 2 hours after chewing nicotine gum. The blood taken was 2x3cc K3EDTA vacuette tubes. The blood was centrifuged 3000rpm for 15 minutes, then stored in a -20 ^{0}C freezer.

Preparation of Samples and Reagents

Plasma samples were added acetonitrile, then homogenized and centrifuged 2000 rpm for 10 minutes. Then evaporated with nitrogen gas and re-dissolved with acetonitrile and then injected into the device.

Sample Checking

The samples obtained were then analyzed using Liquid Chromatography-Waters Alliance 2695® Separation System Mass Spectrometry which was previously calibrated

Results and Discussion

The study was conducted by determining the cotinine/nicotine ratio of 27 male respondents with an age range of 19-45 years who were included in the inclusion criteria and were screened through exclusion criteria. Each respondent was taken a K3EDTA blood sample. Respondents consisted of 6 smokers (22%) and 21 nonsmokers (78%). The data obtained were then analyzed statistically using the SPSS for Windows v.16 program [15].

Data on the general characteristics of respondents in this study were used to find out that, among smokers and non-smokers, the respondents had the same conditions before the examination. This could be seen from the values of screening parameters, were among 27 respondents had a liver function, kidney function, glucose levels, and blood pressure that did not differ much (uniformly), which can be seen in Table 1.

Table 1: General Characteristics of Respondents

Characteristics Respondent	Non-smoking	Smoking
Age (years)	29.43±6.23	30,00±7,239
BMI (Kg/m^2)	21.96±1.99	21,15±2.27
Smoking time (years)	-	12.33±6.38
Systolic (mmHg)	111.43±11.08	106.67±8.16
Diastolic (mmHg)	72.14±7.51	75±5.48
GOT (U/L)	25.72±11.42	25.17±8.03
GPT (U/L)	29.82±17.78	34.17±18.26
Fasting glucose (mg/dL)	85.73±7.16	85.83±6.55
Creatinine (mg/dL)	0.82±0.11	0.88±0.12
Passive smokers (men)		
0 day	18%	16.67%
1-4 days	55%	33.33%
5-7 days	23%	50%
Physical activity:		
High activity	61.90%	50%
Low activity	38.1%%	50%

Nicotine and cotinine concentrations 2 hours after chewing nicotine gum were analyzed using LC-MS. Nicotine was detected in plasma 27 respondents with a concentration range of 2.24-60.66 ng/mL (16.24 ± 12.55ng/mL), while cotinine concentration was 1.5-91.72 ng/mL (23.92 ± 10.17ng/mL). From these results, to determine nicotine metabolism by calculating the ratio of cotinine/nicotine, the ratio of cotinineotinin/nicotine was obtained from 0.08 to 4.9 (1.83 ± 1.10)

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Statistical analysis to test the normal distribution of nicotine concentration, cotinine concentration and the ratio of cotinine/nicotine concentration was carried out using Kolmogorov-Smirnova. The Kolmogorov–Smirnov test (K–S test or KS test) is a nonparametric test of the equality of continuous (or discontinuous, see Section 2.2), one-dimensional probability distributions that can be used to compare a sample with a reference probability distribution (one-sample K–S test), or to compare two [16]. The test results showed that the ratio of cotinine/nicotine was normally distributed (p = 0.200). To test the homogeneity of the population, a Scatter Plotin/nicotine ratio plot was made (Fig. 1). The results obtained showed that the cotinine/nicotine ratio among respondents in this study was heterogeneous (not homogeneous), meaning that there were variations in the cotinine/nicotine ratio among 27 respondents.

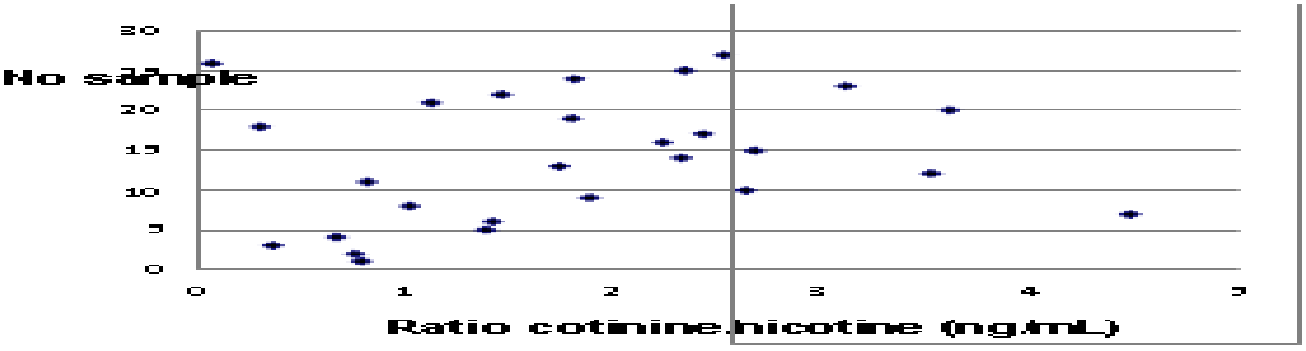

Fig. 1: Scatter Plot cotinine/nicotine ratio in population

This research was conducted on smokers and nonsmokers. However, both types of respondents had been uninformed under the same conditions, where smokers got interventions to fast smoking and drink coffee for 2 weeks. The fasting aimed to eliminate the bias from nicotine that had been present in the body before [17]. This could happen because nicotine contained in the body would be stored in lipids, so for the measurement of nicotine and cotinine levels in smokers, smokers should not smoke for 2 weeks [6, 13]. After adjusting the conditions, the two types of respondents were drawn blood before and after getting treatment. The treatment, in this case, was to chew nicotine gum (Nicorette®, 2mg nicotine). Taking blood before chewing nicotine gum aimed to find out how much nicotine exposure in the body of the respondent before taking the study, so that it could be corrected when taking blood after chewing nicotine gum [5].

After preparation, then analyzed, the results of nicotine metabolism were obtained or symbolized by the ratio of cotinine/nicotine, which varies. The population in this study was classified into several categories to see the difference in nicotine metabolism that occurred. The classification, among others, was based on smoking status, physical activity, and passive smoking. The average cotinine/nicotine ratio in smokers and nonsmokers were different (Fig. 2), which meant that the metabolic response was different. Even though there had been uniformity of conditions between smokers and nonsmokers, there were still differences. In smoker respondents, the ratio of cotinine/nicotine concentration was higher than that of nonsmokers. This meant that metabolism in smokers responders occurred faster than non-smokers because the nicotine would be metabolized faster to cotinine.

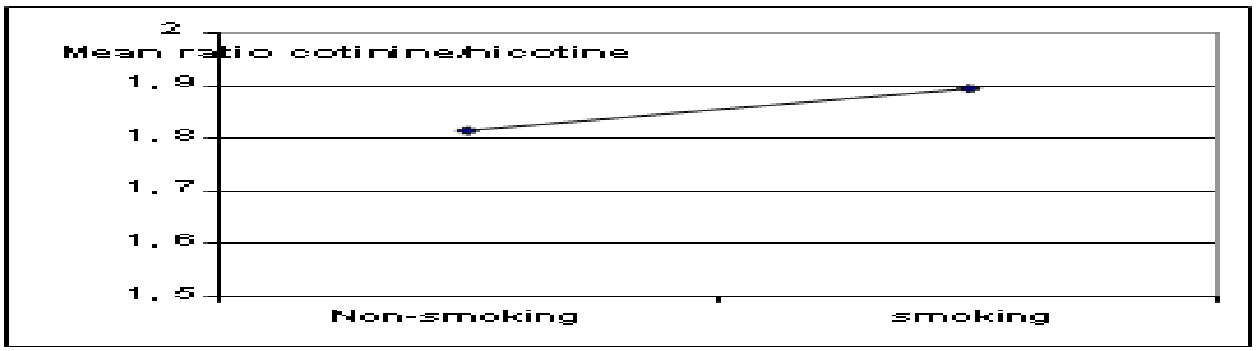

Fig. 2: Cotinine/nicotine ratio in Smokers and Non-Smokers

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When nicotine was sucked into the body, it directly stimulated the secretion of dopamine hormone on the nicotine acetylcholine α4ß2 receptor in the brain from 10 seconds. This dopamine hormone would cause smokers to feel comfortable and calm. Then nicotine was metabolized to become cotinine by the CYP2A6 enzyme. After the presence of nicotine in the receptor was reduced or even the availability was gone, smokers would return to smoking cigarettes until the levels of nicotine in the brain were fulfilled so that the comfort and calm emerge [18].

The difference in nicotine metabolism was also seen in passive smoking status in the past week. In heavy passive smokers, nicotine metabolism occurred faster than mild passive smokers. It was said that the more severe the passive smoking status, the higher the concentration of cotinine [19]. A person was more often exposed to nicotine, so regulation in his body would more quickly metabolize nicotine into cotinine. In this study, respondents were said to be heavy passive smokers, if the respondent was exposed to cigarette smoke more than 3 days in the past week before the analysis. The high concentration of cotinine would cause the ratio of cotinine/nicotine concentration to increase and this was said to be a fast metabolism (Fig. 3). All these differences were made possible by variations in nicotine metabolism that were influenced by genetic variation [5, 20, 21]. The occurrence of the CYP2A6 enzyme polymorphism, which in this case acts as an enzyme to metabolize nicotine into cotinine can result in a metabolic response that varies between individuals. The population in this study was different from other studies so the results were also different, as mentioned earlier, that nicotine metabolism was influenced by ethnicity [22].

Besides that the method of measuring nicotine and cotinine used was different, so the value was also different, it happens because, with different methods, the detection limits were different, the quantification limits and the sensitivity and specificity of the tool were different. Based on [5], there appeared to be a variation in nicotine metabolism among the respondents we studied. By doing a ratio analysis using 5 and 95 percentiles, we could see the range of nicotine metabolic index which could be categorized as a slow metabolic index (cotinine/nicotine ratio <0.22), moderate metabolic index (cotinine/nicotine ratio 0.22 - 4.19) and fast metabolic index (cotinine/nicotine ratio> 4.19) [23].

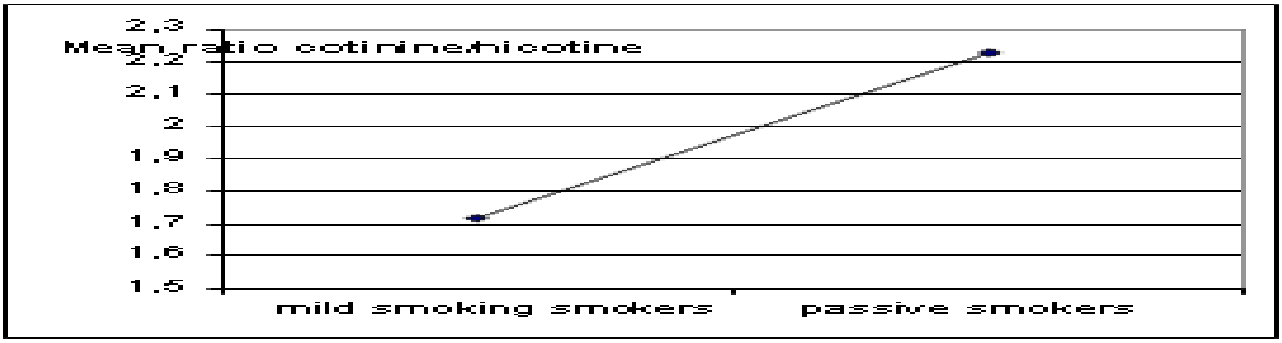

Fig. 3 Cotinine/nicotine ratio in respondents with Light and heavy passive smokers

Conclusion

Based on the results of a study of variations in nicotine metabolism in adult males it can be concluded that, the cotinine/nicotine ratio among 27 heterogeneous respondents (not homogeneous). This means that there are variations in nicotine metabolism among 27 male respondents from 19 to 45 years which can be categorized based on the ratio of cotinine/nicotine, namely mild nicotine metabolic index (<0.22), moderate (0.22-4.19), and weight (> 4.19). This study recommends doing further research where it is necessary to associate the influence of smoking on diseases caused by smoking so that it can be more clearly seen the effect of nicotine metabolism on these diseases.

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