Determination of Malondialdehyde (MDA) Levels In The Saliva of Man Smokers Age 45 - 59 Years

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Abstract.

Background: Smoking can cause health problems. In elderly smoking has become a habit in continuing the previous habit, even smoking habit tends to simply continue without the accompaniment of strong reasons, and these habits become addictive. Riskesdas research results showed that 65.6% of men smoked in 2007 and other research showed that 67% of men smoked in 2011, the prevalence of smoking continues to increase both men and women. Cigarette smoke can cause oxidative stress which is characterized by increased levels of MDA (malondialdehyde) as a marker of oxidative stress in the cell membrane. Objective: This study aimed to determine the effect of smoking on salivary MDA levels in smokers aged 45-59 years. Methods: The study was descriptive cross sectional analytic. The primary data used in this study. Determination of the population in the sample using quota sampling. The data obtained were analyzed with SPSS ver24 32bit for windows to see a comparison of MDA levels between smokers and nonsmokers. Results: From the analysis results data obtained in male smokers 0.503 ± 0.108, while nonsmokers 0.190 ± 0.047. Based on the results seen that MDA levels of salivary male smokers are higher than male nonsmokers with p value = 0.001. Conclusion: Increased levels of MDA can be found in smokers with the influence of smoking behavior conducted in men aged 45-59 years. This can be proven by the P value 0.001 that shows these results significantly, where there is a difference between MDA levels in smokers and nonsmokers.

Keywords: Cigarette; Malondialdehyde; Man and Saliva.

I.   INTRODUCTION

The smoking behavior of the population in Indonesia has not decreased from 2007 to 2013, in fact it increased from 34.2% to 36.3%. 64.9% of men and 23.55% of women still smoked cigarettes in 2013. The average cigarettes smoked per day by the population aged ≥10 years in Indonesia is 12.3 cigarettes, equivalent to one pack. The largest number of cigarettes smoked was found in Bangka Belitung, namely 18 cigarettes. The largest proportion of active smokers every day is more among male smokers than female smokers. (Riskesdas, 2013) Smoking can cause health problems. The nicotine content in cigarettes has a direct effect on the brain and causes dependence. Smokers also tend to experience behavioral changes such as increased stress levels, which are higher in smokers than non-smokers. When compared to non-smokers, brain cells in the receptor area show that smokers have fewer dopamine receptors, which are believed to play a role in addiction. (Doe J, 2009) In old age, smoking often becomes a habit, continuing a previous habit. Smokers often continue the habit without a compelling reason.

For example, when young, they smoked to gain the attention of the opposite sex or because of life's burdens, and then because the habit became addictive in old age.Smoking habitually can continue to change brain cells, resulting in stronger cravings and the risk of addiction, these cell changes can also cause changes in cognitive function, memory and intelligence. (Doe J, 2009) In one cigarette puff contains oxidant molecules in the form of short-lived ROS (Reactive Oxygen Species) which can change endogenous macromolecules and lipid phases into peroxynitrite, long-lived hydroquinone forming superoxide radicals that produce persistent oxidative stress. They enter cells up to the nucleus and cause oxidative damage. Cigarette smoke can also release iron from ferritin which has the potential to cause oxidative stress. (Aoshiba K, 2003; Doe J, 2009) Cigarette smoke also produces extracellular and intracellular antioxidants and increases blood antioxidant levels. (Aoshiba K, 2003) Oxidative stress is the level of imbalance between oxidants and antioxidants in cells or individuals. Changes in MDA, a biomarker of oxidative stress, will reflect changes in lipid oxidation levels. (Doe J, 2009).

II.   METHODS

This study is observational with descriptive analytical methods using a cross-sectional design. The sampling technique in this study was Nonprobability Sampling using Quota Sampling. The sample population in this study were men aged 45-59 years who met the inclusion and exclusion criteria of security guards, staff, and employees at Yarsi University. The type of data used was quantitative data obtained directly through laboratory tests. Data were collected through direct interviews using a questionnaire containing multiple choices in men aged 45-59 years who smoked and non-smokers. Then continued with saliva tests in the laboratory to compare MDA levels. The data obtained were first arranged in tabular form for further data analysis.

To measure lipid peroxidation levels, serum MDA is calculated based on a standard protocol with a method. This method is based on the reaction of MDA with TBA which forms a complex that can be measured by spectrophotometry. The method is 2 ml of saliva is collected in a tube and centrifuged to remove cell debris. 0.2 ml of the sample is added with 0.8 ml of phosphate buffered saline (pH 7.4). After that, 0.5 ml of 30% trichloroacetic acid (TCA) is added, then the sample is placed on ice for 2 hours and centrifuged at 2000 x g at 25ºC for 15 minutes. One ml of the supernatant is mixed with 0.075 ml of 0.1M EDTA and 0.25 ml of 1% barbituric acid in 0.05 N NaOH. Then the sample is boiled for 15 minutes and cooled to room temperature, then the absorbance is measured at λ 532 nm. (Canacki C, 2009)

III.   RESULTS AND DISCUSSION

The number of samples that met the inclusion and exclusion criteria and were willing to be research respondents was 30 people. In this study, respondents were divided into two groups: smokers (15 people) and non-smokers (15 people).

Table 1. Respondent Characteristics Based on Age

1.	Characteristics		Sample By Age		
2.	Age	3.	Amount	4.	%
5.	< 55 years	6.	28	7.	93.33
8.	> 55 years	9.	2	10.	6.67

Based on the table above, it can be seen that the majority of respondents are aged under 55 years.

Table 2. Classification of Smoking Degree Based on Age

	Smoker Classification		
Age	Light	Currently	Heavy
< 55 years	3	5	6
> 55 years	-	-	1

The classification referred to in this table is the division of the degree to which respondents smoke per day, namely:

1. Light (1 – 10 sticks)
2. Medium (11 – 20 stems)
3. Heavy   (> 20 sticks)

Figure 2 shows the distribution based on how long respondents have been smokers. The dominant data were those who have smoked for 11-20 years (47%) and those who have smoked for more than 20 years (33%).
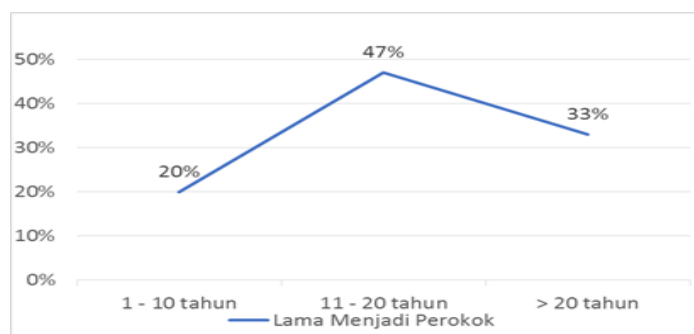

Fig 2. Distribution of Smokers Based on Length of Time Being a Smoker

Based on the survey results, it was found that respondents' reasons for smoking included habit, sensation seeking, atmosphere, and other factors. The survey of respondents showed that the most common reason for smoking was habit (53%).%), this reason is supported by the presence of a family history of smoking.From the habit (53%) of smoking respondents have a normal feeling (47%) after smoking. Respondents also have an opinion on the view of people who smoke is dangerous for health, it is okay to socialize, helps find inspiration and is normal. Surveys of respondents' opinions found it is dangerous for health (60%). This results in smoking behavior at the age of 45-59 years being found to have become a habit to smoke without any strong reason to smoke even though respondents think that smoking is dangerous for health.
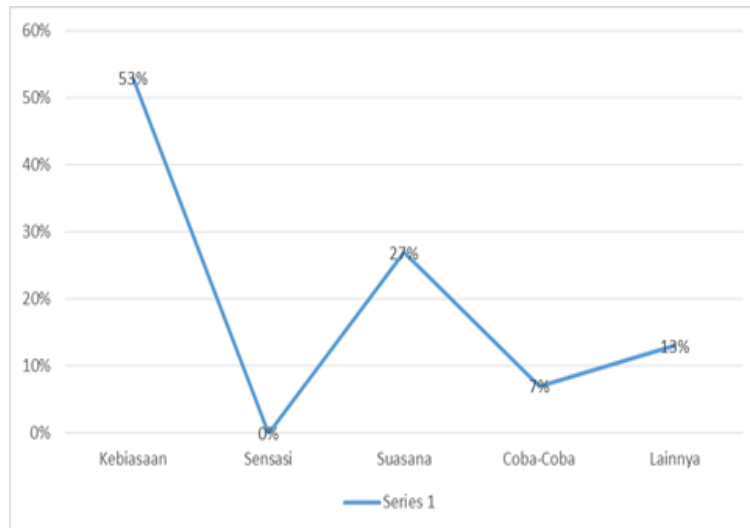

Fig 3. Distribution of Reasons Among Smoker Respondents

The determination of MDA levels in smokers and non-smokers was obtained from spectrophotometric absorbance measurements with $\lambda = 532$ nm in the respondents' saliva. MDA levels were measured on the same day as the saliva was collected to avoid any damage to the saliva. From the analysis results of men aged 45-59 years, it was found that the highest salivary MDA levels were in the smoker sample, namely 0.640 µM, while the lowest was 0.298 µM. Variations in MDA levels can be caused by the degree of smoking, which is divided into light, moderate, and heavy smokers. In this case, in It is suspected that the more cigarettes smoked by respondents in a day indicates high MDA levels.

Table 3. Data on MDA Levels of Smokers by Spectrophotometry

No	Sample	Frequency (%)	MDA (µM)
1	Light	20	0.320± 0.030
2	Heavy	33.33	0.498 ± 0.004
3	Currently	46.67	0.585 ± 0.048

Based on the data, the percentage of salivary MDA levels increased in smokers according to the degree of smoking where heavy smokers had a higher percentage of MDA levels than light and moderate smokers. Based on the Shapiro-Wilk analysis test, the normality distribution of MDA levels in smokers and non-smokers was not normally distributed (P < 0.05) therefore another alternative statistical test was used using the Mann-Whitney test.

Table 4. Description of MDA Levels in Smokers and Non-Smokers Based on the Mann-Whitney Test

Sample	MDA	p-value	N
Smoker	0.503 ± 0.108	0.001	15
Non-smoker	0.190 ± 0.047		15

The table shows the Mann-Whitney test results using SPSS version 24 32-bit for Windows, which showed a significant difference (P-value 0.001). Thus, there is a significant difference in salivary MDA levels between smokers and non-smokers.In this study, 30 respondents were obtained, with an age range of <55 years amounting to 28 people (93%), while the age range >55 years amounted to 2 people (66%). Respondents were separated into groups of 15 smokers and 15 non-smokers. The distribution of smoker data was distinguished by the degree of smoking per day, namely 3 light smokers, 5 moderate smokers and 7 heavy smokers. The distribution of smokers based on age was found in men aged <55 years, there were 3 light smokers, 5 moderate smokers and 6 heavy smokers. In smokers >55 years, 1 person was a heavy smoker. Measurement of salivary MDA levels of smoker respondents found the highest saliva sample was 0.629 µM while the lowest was 0.298 µM. Variations in MDA levels are caused by the degree of smoking which is divided into light, moderate and heavy smokers.This study also found MDA levels in smokers and non-smokers with a p-value of 0.001. The MDA levels in smokers' saliva of 0.5 µM were higher than the MDA levels in non-smokers' saliva of 0.1 µM. Previous research also explained that the calculated MDA levels significantly increased in smokers' saliva of 0.5 µM compared to non-smokers' saliva of 0.3 µM. (Lykkesfeldt, 2007) Smoking is also known to induce oxidative stress due to the production of reactive oxygen and lipid peroxidation caused by smoking.

 (Celec P, 2005) MDA levels are an indicator of lipid peroxidation, which were found to be elevated in this study. Cigarette smoke inhaled through the normal respiratory tract disrupts the oxidant/antioxidant ratio, leading to the formation of free radicals in exposed organs. Free radicals found in cigarettes or those caused by cigarette smoke can cause oxidative stress. Increased smoking can cause oxidative stress and lead to increased MDA levels. (Demirtas M, 2014) The research was conducted using spectrophotometry and TBA reagents based on the formation of a complex that can be measured spectrophotometrically at λ 532 nm. (Canacki C, 2009) Smoking can cause a temporary increase in salivary flow. In previous studies,Men who smoke have higher salivary flow than nonsmokers. Tobacco also increases glandular secretions, while nicotine can cause abnormalities in the glands that produce saliva. (Almeida P, 2008) Saliva is necessary because it contains proteins and enzymes that are beneficial to the body, one of which is the digestive enzyme amylase, which functions to break down carbohydrates into maltose, maltose, and dextrins. (Almeida P, 2008) However,Chronic smokers have been found to have very low salivary flow and increased dental and oral abnormalities. This low salivary flow can also reduce the enzymes and proteins contained in saliva, thus diminishing its beneficial effects on the body.

IV.   CONCLUSION

Based on the results of this study, it can be concluded that there is a difference in Malondialdehyde levels in smokers and non-smokers from men aged 45-59 years.The salivary MDA levels of male smokers aged 45-59 years were (0.503 ± 0.108) while in non-smoking men it was (0.190 ± 0.047) with a p value of 0.001. This shows that the salivary MDA levels of male smokers were higher than the salivary MDA levels of non-smoking men.Based on the survey results, it was found that the reason respondents smoked (n=15) was habit (53%). Regarding the smoking habit, respondents felt it was normal (47%) even though respondents knew that smoking was harmful to health (60%).

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