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Der Pharmacia Lettre, 2017, 9 (1):93-100
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Smoking Habit and Telomere Length Relationship in Minangkabau Ethnicity Males

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ABSTRACT

Introduction: Smoking habit is one of major health-related social problem in Indonesia. It is predicted that smoking could accelerate individual ageing based on procuring oxidative stress. Toxic components within cigarette smoke act as exogenous exposure which is responsible for increasing Reactive Oxygen Species (ROS) that lead to telomere shortening. The aim of this research is to know the relationship between Smoking habit and telomere length in Minangkabau ethnicity males

Method: This cross-sectional study included 130 males, Minangkabau ethnicity, ages 30-40-year-old. The characteristics and smoking habit data were collected by using questionnaire, while the blood was taken to measure telomere length by using O'Callaghan & Fenech. The data was analyzed by Student's T test, One Way- Anova and correlation's test.

The result: This research indicates that the mean length of telomeres was 579,75 + 314,75 %. The average length of smoked cigarettes was 14,96+13,67 years and types of cigarette the most widely used is filter cigarettes as 64%. The average length of the telomere subject does not smoke are 638,07 + 380,11 bp, smoking filter cigarettes 582,74 + 279,83 bp and smoking non-filter cigarettes as 516,32 + 241,13 bp. There was a significant negative correlation between length of smoked with telomere length ($r = -0,206$, $p=0,04$). There was the average difference of smokers with no smokers ($p=0,03$).

Conclusion: The longer smoke, the shorter the telomeres and telomere length smoking non-filter cigarettes has shorter than non-smokers and smoking filter cigarettes

KEYWORDS: Smoking Habit, Telomere Length, Filter Cigarettes

INTRODUCTION

The success of development of a nation, especially health development, can be seen from several health indicators, including life expectancy. Life expectancy in developing countries is still low compared to developed countries. According to WHO data in 2015, the average life expectancy worldwide has increased from 67 years in 2009 to 71 years in 2013. Nevertheless, there is a gap in life expectancy between developed and developing countries. Currently in Indonesia has been an increase in the average life expectancy to 70.1 years, but still below the national target of 72 years and life expectancy in West Sumatra Province reached 67.9 years [1].

Various factors can lead to fail to achieve the national target on the average life expectancy. Smoking, unhealthy diet, lack of physical activity, increased fat mass and obesity, and stress can lead to increased oxidative burden and the rate of telomere shortening [2].

Telomere length is emerging as a biomarker for aging and survival. TL varies between individuals of the same age, is influenced by DNA-damaging factors such as oxidative stress, and is heritable. Moreover, TL variations between smoking individuals are the important risk factors for many age-related diseases. Both are states of heightened oxidative stress, which increases the rate of telomere erosion per replication, and inflammation [3].

Telomere dynamics is pertaining to the length and the cutting of telomere which is play role in the normal aging process. Normally, the shortening of telomere take place from the lost during normal diploid cells division. Cell culture, based on telomere length which is truncated, showed its limited lifetime. The lengths of telomeres in humans are normally reduced by approximately 24.8 to 27.7 base pairs per year. Progressive telomere shortening causes aging, apoptosis, or oncogenic transformation of somatic cells. Shorter telomere has been associated with an increased incidence of disease and poor survival [4].

Reactive oxygen species (ROS) are of primary importance as they cause damage to lipids, proteins, and DNA either endogenously by cellular mechanism, or through exogenous exposure to environmental injury factors, including oxidation insult factors, such as tobacco smoke. Currently 46.3 million adults (25.7 percent of the population) are smokers. This includes 24 million men (28.1 percent of the total). Smoking can accelerate individual ageing based on oxidative stress. The toxic components in cigarette smokes act as exogenous exposure that will lead to increase *Reactive Oxygen Species* (ROS) [5].

Research on smoker blood sample provided the evidence on telomere shortening acceleration. Smoking doses had shown to have a negative correlation with TL. It is supported by another research on female smoker leukocyte sample that investigate the loss of telomere DNA. The average of DNA loses is 25.7 – 27.7 bp per year. Therefore, it is suggested that telomere erosion that caused by smoking a pack per day for forty years is equivalent to 7.4 years of life. Ultimately, smoking does increase oxidative stress, accelerate telomere shortening and increase the rate of ageing process [5].

Based on the consumption of cigarettes, Indonesian is the biggest smokers in the ASEAN country after the Philippines and Vietnam. The number of smokers, including children, teenagers, and adults, in Indonesia is around 27.6% which means every four Indonesian, there was one smoker. This percentage is much larger than America nowadays which is only about 19% or one smoker of every five Americans. Although in 1965 the number of smokers in the United States is 42% of the population. Basic Health Research (Riskesmas) in 2013 showed that the prevalence of smokers is 29.3% in Indonesia and 26.4% of West Sumatra [7].

METHODS

This cross-sectional study was conducted in Padang City. This study was performed on 130 Minangkabau ethnic males, aged 40-50 years, and worked as the district civil servant. Minangkabau ethnic meant if both the parents and the ancestors are Minangkabau people. The sample size was calculated using the formula for continuous data on population.

This study was approved by the Ethical Committee of Medical Faculty, Andalas University. The collection of primary data obtained directly from the respondents by conducting interviews using a questionnaire about the characteristics and smoking habits. Blood samples were drawn from all subject (5ml) and stored into EDTA-containing tubes for lab transfer. All samples were centrifuged at 1000×g for 10 minutes at 4°C and stored at -70°C for DNA extraction. Genomic DNA was extracted from buffy coats with Qiagen (QIAamp DNA Blood Mini Kit, Germany) and quantified by spectrophotometer (Hitachi 1800, Japan). All samples were run by Multiplex Real Time PCR Biorad CFX 96 TM detection system with TM Software CFX manager. Telomere length was measured using O'Callaghan & Fenech technique (2011) [8].

The quantitative variables were recorded as mean \pm SD, median and percentage. Differences mean between lifetime smoking habits and types of cigarettes with telomeres length were tested using the sstudent's t test, one way- anova and analysed on test was used to obtain the differences mean between the degree of smoke with telomeres length and their correlation was analyzed by using Pearson's correlation or non-parametric Spearman Ranks' correlation. P values less than 0.05 were considered significant. Data were analyzed using SPSS for windows (version 22.00, IBM).

RESULTS

Padang is the capital city of West Sumatra Province with an area of 694.96 km², has a population of 784 740 inhabitants with a population density of about 1,129 inhabitants / km². The city consists of 11 districts and 104 village (BPS West Sumatra Province, 2004).

The research was conducted in 11 districts in the city of Padang. 130 males, districts civil servants aged 40-50 years, and have fulfilled the inclusion and exclusion criteria, were included. Taken from the selected subjects, the blood samples were taken for telomere examination. The interview, including characteristics, smoking habits, and anthropometric measurement, was carried out at the same day.

Telomeres are essential for maintaining chromosome stability by protecting the chromosome ends from recombination, fusion and degradation. Therefore, the loss of telomeres will have a profound effect in the maintenance and integrity of chromosomes. In Table 1 it can be seen the average telomere length of research subjects.

Table-1: Average Telomere Lengths of Research Subjects

Variable	N	Mean \pm SD(bp)	Min	Max
Telomer Length	130	579.75 \pm 314.75	202	2117

In table 1: we can see the mean telomeres length is 579.75 \pm 314.75 bp, the shortest telomeres are 18.56 bp and the longest telomere is 2117 bp.

Table-2: Mean and Distribution of Smoking Habit on Subject Research.

Variable	n	%
Smoking habits		
* nonsmokers	77	59
* smokers	54	41
Degree of smoke		
* mild	65	50
* moderate	45	35
* heavy	20	15
Types of cigarettes		
* non-filter	27	36
* filter	50	64

In Table 2, it can be seen that more than half of the study subjects had a smoking habit. Mild smokers are higher than both moderate and severe smokers, most of the smoker subjects are smoking filter cigarettes (64%). The average of cigarette consumption is 271.63 \pm 341, 24 bars and the Span of cigarettes smoking was 14.96 \pm 13.67 years.

Lifetime smoking habits were assessed from the number of the smoked-cigarettes per day, span of smoking, cigarette type and degree of smoke. All these factors associated with telomere length. In the table below we can see a correlation between the number of cigarettes smoked and the telomere length of the research subjects.

Table-3: The Correlation between The Number and Span of Smoking Cigarette with Telomere length

Telomer Length	number of cigarettes	span of cigarettes smoking
r	-0,159	-0,206
p	0,07	0,04*
*Pearson correlation test		

Table 3 shows that there is a significant negative correlation with mild strength between span of cigarettes smoking with telomere length, whereas the number of cigarettes nearly as significant negative correlation with telomere length.

Table-4: Differences in Mean Telomere Length by Smoking Habit, Degree, and Type Cigarette Smoking

Variable	Mean \pm SD	p value
Smoking habits		
* never Smoke	638.07 \pm 380.11	0.03*
* smoker	516.32 \pm 241.13	
Degree of smoke		
* mild	630.14 \pm 366.43	0.69**
* moderate	521.22 \pm 245.24	
* heavy	553.15 \pm 259.59	
Types of cigarettes		
* filter	516.32 \pm 241.13	0.125*
* non-Filter	582.74 \pm 279.83	
* T-test ** one way-anova test		

It can be seen in Table 4 that the telomeres in subjects who smoke a filter cigarettes were longer than smoking non filter and there is a significant difference in telomere length between the smoking and non-smokers ($p = 0,03$) and there is no significant difference in telomere length by type of smoking ($p = 0.125$). The heavy smokers have shorter telomeres than mild smokers. There is no significant difference between telomere length based on the degree of smoking ($p = 0.69$).

DISCUSSION

Telomeres are DNA structures found at the ends of each chromosome. Telomeres are essential for maintaining chromosome stability by protecting chromosome ends from recombination, fusion and degradation. Therefore, the loss of telomeres will have a profound effect in the maintenance and integrity of chromosomes. Aging at the cellular level attributed to the loss of telomere DNA during replication of somatic cells and is regarded as the biological clock in the aging process of cells. Eukaryotic somatic cells *in vitro*, normally can only divide a limited number. Biologically, aging occurs within ageing of which telomeres shortened has been programmed in every cell division [3].

Genetic and environmental factors modulate the difference in telomere lengths between individuals. But the aging process that occurs outside the pre-programmed process can occur as a result of oxidative stress. Various factors can cause oxidative stress which ended with telomere shortening, so the cells fail to grow and continues with organ damage. Cell proliferation capacity, cellular environment, and epigenetic factors are some elements that affect this telomere heterogeneity. Several studies have been conducted to look at telomere length in human [8]:

In this study, the average telomere lengths (TL) were 579.75 ± 314.75 , whereas telomere length about smoking was 516.32 ± 241.13 bp that is shorter than non-smoker subjects 638.07 ± 380.11 bp. The TL in this study is shorter than Njajou finding (2007). The study included 356 men and 551 women, aged 18-92 years, from large Amish families. Mean TL in leukocytes that was measured by quantitative PCR were 6198 ± 1696 bp. Telomer Length was negatively correlated with age ($r = 0.40$; $P < 0.001$) [9]. Different finding was reported by Weischer (2014) which is conducted on 4,576 adult Danish ranging from age 20 to 100 years. The study resulted in TL that is more 4000 bp [11].

The different results among these studies may be caused by race and subject's age variation. This present study was carried out to Minangkabau race males of ages 40-50 years old, while Njajou (2007) and Weischer (2014) were taken place in America within range of age 18-100 years old [10].

A person's genetic makeup is not the only determinant of telomere length; environmental factors such as smoking is responsible for premature telomere shortening. Smoking can be harmful to health because cigarettes contain a variety of toxic substances that are harmful, for instance nicotine, tar, carbon monoxide (CO), arsenic, ammonia, formic acid, acrolein, hydrogen cyanide, nitrogen oxides, formaldehyde, phenol, acetol, hydrogen sulfide, pyridine, methyl chloride and methanol. Those are considered as the main toxins in cigarette smoke: nicotine, tar, and carbon monoxide [5].

The mechanisms involved in the pathological consequences of smoking are still elusive. Cigarette smoke was released by the smoker can be grouped into two phases, tar phase (particle size > 0.1 μ m) including nicotine and gas phases. Cigarette smoke contains tar phase > 1017 free radicals/g and >1015 free radicals/puff. Free radicals from smoke tar phase has a longer half-life (a few hours to months), while the radicals of the smoke gas phase only has a half seconds [12].

Oxidative stress is a key factor in all the processes associated with telomere shortening. Conditions of oxidative stress was caused by smoking can cause a factor in short telomeres and aging. Increased oxidative stress not only accelerates telomere shortening, but has been shown to decrease the activity of telomerase in vitro (in the smooth muscle cells of blood vessel endothelial cells) and in vivo [13].

This study result shows the significant TL different between smokers and non-smokers ($p=0.04$). There is significant negative correlation between duration of smoking and telomeres length. Cigarettes consumption seems to have no significant relation with TL, however, the higher tobacco consumption the shorter telomere tends to be. The same result was reported by Weischer (2014) of which resulted in significant relationship between smoking and telomere length ($p=8 \times 10^{-3}$). In addition, Broberg K (2005) reported that current smokers with short telomeres had more than six times as higher risk as non-smokers/former smokers with long telomeres (OR = 6.3, 95% CI 1.7–23) [14]. The authors observed a statistically significant difference in TL among men and women ($p < 0.001$). McGrath (2007) also observed a significant difference in TL across categories of pack-years of smoking ($p = 0.01$). Huzen (2014) studied on 8074 participants from the Prevention of Renal and Vascular End-stage Disease (PREVEND) study. It has reported that there was significant relationship between lifetime smoking habits with telomere length [15,16]

CONCLUSION

The longer smoke, the shorter the telomeres and telomere length smoking non filter cigarettes has shorter than non-smokers and smoking filter cigarettes.

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