http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2017.516.522

Research Article
Relationship Between Body Composition and Smoking Habit with Telomere Length of Minangkabau Ethnicity Men, in West Sumatera, Indonesia

[1]Delmi Sulastri, [2]Yuniar Lestari, [3]Afriwardi and [1]Desmawati

[1]Department of Nutrition, Faculty of Medical, University of Andalas, 25163 Kota Padang, Sumatera Barat, Indonesia
[2]Department of Public Health, Faculty of Medical, University of Andalas, 25163 Kota Padang, Sumatera Barat, Indonesia
[3]Department of Physiology, Faculty of Medical, University of Andalas, 25163 Kota Padang, Sumatera Barat, Indonesia

Abstract

Background and Objective: An increase in body composition and smoking habit are risk factors for high free radical levels in the body. This can lead to oxidative stress, due to the imbalance of pro-oxidants and oxidants in the body that leads to telomere shortening. The purpose of this research was to investigate the correlation between body composition and smoking habit with telomere length of men in West Sumatera, Indonesia. **Materials and Methods:** This cross-sectional study was conducted in Padang City using a sample of 130 Minangkabau ethnic district civil servant men aged between 40-50 years. The smoking habit were collected using a questionnaire, body composition data with bioelectrical impedance analysis (BIA) and blood sample analysis using O'Callaghan and Fenech's technique to measure telomere length. **Results:** This research indicated an average telomere length was 580.37±323.58 bp, BMI was 25.01±4.15 and percentage body fat was 22.06±6.16%. The proportion of subjects who smoke is 58.5% with heavy smoker 26.3%. The average length of smoked cigarettes was 25.2±7.3 years and the average of cigarette consumption is 270.58±343.18. There were no correlations between BMI and body fat percentage with telomere length ($p>0.005$). There is a negative correlation were significantly between smoking duration with telomere length ($r = -0.270$, $p = 0.020$). Telomere loss was 94.39 bp throughout the life-span equivalent to losing 3.4-3.8 years. **Conclusion:** Body composition is not a risk factor but smoking duration is a risk factor for telomere shortening in ethnic Minangkabau men.

Key words: Telomere length, body composition, smoking habit, life span of smoker, telomere shortening

Citation: Delmi Sulastri, Yuniar Lestari, Afriwardi and Desmawati, 2017. Relationship between body composition and smoking habit with telomere length of Minangkabau ethnicity men, in West Sumatera, Indonesia. Pak. J. Biol. Sci., 20: 516-522.

Corresponding Author: Delmi Sulastri, Department of Nutrition, Faculty of Medical, University of Andalas, 25163 Kota Padang, Sumatera Barat, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Life expectancy is one important indicator of a nation population's health status. This is still relatively low in developing countries compared to developed countries. The average life expectancy worldwide has increased from 67 years in 2009 to 71 years in 2013[1]. Nevertheless, in Southeast Asia, which is composed of mainly developing countries the average life expectancy is 71 years in 2013. Average life expectancy Indonesia has reached 70.1 years, still falling short of the national target of 72 years. In the province of West Sumatra life expectancy is only 67.9 years[1,2].

One of the biomarkers indicating aging and predicting life expectancy is telomere length. Telomeres are nucleoprotein complexes at the ends of eukaryotic chromosomes and DNA molecules[3]. Telomere consists of a 5'-TTAGGG-3 'base sequence that repeats over 2-20 kb Telomeres are essential for maintaining chromosome stability by protecting the end of chromosome from recombination, fusion and degradation[4]. Therefore, the loss of telomeres will have a profound effect on the maintenance and integrity of chromosomes. Telomere shortening occurs due to the failure to synthesize the most part ends in a linear DNA molecule during ordinary DNA replication. Hence, telomeres gradually become shorter with age. Cell culture studies show life span is limited by telomere shortening, a natural process that begins with the onset of aging at the cellular level and occurs as diploid cells lose telomeres at cell division due to the failure of this process to synthesize the further most ends in a linear DNA molecule[3].

The lengths of human telomeres are normally reduced by approximately 24.8-27.7 base pairs per year[5]. Progressive telomere shortening is caused by aging, apoptosis, or oncogenic transformation of somatic cells. Shorter telomere length has been associated with an increased incidence of disease, organ malfunctions, poor recovery from illness and premature death[3].

Obesity as measured by a high body mass index and body fat percentage has been shown to be a significant risk factor for many diseases. A diet high in fat and low in vegetables can cause fat accumulation in the body including in the abdomen and upper arms. High body fat percentage will ultimately increase the release of reactive oxygen species (ROS), which are responsible for the oxidative stress. Furukawa *et al.*[6] showed that waist circumference and body mass index (BMI) was significantly correlated with increased plasma and urine levels of reactive oxygen species. Song *et al.*[7] has shown that BMI is highly correlated with biomarkers of DNA damage, according to age. Prolonged oxidative stress will cause damage to cells, tissues, organs and the emergence of degenerative diseases[7].

There is a theoretical relationship between obesity and telomere length. Obesity is associated with inflammation in adipose tissue resulting from chronic activation of the immune system. Inflammation of adipose tissue will be a major source of inflammatory cytokines which may eventually result in insulin resistance, impaired glucose tolerance and diabetes. Increased inflammatory cytokines stimulate changes in leukocyte cells and mitochondrial activity which increase the production of reactive oxygen species. This in turn will increase the erosion of telomeres and therefore telomere shortening[8,9].

Globally, the prevalence of obesity is increasing. Data from WHO shows that 10% of men and 14% of women are obese. At 3%, the incidence of obesity is significantly lower in the Southeast Asia region and of less concern than in North American where a quarter of the population are obese. The results of Indonesia's 2013 Basic Health Research project, the prevalence of obesity in adult men was 19.7%, a dramatic increase from 7.8% in 2010[10,11].

Increased prevalence obesity is also evident in children. Based on the data from West Sumatra Provincial Health Office in 2012, 5.4% of children in this province are obese. This figure is 8% higher than the national average[11].

An unbalanced diet is a risk factor for increased body mass index and body fat percentage causing obesity. The Minangkabau ethnic group who make up most of the population of West Sumatra have a diet higher in saturated fat and lower in fiber and antioxidant rich vegetables and fruits than other ethnic groups in Indonesia[12]. High consumption of coconut oil and coconut milk is a major source of high levels of unsaturated fatty acids in the blood. Sulastri *et al.*[13] found that the saturated fat intake of ethnic Minangkabau society was higher than the recommended rate. This excess saturated fatty acid (SAFA) increases BMI and body fat percentage[7].

Smoking can accelerate the aging individual predicted by the effects of oxidative stress caused. Components of cigarette smoke is toxic exposure to exogenous will increase reactive oxygen species (ROS)[14]. Research regarding the blood of smokers find that an accelerated telomere shortening. Smoking dose shown to correlate negatively with telomere length [9]. Another study on leucocytes female smokers showed that telomere DNA is missing an average of 25.7-27.7 base pairs per year. Therefore, telomere erosion caused by smoking one pack of cigarettes a day for 40 years is equivalent to 7.4 years of life[5].

Based on the consumption of cigarettes, Indonesia is the biggest smokers in the ASEAN country after the Philippines and Vietnam. The number of smokers in Indonesia around 27.6%, every 4 Indonesia, there was a smoker[11]. This percentage is much larger than America today which is only

about 19% or there is a smoker of every 5 Americans[1]. Basic Health Research in 2013 showed an increased prevalence of smokers in Indonesia from 27.2-29.3 and 26.4% smokers in West Sumatra[11].

This study was conducted to determine whether obesity as measured by BMI and percentage body fat and smoking habit were definitive factors in telomere shortening amongst Minangkabau middle aged males.

MATERIALS AND METHODS

This cross sectional study was conducted subdistrict of Padang City from March to December 2016. This study was performed on 130 Minangkabau ethnic men, aged 40-50 years and specifically worked as the district civil servant. Minangkabau ethnic means 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 with registration number 051/KEP/FK/2016.The collection of primary data was obtained directly from the respondents by conducting interviews using a questionnaire about the characteristics to assess body composition using Tanita Bioelectrical Impedance Analysis (Model TBF-310, Tanita Corporation of America, Tanita BIA). Blood samples were drawn from all subject (5 mL) and stored into EDTA containing tubes for lab transfer. Blood samples were centrifuged at 1000 rpm for 10 min at 4°C and stored at -70°C for DNA extraction. Genomic DNA was extracted with Qiagen (QIAamp DNA Blood Mini Kit, Germany) and quantified by spectrophotometer (Hitachi 1800, Japan). Samples were run by Multiplex Real Time PCR BioradCFX 96 TM detection system with TM Software CFX manager. Telomere length was measured using O'Callaghan and Fenech[15] technique.

Statistical analysis: Data were analyzed using the SPSS software (version 22.0 for Windows, IBM Corp., NY, Armonk). The quantitative variables were recorded as Mean±SD, median and percentage. The correlation was analyzed by using Pearson's correlation or non-parametric Spearman Ranks' correlation. p-values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Average telomere length and body composition are shown in following Table 1.

Table 1: Average telomere length, body mass index and body fat percentage of Minangkabau men

Variables	Mean±SD	Minimum value	Maximum value
Telomere length (bp)	580.37±323.58	202.00	2117.00
Body fat (%)	22.06±6.16	3.90	37.80
BMI	25.01±4.15	16.30	37.90

The results shows that the mean absolute telomere length of these Minangkabau men ranged from 202-2117 bp with mean 580.37±323.58 bp or total telomere length per diploid genome between 18.56-194.74 kb with mean of 53.73 kb. This result is shorter than that found by O'Challagan and Fenech[15] (for 65-75 year old subjects (regardless of gender) of between 380.43-1891 bp with mean 941.30 bp or total per diploid genome telomere length between 35-174 kb with an average of 86.6 kb.

This discrepancy in length reflects the difficulty in comparing telomere lengths between studies. Telomere length measurements are highly dependent on method used One of the oldest methods is using the terminal restriction fragmentation (TRF)[16]. However this method requires a large amount of DNA and the resulting value measures not only the telomere length but also sub telomere lengths. More recently qPCR type methods such as qPCR, MMqPCR and aTLqPCR, that requires little DNA and only calculates the length of telomeres has been more commonly used. This study uses aTLqPCR which measures an absolute telomere length. While the other qPCR methods measure relative telomere length. The results of this study, shows that telomere length is comparable to O'Challagan and Fenech[15] results. It can be estimated that the relative telomere length on these results in an average range of 4000 bp compared to O'Challagan's average of about 5000 bp telomere lengths (4806-12132 bp) than the results of this study. Njajau *et al.*[17] (found the length of telomeres in 18-92 year old Amish males Caucasian. Weischer *et al.*[18] study of 4.576 Danish women aged 20-100 years found longer relative population) to be 6.158±1.663 bp. Differences between these results and those of our study could be due to, not only the problems related to the equipment and methods used but also variations of race and the differing ages of the sample groups. The sample in this study were 40-50 years old while a wider range of ages were included in the other studies. It is known that genetic factors play a role in telomere length, as show by Njajau *et al.*[17], who found a significant correlation between a Person's telomere length and his father's ($p = 0.006$)[18]. The enzyme telomerase which affects telomere length but only a few cells are exposed to telomerase activity and the amount of this activity appears to have a genetic basis. Gender is also a significant factor. Several studies have shown that women's telomeres are biologically

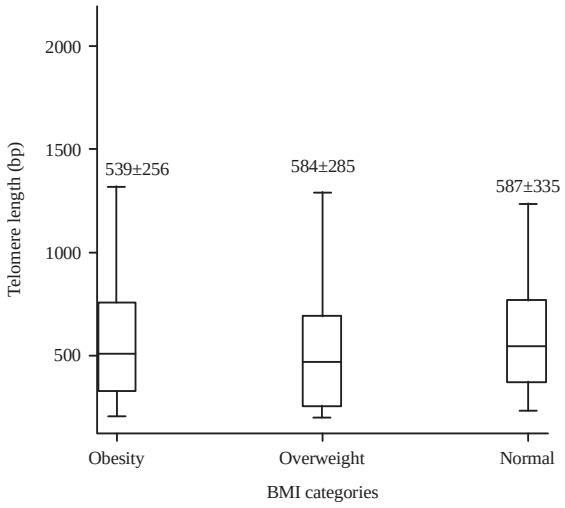

Fig. 1: Difference of means of telomere length based on BMI category
Data expressed as Mean±SD

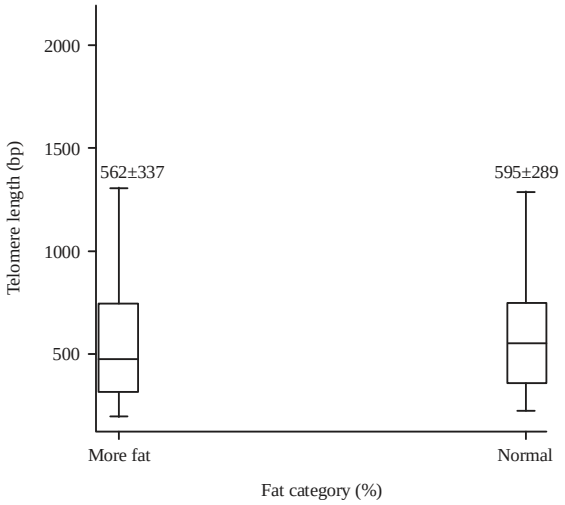

Fig. 2: Difference of means of telomere length based on fat percentage category
Data expressed as Mean±SD

longer than men's. One study on three ethnic groups (European whites, African Americans and Hispanics) found that the leukocyte telomeres in newborn females is on average 57 bp longer than in males. Telomere length can be influenced by a combination of factors such as genetic and environment[19].

Telomere length differences based on BMI categories can be seen in the figure below.

The average telomere length in the subject of obesity is 514±283 bp, overweight is 584±285 bp and normal is 587±335 bp as seen in Fig. 1. Although, there is no difference in telomere length in all three groups of BMI (p = 0.66) but the telomere length of the subject with normal BMI is longer than the subject of obesity and overweight.

The average telomere length in the subject with more fat percentage is 562±337 bp and normal is 595±289 bp as shown in Fig. 2. Although, there is no difference in telomere length in groups of fat percentage (p = 0.56) but the telomere length of the subject with normal fat percentage is longer than the subject with more fat percentage.

More than half of respondents in this sample were in the overweight or obese categories (66.2%) and carried an excess percentage of body fat (53.8%). However, there was no significant relationship between body composition with telomere length. This result is at odds with other studies. Lee *et al.*[20] found a statistical significance (p = 0.03) negative association between BMI with telomere length in 309 non Hispanic whites. Ornish *et al.*[9] studied 70 obese people found that telomere erosion increases with weight. Song *et al.*[7] found a high correlation between BMI and DNA damage. However, a more recent study, Valdes *et al.*[5] suggests that while obesity is a clear factor in telomere shortening it is better measured by leptin concentration rather than BMI or percentage body fat which do not correlate so clearly with telomere length[7,9].

This study failed to find a significant correlation may due to other more significant lifestyle factors masking the correlation. Literature shows that short telomeres are not only found in respondents, who are obese or have excess body fat but also in smokers and those with low levels of physical activity. Someone with weight within the normal range whose lifestyle includes other high risk factors such as smoking and less physical activity will also have short telomeres.

This study has shown that body composition is not the only factor associated with telomere length. There was an extremely high number of smokers across all BMI in this sample with almost 83% describing themselves as moderate to heavy smokers. It is well known that smoking can affect telomere length and accelerate aging. Exposure to toxic components of cigarette smoke increases reactive oxygen species (ROS)[15].

It would appear that this higher impact of smoking masks any correlation between body composition with telomere length. These extraordinary high rates of smoking could also partly explain the higher levels of telomere erosion found compared to in the American samples where the rate of smoking is only 15%[2].

Smoking habits of respondents was categorized by smoking and non-smoking and can be seen in the Table 2.

About 58.5% of respondents are smokers as shown in Table 2 and difference of means of telomere length based on smoking habit we can see in the Fig. 3.

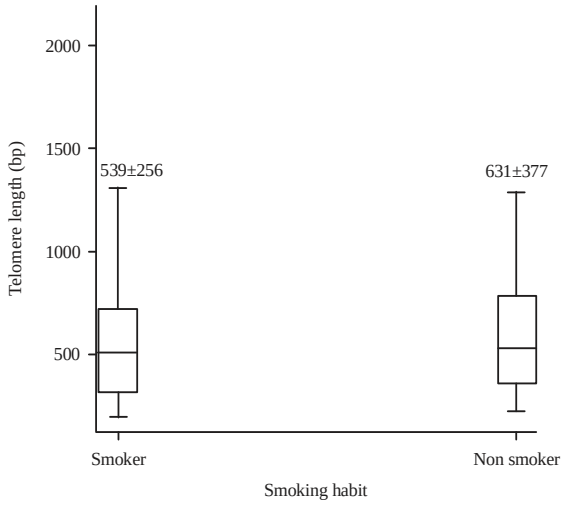

Fig. 3: Difference of means of telomere length based on smoking habit
Data expressed as Mean±SD

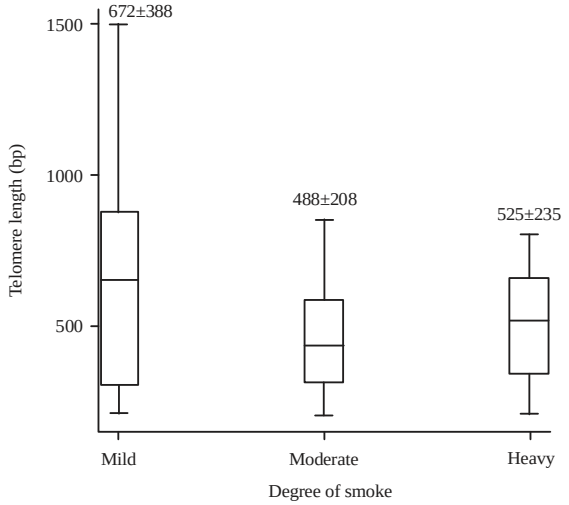

Fig. 4: Difference of means of telomere length based on degree of smoke
Data expressed as Mean±SD

The average telomere length in the subject with smoker is 539±256 bp and non smoker is 631±377 bp as shown in Fig. 3. There is a significant mean in telomere length based on smoking habit (p = 0.03). As for the degree of smoking among respondents who smoke seen in Table 3.

Based on the degree of smoking, it was found that 26.3% had heavy smoking.

There is no difference average in telomere length based on degree of smoke (p = 0.08), as shown in Fig. 4.

A negative correlation (r = -0.270) were significantly (p = 0.020) between smoking duration with telomere length

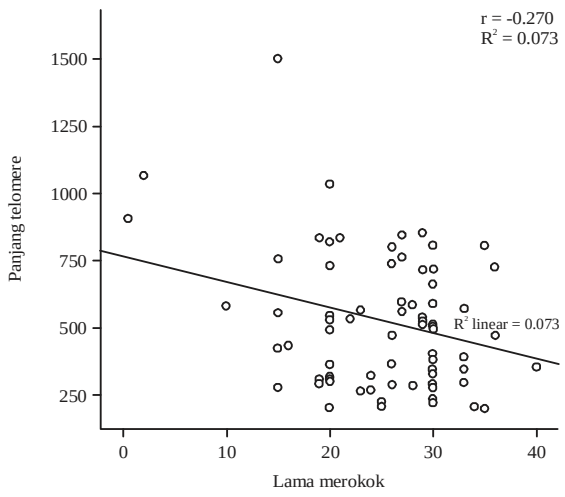

Fig. 5: Correlation duration of smoked with telomere length (p = 0.020)

Table 2: Distribution of respondents by smoking habit

Smoking habit	Numbers	%
Smoker	76	58.5
Non smoker	54	41.5
Amount	130	100.0

Table 3: Distribution of respondents by degrees of smoking

Degree of smoke	Numbers	%
Mild	12	15.8
Moderate	44	57.9
Heavy	20	26.3
Amount	76	100.0

shown in Fig. 5. $R^2 = 0.073$ showed that 7.3% telomere length is determined by factors of smoking duration.

The results of correlation test showed that 7.3% long telomere length is determined by the smoke, others are caused by other factors. Prediction of regression tests to get the results that each additional one year old smoking would shorten telomeres at 9.439 bp (regression equation Y = 766.28-9.439X). Normal telomere loss in one year is 24.8-27.7 bp[6]. If someone smoked for 10 years, he will lose telomere along 94.39 bp or predictable reduce age 3.4-3.8 years of life. Weischer *et al.*[18] reported no significant association between smoking and telomere length (p = 8×10-3). In addition, Broberg reported that smokers have a risk six times shorter telomeres compared with non smokers (OR = 6.3).The mechanisms involved in the pathological effects of smoking on telomere length remains elusive. Cigarette smoke contains free radicals are very high and is divided into phases nicotine and gas phase. Oxidative stress is known to be a key factor in all processes related to telomere shortening[21,22].

This study has several limitations, including researchers do not put attention to internal factors such as levels of enzyme that can improve telomere length and levels of endogenous antioxidants to reduce oxidative stress.

This study suggest that maintaining a healthy weight is not sufficient to slow the telomere shortening process and for Minangkabau males smoking factors may pose greater risks. Further research could examine internal factors such as levels of telomerase enzyme and genetic variation.

CONCLUSION

Based on this study, the conclusions are: Minangkabau men have significantly shorter telomeres than those found in other samples. No significant relationship between body composition (percent body fat and BMI) with telomere length could be established in this sample so it appears that other factors such as smoking may play a larger role.

SIGNIFICANCE STATEMENT

This study demonstrates that obesity, while a risk factor for many diseases, is not as significant a factor in telomere length in middle age Minangkabau men. It is not sufficient to encourage these men to reduce weight to prevent premature death but other factors, particularly smoking, must also be addressed.

ACKNOWLEDGMENT

We would like to thank the Medical Faculty of Andalas University for funding this research project (Grant no 33/UN.16/HKRGB/LPPM/2016) and special thank to all respondents who participated in this study.

REFERENCES

1. WHO., 2015. WHO global health observatory data repository: Life expectancy-data by country. World Health Organization, Geneva.
2. MHR., 2013. Overview of health elderly in Indonesia. Data and Information Center, Jakarta.
3. Shammas, M.A., 2012. Telomeres, lifestyle, cancer and aging. Curr. Opin. Clin. Nutr. Metab Care, 14: 28-34.
4. Dickson, M.A., W.C. Hahn, Y. Ino, R. Vincent and J.Y. Wu *et al*., 2000. Human keratinocytes that express hTERT and also bypass a $p16^{INK4a}$-enforced mechanism that limits life span become immortal yet retain normal growth and differentiation characteristics. Mol. Cell. Biol., 20: 1436-1447.
5. Valdes, A.M., T. Andrew, J.P. Gardner, M. Kimura and E. Oelsner *et al*., 2005. Obesity, cigarette smoking, and telomere length in women. Lancet, 366: 662-664.
6. Furukawa, S., T. Fujita, M. Shimabukuro, M. Iwaki and Y. Yamada *et al*., 2004. Increased oxidative stress in obesity and its impact on metabolic syndrome. J. Clin. Invest., 114: 1752-1761.
7. Song, Z., G. Figura, Y. Liu, J.M. Kraus and C. Torrice *et al*., 2010. Lifestyle impacts on the aging associated expression of biomarkers of DND damage and telomere dysfunction in human blood. Aging Cell, 9: 607-615.
8. Susantiningsih, T., 2015. [Obesity and oxidative stress]. J. Kedokteran Univ. Lampung, 5: 89-93.
9. Ornish, D., J. Lin, J.M. Chan, E. Epel and C. Kemp *et al*., 2013. Effect of comprehensive lifestyle changes on telomerase activity and telomere length in men with biopsy-proven low-risk prostate cancer: 5-year follow-up of a descriptive pilot study. Lancet Oncol., 14: 1112-1120.
10. WHO., 2011. WHO Obesity health topic. World Health Organization, Geneva.
11. Kemenkes, R.I., 2013. Basic health research. Balitbangkes, Jakarta.
12. Hatma, R.D., 2011. Lipid profiles among diverse ethnic groups in Indonesia. Acta Med. Indones., 43: 4-11.
13. Sulastri, D., S. Rahayuningsih and Purwantyastuti, 2005. Patterns of fat, fiber and antioxidant intake, and its relationship to lipid profile in minangkabau ethnic men. Maj. Kedokt. Indon., 55: 61-66.
14. Babizhayeva, M.A. and Y.E. Yegorov, 2010. Smoking and health: Association between telomere length and factors impacting on human disease, quality of life and life span in a large population-based cohort under the effect of smoking duration. Fundam. Clin. Pharmacol., 10.1111/j.1472-8206.2010.00866.x .
15. O'Callaghan, N.J. and M. Fenech, 2011. A quantitative PCR method for measuring absolute telomere length. Biol. Proced., 13: 1-10.
16. Fojtova, M., P. Fajkus, P. Polanska and J. Fajkus, 2015. Terminal restriction fragments (TRF) method to analyze telomere lengths. Bio. Protocol., Vol, 5 10.21769/BioProtoc.1671.
17. Njajou, O.T., R.M. Cawthon, C.M. Damcott, S.H. Wu and S. Ott *et al*., 2007. Telomere length is paternally inherited and is associated with parental lifespan. Proc. Natl. Acad. Sci. USA., 104: 12135-12139.
18. Weischer, M., S.E. Bojesen and B.G. Nordestgaard, 2014. Telomere shortening unrelated to smoking, body weight, physical activity and alcohol intake: 4,576 general population individuals with repeat measurements 10 years apart. PLoS Genet., Vol. 10, No. 3. 10.1371/ journal.pgen.1004191.

19. Dalgard, C., A. Benetos, S. Verhulst, C. Labat and J.D. Kark *et al*., 2015. Leukocyte telomere length dynamics in women and men: Menopause vs age effects. Int. J. Epidemiol., 44: 1688-1695.
20. Lee, M., H. Martin, M.A. Firpo and E.W. Demerath, 2011. Inverse association between adiposity and telomere length: The fels longitudinal study. Am. J. Hum. Biol., 23: 100-106.
21. Broberg, K., J. Bjork, K. Paulsson, M. Hoglund and M. Albin, 2005. Constitutional short telomeres are strong genetic susceptibility markers for bladder cancer. Carcinogenesis, 26: 1263-1271.
22. Ambrose, J.A. and R.S. Barua, 2004. The pathophysiology of cigarette smoking and cardiovascular disease: An update. J. Am. Coll. Cardiol., 43: 1731-1737.