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The Effect of Leukocyte Telomere Length on Blood Pressure among Thai Adolescents

Phennapha Luealai[1], Tippawan Pongcharoen[2], Wantanee Kriengsinyos[3], Uthaiwan Suttisansanee[4], Nattira On-Nom[5], and Chaowanee Chupeerach[6]
[1,2,3,4,5,6]Institute of Nutrition, Mahidol University, 999 Phutthamonthon 4 Rd., Salaya, Phutthamonthon, Nakhon Pathom, Thailand
Email: chaowanee.chu@mahidol.ac.th

Abstract

Background: Leukocyte telomere length (LTL) is a biomarker of biological aging that has a significant relationship with cardiovascular disease, cancer, diabetes, and hypertension. However, the relationship between relative leukocyte telomere length and blood pressure has been unclear among adolescents which may provide early biological information about cardiovascular health and the likelihood of developing related diseases later in life. Therefore, this study aimed to examine the effect of LTL on blood pressure among Thai adolescents.

Methods: A cross-sectional study was conducted among 59 Thai adolescents aged between 13-15. The blood sample, anthropometric, and clinical data were collected following an approved protocol. Relative telomere length (RTL) was measured using the qPCR method. The short and long telomere length was classified by mean RTL. The differences in blood pressure between short and long telomere length were analyzed using the Mann-Whitney U test.

Results: The results showed a significant difference in systolic and diastolic blood pressure (SBP and DBP, respectively) between short and long relative telomere length (RTL), but it was only found in the male group. The data indicated subjects with shorter RTL had a significantly lower SBP and DBP compared to those with longer RTL (P=0.031, 0.041, respectively).

Conclusions: Our findings revealed that differences in leukocyte telomere length were related to blood pressure among Thai adolescent

Keywords: Adolescent, Leukocyte Telomere Length, Biological Aging, Blood pressure, Polymerase chain reaction

1. Introduction

Telomeres are structures located at the end of chromosomes in eukaryotic cells which compose of tandem TTAGGG repeats of DNA by working together with six specific proteins known as shelterin complex (Blackburn, 2010). Telomeres play a critical role in maintaining genomic stability by protecting the chromosome ends from degradation and fusion with neighboring chromosomes through several mechanisms (Chow et al., 2012). During cell division, the end-replication problem occurs at the ends of linear chromosomes, which leads to the progressive shortening of telomeres with each cell division. Eventually, telomeres become critically short, accelerating the cellular aging process known as senescence, where the somatic cells stop or limit dividing and go through programmed cell death or apoptosis (Heidinger et al., 2012)

Leukocyte telomere length (LTL) has been suggested as a biomarker of biological aging (Factor-Litvak et al., 2016). Moreover, telomere shortening has been associated with the risk of age-related diseases such as cardiovascular disease, cancer, diabetes, and hypertension (Shammas & care, 2011; Tellechea & Pirola, 2017; Willeit et al., 2014). Furthermore, telomeres are sensitive to oxidative stress, inflammation, and aging-related hydroxyl radicals. The build-up of inflammatory cytokines can cause cellular dysfunction, leading to shortened telomere length and an increased risk of cardiovascular disease (CVD) in humans as they age (O'Donovan et al., 2011). Hypertension has been recognized as a significant global publish health issue due to its direct connection with cardiovascular and cerebrovascular disease events (Kearney et al., 2005).

In recent years, several epidemiological studies have investigated the association between telomere length and elevated blood pressure in adults (Tellechea & Pirola, 2017). However, few adolescent studies have focused on the relationship between leukocyte telomere length and blood pressure (Masi et al., 2012; Todendi et al., 2020). In addition, the associations are not well characterized in adolescents. Therefore, enhancing our knowledge about the factors related to LTL in adolescents is helpful for future research on the determinants of biological aging and valuable

for developing health recommendations for preventing age-related disease from the early stage of life. Hence, this study aimed to examine the difference in blood pressure between short and long LTL among Thai adolescents.

2. Purposes

To examine the effect of leukocyte telomere length (LTL) on blood pressure and anthropometric measurement in Thai Adolescents

3. Research Methodology

3.1 Study design and subjects

This study was designed as a cross-sectional study that included 59 Thai adolescents aged 13-15 years recruited from the previous study "Association of early life exposure and long-term health and cognitive development outcome in adolescents in northeast Thailand" project, a community-based study of adolescent nutrition and health from Khon Kaen province in 2013. This study was approved by the research ethics committee of the human research internal review board, Mahidol University (MU-IRB), the protocol number: 2022/062.1603.

3.2 Anthropometric measurement

Anthropometric data including weight, height, and waist circumference were measured by approved protocol from each subject at the study site. Weight was measured in kilograms with a digital scale (Seca digital scale model 813, Seca Corporation, Hamburg, Germany). Height was measured in centimeters with measuring tape. Waist circumference was measured in centimeters with an inelastic measuring tape. BMI was calculated using the weight and height of each participant by the same method as adults to calculate BMI-for-age Z-score to determine nutritional status using the WHO growth reference (2007) criteria for 5-19 years. Body fat percentage was measured using the deuterium dilution technique and calculated following the IAEA method (Malone, 2009).

3.3 Clinical measurement

Clinical measurements including systolic and diastolic blood pressure (SBP and DBP, respectively) were obtained using an automatic blood pressure monitor (Omron Automatic Blood Pressure Monitor HEM 7200). Each participant was asked to sit on the chair in a sitting position before the measurements.

3.4 Blood collection

Before the blood collection, all the participants were instructed to fast for at least eight hours and only drink water. A total of 10 ml of fasting blood was collected from each participant at the study site in Khon Kaen province. EDTA-treated blood was kept at -70˚C for further DNA analysis.

3.5 Telomere length analysis

3.5.1 DNA extraction

Genomic DNA was extracted from the peripheral leukocytes in EDTA-treated blood using the Flexi gene DNA kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The extracted DNA concentration and purity were confirmed through measurement by NanoDrop Lite Spectrophotometer (Thermo Scientific USA). DNA purity was determined by the ratio of the absorbance values of 260nm vs. 280nm (A260/A280) and 260 nm vs. 230 nm (A260/A230). Values should be in the normal range (1.8-2.0 and 2.0-2.2, respectively), which was clean and suitable for further analysis.

3.5.2 Relative telomere length measurement

Relative telomere length (RTL) was measured on genomic DNA using a quantitative polymerase chain reaction (qPCR) method as described by Cawthon (Cawthon, 2002; O'Callaghan & Fenech, 2011). In brief, this method measures the average relative telomere length from the ratio between telomere length (T) amplification product and single-copy gene (S) products (Acidic ribosomal phosphoprotein PO (36B4)) for each sample. For each reaction, the total volume of 10 ul was run separately in a 96-well plate in the QuantStudio™ 5 Real-Time PCR machine (Thermo Fisher, USA). The total volume of 10 ul contained 20 ng DNA template, 1xPowerUp SYBR Green Master Mix (Applied Biosystems™, Thermo Fisher, USA), 10 µM telomere forward primer, and 10 µM telomere reverse primer. The primer sequences were telomere forward (5'-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGG GTTTGGGTT-3'), telomere reverses (5'- GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3'). The number of diploid genomes was determined using the 36B4 (a single copy gene) amplicon with the same reagents as the telomere. The primer sequences were 36B4 forward (5'- CAGCAAGTGGGAAGGTGTAATCC-3'), 36B4 reverses (5'- CCCATTCTATCATCAA CGGGTACAA-3'). The cycling conditions are the initial holding stage at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 sec and 60 °C for 1 min.

Duplicate samples were used to maintain quality control, and the results were verified for consistency between the two samples. Samples that exhibited high variability levels (>10%) were subjected to a second analysis to confirm the results. To assess the efficiency and specificity of qPCR, negative control and melting curve analysis were carried out for Tel and 36B4 primers.

The T/S ratio for each sample was calculated using the formula as follows

$$\text{Calculated } \Delta Ct = Ct(\text{Telo}) - Ct(36B4)$$

$$\text{Calculated T/S ratio} = 2^{-\Delta Ct}$$

The relative T/S ratio was determined by dividing the T/S ratio of each sample by the mean T/S ratio of the entire participants, and it was expressed as relative telomere length (RTL).

3.6 Statistical analysis

Data were tested for normal distribution using the Kolmogorov-Smirnov test. Most of the data not be verified as a normal distribution, the non-parametric tests were used. Subject characteristics were analyzed using descriptive data and presented as mean ± SD. Mann-Whitney U test was used to examine the difference between two gender-independent groups as well as the difference in blood pressure between the short and long LTL group. All statistical analyses were performed using SPSS Ver. 26 for Windows (Chicago, USA). $P < 0.05$ was considered statistically significant.

4. Results

Table 1 shows the baseline characteristics of the 59 participants stratified by gender. The number of males and females was 29 and 30 people, respectively. All the subjects were on the same age level, and the mean age was 14.77 ± 0.31 years old. Anthropometric characteristics consist of weight, height, BMI, BMI-for-age Z-score, waist circumference, and body fat percentage. There are significant differences in weight and height between the male and female groups (P = 0.008 and 0.000, respectively). This result indicates that the male's status was bigger than the female group. However, BMI and BMI-for-age Z-score showed similar overall nutritional status of both genders and waist circumference. Meanwhile, females presented a higher body fat percentage (27.70 ± 5.95; mean ± SD) when compared to males (16.51 ± 5.17, P=0.000)). Clinical characteristics, males presented higher SBP (108.26 ±10.88 mmHg) compared to females (103.72 ±8.80 mmHg), P = 0.048). On the other hand, when compared with males, females had higher DBP (63.13 ± 6.82 mmHg) versus (60.12 ±7.13 mmHg), P=0.034).

Table 1 Baseline characteristics of subjects stratified by gender

Variables	Total N=59	Male N=29	Female N=30	P-value
Age (y)	14.77 ± 0.31	14.81 ± 0.32	14.73 ± 0.30	0.336
Weight (kg)	50.66 ± 0.47	53.55 ± 9.97	47.87 ±10.33	**0.008***
Height (cm)	160.38 ±7.37	165.45 ± 5.67	155.47 ±5.17	**0.000***
BMI (kg/m^2)	19.60 ± 3.32	19.48 ± 3.03	19.72 ±3.63	0.988
BMI-for-age, Z-score	-0.31 ± 1.18	-0.27 ± 1.16	-0.34 ± 1.21	0.739
WC (cm)	68.03 ± 8.06	68.40 ± 6.79	67.67 ± 9.23	0.289
Body Fat (%)	22.40 ± 7.91	16.51 ± 5.17	27.70 ±5.95	**0.000***
SBP (mmHg)	105.95 ± 10.05	108.26 ± 10.88	103.72 ± 8.80	**0.048***
DBP (mmHg)	61.65 ± 7.08	60.12 ±7.13	63.13 ± 6.82	**0.034***

BMI: body mass index; WC: waist circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure. Mann-Whitney Test for differences between gender. *Significantly different ($p<0.05$). All values are mean±SD unless otherwise noted.

Figure 1 presented the baseline telomere length of participants, the median relative telomere length of males was 0.68 T/S ratio, and the median relative telomere length of females was 0.73 T/S ratio. There was no significant difference in relative telomere length between gender was observed.

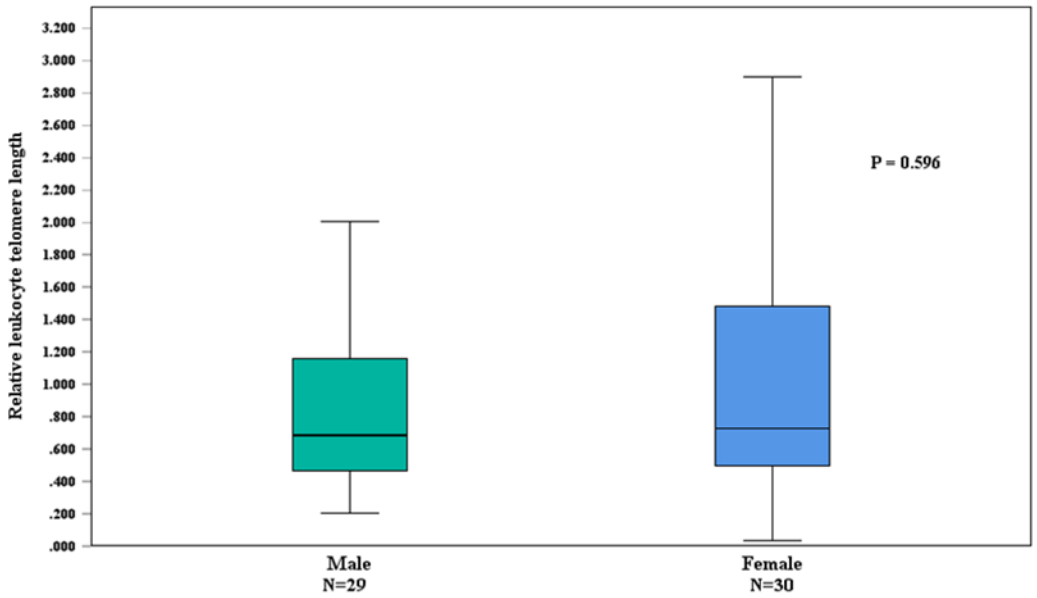

Figure 1 Relative leukocyte telomere length (T/S ratio) for males and females. * Mann-Whitney U-test.

Table 2 presented the comparison of anthropometric measurement between short and long-relative telomere length (RTL) groups. The results show no statistically significant differences in the distribution of anthropometry measurements between short and long RTL.

Table 2 Comparison of anthropometry measurement between the short and long RTL groups

Parameters	Males		P-value	Females		P-value
	Short TL N = 19	Long TL N = 10		Short TL N= 18	Long TL N= 12	
Weight (kg)	53.28 ± 11.34	54.06 ± 7.19	0.435	45.91 ± 6.65	50.82 ±14.04	0.672
Height (cm)	164.37 ± 4.19	167.52 ± 7.60	0.383	154.71 ± 5.13	156.62 ± 5.25	0.459
BMI (kg/m^2)	19.63 ± 3.60	19.19 ± 1.57	0.714	19.16 ± 2.63	20.56 ± 4.77	0.472
BAZ	-0.27 ± 1.35	-0.28 ± 0.73	0.945	-0.47 ±1.04	-0.14 ± 1.46	0.553
WC (cm)	68.93 ± 8.04	67.40 ± 3.50	0.982	65.66 ± 5.28	70.67 ± 12.86	0.446
Body Fat (%)	16.28 ± 5.26	16.91 ± 5.29	1.000	26.85 ± 4.98	28.97 ± 7.22	0.189

BMI: Body mass index; BAZ: BMI-for-age Z-score; WC: Waist circumference; SBP: Systolic blood pressure; DBP: Diastolic blood pressure. Mann-Whitney Test for differences between gender, *Significantly different with p<0.05. All values are mean±SD unless otherwise noted.

Figures 2 and 3 show that there was a significant difference in systolic and diastolic blood pressure between the short and long RTL groups, but it was only found in the male group. The data indicated male subjects with shorter RTL tended to have lower SBP and DBP compared to those with longer RTL (P=0.031, 0.041, respectively).

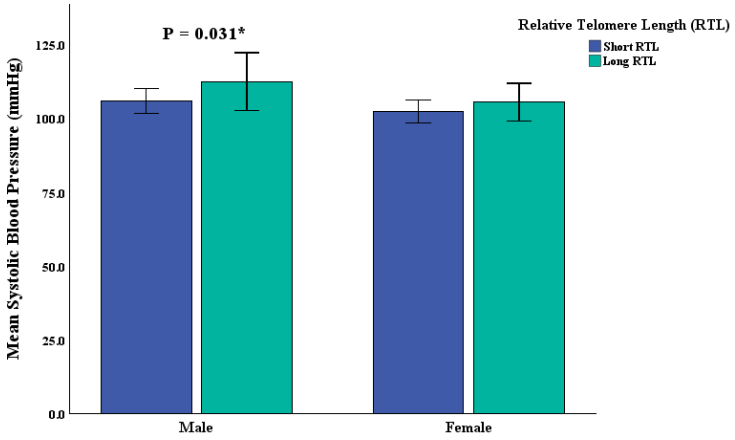

Figure 2 Comparison of systolic blood pressure between short and long relative telomere length (RTL) stratified by gender

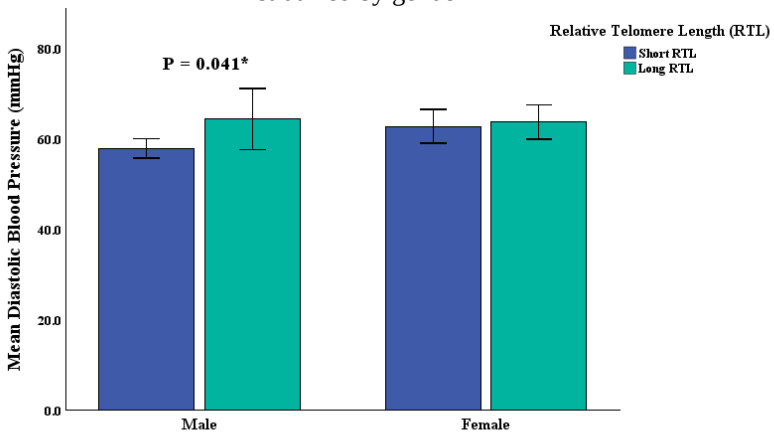

Figure 3 Comparison of diastolic blood pressure between short and long relative telomere length (RTL) stratified by gender

5. Discussion

This cross-sectional study investigated the effect of leukocyte telomere length on anthropometric assessment and blood pressure among Thai adolescents. The results showed that male adolescents with shorter RTL presented significantly lower blood pressure than those with longer RTL. However, the mean systolic and diastolic blood pressure values observed among the study participants were found to be within the range considered normal. Therefore, drawing a definite conclusion regarding the association of RTL differences with high blood pressure was challenging based on these results. It was possible that the longer RTL of the young and healthy population was a marker of healthier cells. These healthier cells may have better cardiovascular health overall through the regulation of endothelial function and kidney function which could lead to higher blood pressure but still within the normal range (Blackburn, 2005; Nakao et al., 2017). While some studies suggest a link between telomere length and blood pressure regulation in healthy adolescents, more research is needed to understand this relationship. Additionally, blood pressure regulation is a complex process influenced by genetic, lifestyle, and environmental factors, requiring a comprehensive approach to studying hypertension in adolescents (Kesornpikul et al., 2021).

Blood pressure is also associated with LTL in an adult investigation. The recent study of NHANES, 1999-2002 found the relationship between mean telomere length and the levels of systolic and diastolic blood pressure as well as the prevalence of hypertension. The possible mechanism is shorter telomere length contributes to cellular

senescence or the state of irreversible arrest, in endothelial cells, impairing their ability to produce nitric oxide and leading to endothelial dysfunction. Moreover, short telomere length is related to insulin resistance leading to the development of high blood pressure (Huang et al., 2020). On the other hand, studies on the younger population found no association between telomere length and blood pressure or hypertension among children and adolescents. The possible explanation for this finding is telomeres shorten gradually with time and may take years to affect blood pressure. Younger people have longer telomeres than adults as their cells have divided fewer times. Hence, the influence of telomere shortening on blood pressure may not be as significant in younger individuals (Masi et al., 2012; Todendi et al., 2020).

In the present study, we observed that the sex differences were not affected leukocyte telomere length (LTL) among Thai adolescents. The population-based cross-sectional survey of Australian children aged 11-12 also suggested this finding. They found that telomere length did not differ by gender in children and the authors suggested that sex differences might be associated with telomere length later in life. In contrast, results from another study indicated that sex difference was associated with telomere length during early adolescence, but it was not explained by sex steroid hormones or body size. Therefore, further longitudinal studies are needed to justify the mechanism of telomere attrition across lifespans. (Nguyen et al., 2019).

Furthermore, after comparing the anthropometric data between short and long RTL groups. We found that the median of anthropometry data was not significantly different across short and long RTL groups. This finding was correlated with the cross-sectional study among Caucasian and African-American adolescents. They discovered that LTL was unrelated to BMI, waist circumference, and body fat percentage at this age (Todendi et al., 2020). The influence of body size and adiposity on telomere length may not be evident until later in life, or other factors such as physical activity and diet may have a greater impact during adolescence. In contrast, a cross-sectional study conducted on children and adolescents aged 9-13 years found that subjects who were overweight or obese had a significantly shorter LTL compared to those who were normal weight (Buxton et al., 2011). The contrasting result could be due to the different ethnicity, small sample size, and the methods of anthropometric measurement that affect the variation of leukocyte telomere length of the participants which may restrict the ability to make definite conclusions about the causal direction of the relationship.

However, some limitations should be taken into consideration for the present study. Firstly, we have not explored the inflammation and oxidative stress markers, as well as other environmental or behavioral factors such as diet, physical activity, smoking status, and stress, which might clarify the correlation between LTL and blood pressure. Secondly, the scope of the study population is limited to the Thai population, and the results may not be generalizable to other populations. Finally, the causal relationship between LTL and blood pressure cannot be deduced from the cross-sectional design study, and further longitudinal investigations are necessary to verify the correlation.

6. Conclusions

This present study found a significant difference in systolic and diastolic blood pressure between short and long relative telomere length (RTL), but it was only found in the male group. The data indicated subjects with shorter RTL had a significantly lower SBP and DBP compared to those with longer RTL. However, this association needed well-planned prospective studies to elucidate the causal relationship between them.

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7. Recommendations

Given the rising incidence of age-related diseases among adolescents, investigating the relationship between cardiovascular disease risk factors and telomere length could contribute to formulating health recommendations that help prevent multiple diseases in early life. Future research can address some gaps in our knowledge, such as the need to increase sample sizes to enhance statistical power for detecting the effect of leukocyte telomere length differences on the blood pressure in the study population. Furthermore, obtaining additional information about the potential confounding effects of environmental and behavioral factors on this association is essential. Lastly, longitudinal studies are necessary to explore the causal impact of early dietary patterns on LTL.

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