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Research Paper

Association of traffic volume and leukocyte telomere length of Malaysian populations living in urban and rural areas

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ABSTRACT

Telomeres are repetitive sequences (5′-TTAGGG-3′) of deoxyribonucleic acid (DNA) at the ends of eukaryotic chromosomes and play a critical role in maintaining genomic stability. Shortened telomeres are linked to cellular senescence and apoptosis with environmental factors such as traffic volume pollution potentially influencing telomere length. This study examined the variation in leukocyte telomere length (LTL) among adults living in high- and low-traffic areas in Malaysia. A cross-sectional analysis was conducted on 101 adults from a high-traffic area, Kuala Lumpur and 101 adults from Hulu Langat, a low-traffic area, to assess the relationship between exposure to traffic volume and LTL. Healthy, non-smoking, non-alcoholic participants who had resided and worked in their respective locations for the past five years were selected. LTL was measured using the quantitative polymerase chain reaction (qPCR) method from peripheral blood samples, revealing that participants from the high-traffic area had significantly shorter mean LTL (0.77 ± 0.23) than those participants from the low-traffic area (1.09 ± 0.07) ($p < 0.001$). Notably, there is a strong inverse relationship between heavy traffic exposure and LTL, as LTL decreased by 0.38 units [(95 %CI: 0.26, 0.5), $p = 0.01$] and 0.16 units [(95 %CI: −0.16, 0.19), $p = 0.04$] for each increase in a single light vehicle and heavy vehicle, respectively. Individual covariates, outdoor jobs, intake of grilled food, indoor grilling, and passive smoking were also negatively associated with LTL. These findings suggest that high traffic volume may contribute to reduced telomere length and has broader implications on public health.

1. Introduction

Telomeres are repetitive nucleotide sequences located at the ends of chromosomes that protect chromosomes from degradation and prevent their ends from fusing during cell division. In humans, telomeres consist of the sequence 5′-TTAGGG-3′ along with specialized proteins that safeguard essential genetic material during cellular replication [1]. Each time a cell divides, telomeres shorten, acting as a biological clock for cellular lifespan and senescence. Once telomeres reach a critical length, cells either cease dividing (senescence) or undergo programmed cell death (apoptosis). Maintaining telomere length is crucial for genomic stability, tissue regeneration, and overall organismal health [2].

Telomere length (TL) shortening is strongly linked to cellular senescence, tissue damage, and age-related diseases, including cardiovascular disease, diabetes, and certain cancers. Aging tissues lose regenerative capacity due to increased rates of cellular senescence and apoptosis triggered by critically shortened telomeres. Inflammatory responses and oxidative stress accelerate telomere shortening, particularly when telomeric DNA is damaged by reactive oxygen species (ROS) [3]. Oxidative stress arises from both internal sources, such as cellular metabolism, and external factors, including environmental pollutants such as air pollution. As a result, environmental factors may

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significantly impact telomere dynamics [4].

Among environmental stressors, particularly traffic-related pollutants, have emerged as critical factor influencing telomere attrition. Urban transportation is vital to a country's economic and social growth [5], and the need for transportation services in cities is on the rise due to many factors, such as urbanization, population growth, rising living standards, and the expansion of businesses and industries. Elmansouri et al. [6] found that efforts to expand vehicle population and dependence on private transportation have led to significant increases in traffic volume, road crash rates, and traffic-related air pollution [6].

Traffic volume pollution refers to the harmful pollutants emitted by vehicles, particularly in densely populated urban areas with high traffic density. When fossil fuels are combusted in vehicle engines, they release pollutants such as particulate matter ($PM_{2.5}$ and PM_{10}), polycyclic aromatic hydrocarbons (PAHs), nitrogen oxides (NOx), carbon monoxide (CO), and volatile organic compounds (VOCs), all of which pose significant risks to human health [7]. Increases in both automobile use and traffic congestion are directly correlated with a corresponding rise in air pollution levels [8]. The correlation between TL shortening and traffic-related air pollution has attracted considerable interest due to its consequences for human health, aging, and disease vulnerability.

In numerous regions of Malaysia, vehicular emissions have emerged as the primary contributor to air pollution [9]. The escalating intensity and length of traffic volume may significantly elevate pollutant emissions and deteriorate air quality, especially in proximity to major roadways. These emissions pose risks of morbidity and mortality for drivers, commuters, and residents near roadways [8]. Moreover, these pollutants not only affect the respiratory and cardiovascular systems but also contribute to oxidative stress, accelerating biological aging, and promoting chronic disease [10].

Cellular functions such as cellular replication cause TL to shorten; however, air pollution and other environmental factors may speed up this process [11]. Given the potential health implications of TL shortening and its connection to environmental pollution, this study aims to investigate how leukocyte telomere length (LTL) is affected by exposure to traffic volume and related air pollution in Malaysia. By comparing adults living in high-traffic urban areas like Kuala Lumpur to those in rural, low-traffic regions such as Hulu Langat, we seek to answer our primary question: "Is there an association between LTL shortening and living closer to major roads and heavy traffic?", and our secondary question: "Is LTL shortening correlated with individual covariates (e.g. age, sex, BMI, body and visceral fat percentages) and other potential confounders (e.g., socioeconomic status, occupation, diet, passive smoking, and indoor stressors)?"

2. Materials and method

2.1. Selection of study locations

The Klang Valley is situated approximately in the middle region of the West Coast of Peninsular Malaysia [12]. It comprises five areas: the Federal Territory of Kuala Lumpur, Gombak, Petaling, Klang, and Hulu Langat. In our study, Klang Valley is the home of both heavily and less populated areas such as Kuala Lumpur and Hulu Langat, respectively. These two study locations were selected based on the yearly road traffic volume statistics from the Malaysian Ministry of Works [13]. The daily maximum traffic volume of 260,288 cars and the minimum traffic volume of 775 vehicles within a 16-h period have been recorded at Petaling Jaya Traffic Counting Station No.BR807 (N 3.11197 and E 101.656125) and at the Hulu Langat Traffic Counting Station No.BR613 (N 3.13398 and E 101.91613), respectively, over the past seven years, as shown in **Tables S1 and S2** (Supplementary Materials).

The high-traffic volume site is located in Bukit Kerinchi, Kuala Lumpur, and was selected due to its proximity (within one km) to Station No.BR807 that recorded the highest traffic volume in Malaysia (**Table S1**) (Supplementary Materials) [13]. Kuala Lumpur, the capital of Malaysia, functions as the primary center for economic, commercial, and industrial activities in the country. The Bukit Kerinchi area is highly congested and heavily populated. It is next to several significant highways, such as the Kuala Lumpur–Seremban Highway, the North-South Highway, and the New Pantai Highway. The low-traffic volume site is in Sungai Lui Village, Hulu Langat, Selangor, and this area is characterized by limited road traffic because of its far-flung position, approximately 45 km from the high-traffic site and main highways. Sungai Lui Village experiences minimal vehicular traffic and is located 5 km away from Station No.BR613 that recorded the lowest traffic volume in Malaysia (**Table S2**) (Supplementary Materials) [13]. These two study sites are illustrated in Fig. 1.

2.2. Study design, populations, and questionnaire

The study employed a comparative cross-sectional design. The study sample comprises two populations: one from the high-traffic region ($n = 101$) and another from the low-traffic region ($n = 101$). The project has been approved by the Universiti Putra Malaysia (UPM) Ethics Committee for Projects Involving Human Subjects (JKEUPM-2018-285). The screening questionnaire was disseminated to the residents of Bukit Kerinchi and Sungai Lui Village between December 2020 and December 2021 and subsequently retrieved. The screening questionnaire was employed to determine the eligible participants based on the specified criteria for inclusion and exclusion.

The inclusion criteria encompassed individuals between the ages of 18 and 75 who resided and worked within the research regions for a minimum of five years and experienced persistent exposure to the local traffic and its pollutants. The individuals who were excluded from the study were smokers, alcohol drinkers, and those who had chronic conditions such as hypertension, diabetes mellitus, asthma, cancer, and HIV/AIDS. These chronic diseases, smoking, and alcohol drinking were associated with shorter telomere length, and therefore we excluded the smokers, alcohol drinkers, and participants with any of these diseases [1,14–20].

The participating individuals were instructed to complete the main questionnaire on the day of blood collection. This comprehensive methodology enabled us to collect significant data on potential confounding variables that could affect telomere length. Through the analysis of answers, we could identify correlations that could elucidate the effects of dietary practices and everyday life on participants' telomere length. The main questionnaire includes questions about a socioeconomic background, including educational and marital status. There were five categories of education: no education, primary, secondary, pre-university, and tertiary education. The marital status encompassed three categories: single, married, and widowed or divorced. Many studies showed that lower educational attainment and unmarried status were associated with shorter LTL [21–23].

Additionally, we inquired about the nature of the occupation and if the work environment was outdoors or indoors. Since we included only non-smokers, we had to ask if there were any smokers living with them. Lastly, questions concerning the regular consumption of grilled foods and the practice of indoor grilling or frying were incorporated into the primary questionnaire. The ingestion of grilled meals, indoor presence of passive smoking, and indoor grilling are associated with higher exposure to PAHs that can affect the LTL inversely [24].

2.3. Blood sampling

Each participant was provided with a comprehensive explanation of the study's objective before completing a consent form. All participants were interviewed using a standardized questionnaire to gather sociodemographic data. Prior to blood sample collection, height, body weight, BMI, body fat percentage, and visceral fat percentage were assessed using Omron Full Body Composition Monitor and Scale (HBF-516B). The Kuala Lumpur group underwent blood sampling at a clinic in

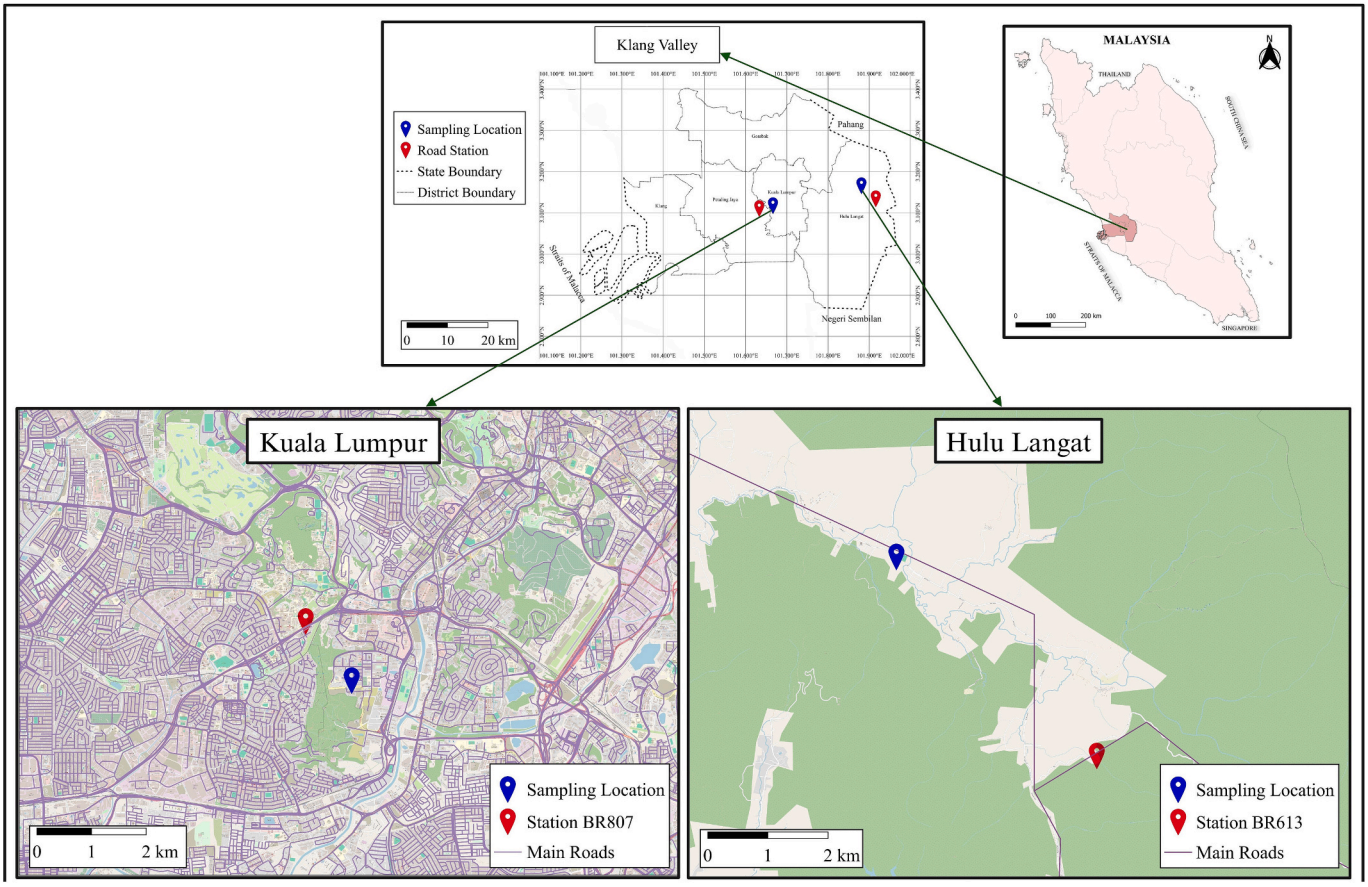

Fig. 1. Map of study locations [The map showed both study areas in Klang Valley (dark pink area), Malaysia. The dark blue symbols referred to the blood sampling areas, while the red symbols referred to the stations of the Ministry of Works that counted the vehicle volumes]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Bukit Kerinchi, Kuala Lumpur, from November 2021 to December 2021. During March and April 2022, blood samples were collected from the Hulu Langat group at the community center of Sungai Lui Village in Hulu Langat, Selangor. Ten milliliters of blood were drawn from each participant using an aseptic technique. Subsequently, 5 mL of blood was collected in BD Vacutainer EDTA tubes for quantitative polymerase chain reaction (qPCR) analysis, while the remaining 5 mL was placed in plain tubes for use in related research.

The blood tubes were labeled with the relevant participant code and kept promptly refrigerated at 4 °C following collection. The blood samples were transported on the same day to the lab and centrifuged at 2500 rpm for 10 min using a refrigerated centrifuge (Eppendorf 5810R, Germany). This process was used to separate the blood in the EDTA tubes into three distinct layers: plasma, buffy coat, and cells. The buffy coat layer was transferred by pipette into 2 mL microcentrifuge tubes that were labeled with the corresponding code of participants and stored at −80 °C for DNA isolation and LTL measurement.

2.4. DNA extraction and telomere length measurement

DNA was extracted from the buffy coat of blood samples using QIAamp DNA Mini Kit (QIAGEN, Germany) by spin protocol according to the manufacturer's guidelines. Buffy coat is a subfraction of whole blood that is enriched with leukocytes and produces approximately 5–10 times more DNA per unit volume than whole blood [25]. The DNA concentration and purity were determined using spectrophotometer (Lambda, PCRmax, United Kingdom) before dilution as per experimental requirements (5 ng μL^{-1}).

LTL was measured using qPCR method according to Cawthon et al. [26]. Firstly, the Telomere (T) to single copy gene (S) ratio (T:S ratio) was calculated for each sample using standard curves. Telomere mean length determines this ratio. Secondly, normalizing each sample's ratio to a reference DNA standardized run discrepancy [26]. After optimization, two different master mixes for T and S were prepared in different tubes with a total reaction of 20 µL added into every well [27]. Except for the oligonucleotide primers, T and S PCRs contained identical components.

The total concentration of reaction mixture for the T reaction consisted of 10 µL of 2× ChamQ Universal SYBR® qPCR Master Mix (Vazyme, China), telomere primers: 1 µL of tel-1 (270 nM), 1 µL of tel-2 (900 nM), 3 µL of 5 ng μL^{-1} of DNA samples and 5 µL of molecular grade water as shown in **Table S3** (Supplementary Materials). The S reaction was almost identical to the T reaction except for the 36B4 primers: 1 µL of 36B4u (300 nM) and 1 µL of 36B4d (500 nM). The T and S PCR reactions were performed in separate plates using LightCycler® 480 (Roche diagnostic).

Both reactions' thermal cycling profiles began with a 15-min incubation at 95 °C. For telomere PCR, 25 cycles of 95 °C for 15 s and 54 °C for 2 min were performed. For the 36B4 PCR, there were 35 cycles of 95 °C for 15 s and 58 °C for one minute. The primers were purchased from Bio Basic (Canada), and all the reactions were performed in triplicate, as illustrated in **Table S4** (Supplementary Materials). The telomere analysis was quantified by using the $2^{-\Delta\Delta CT}$ method. A standard curve was developed to evaluate the PCR reaction's efficiency where one reference DNA was diluted serially with 1.68-fold per dilution, yielding 5 DNA concentrations with a range of (100–10.54 ng μL^{-1}).

2.5. Quality control (QC) and quality assurance (QA)

Due to the sensitivity of nucleic acid amplification technologies, it was necessary to take certain precautions when manipulating QIAamp Mini columns during DNA extraction to prevent cross-contamination between sample preparations. Firstly, gloves were worn throughout the entire procedure and were replaced promptly if they came into contact with the sample. Secondly, the sample was carefully applied to the QIAamp Mini column and pipetted into it without wetting its periphery. Thirdly, pipet tips were replaced between each liquid transmission and did not come in contact with the QIAamp membrane.

The purity of every extracted DNA was checked by the Lambda spectrophotometer (PCRmax, United Kingdom) via the 260/280 and 260/230 ratios, as depicted in **Fig. S1** (Supplementary Materials). The average values of the 260/280 and 260/230 ratios were 1.7 and 2 among all samples, respectively. The DNA was stored in QIAamp DNA Mini reagent 'Buffer AE' at −80 °C. There was an average of one freeze-thaw cycle between DNA extraction and the qPCR assay. This was done to conduct a qPCR dilution. On average, samples were stored for one week between DNA extraction and qPCR analysis.

The two runs (T and S) were always conducted on separate plates on the same day using the same DNA aliquot that was stored at 4 °C for approximately 2.5 h between runs. On a 96-well Roche plate, each cycle contained 25 triplicate samples, 5 standards, 3 positive controls, and 2 non-template controls. The qPCR assays of both groups' DNA samples were randomized together, and the total duration of the qPCR assays was two months.

When the standard deviation of replicate quantification cycle (Cq) values exceeded 25 %, the replicate Cq values were assessed according to their deviation from the mean Cq of the triplicates. If one replicate departed from the mean Cq by more than 15 %, it was deemed an outlier, and the mean Cq was recalculated using two replicates. If the Cq standard deviation for either T or S replicates remained above 25 % after the elimination of a single outlier or exceeded 25 % without a distinctly identified outlier according to the aforementioned criteria, the sample was re-evaluated for both T and S using the same quality control assessment [28]. Hopefully, we did not encounter any of these conditions during the analysis.

The intra-plate correlation coefficients (CV) of the T and S reactions were 2.08 and 1.03, respectively. Whilst the inter-plate CV of the T and S reactions were 1.73 and 2.83, respectively.

The correlation coefficients for the Telomere and 36B4 gene standard curves were $R^2 = 0.99$, as shown in **Figs. S2** and **S3** (Supplementary Materials). Furthermore, the qPCR efficiency for the Telomere gene and 36B4 gene were 99.5 % and 103.1 %, respectively. The qPCR efficiencies should be in the range of 95–105 % and were calculated in this study using the linear regression slope of the standard curve based on the following formula:

$$\text{PCR efficiency} = -1 + 10^{(-1/\text{slope})}.$$

2.6. Statistical analysis

The statistical analysis was conducted using IBM SPSS Statistics version 27 according to the study's objectives and corresponding hypotheses. An analysis was conducted on the dataset to identify any missing data and outliers. The Kolmogorov-Smirnov and Shapiro-Wilk statistics were used in the normality test, which investigates the distribution of study variables. The continuous variables that followed a normal distribution were assessed using descriptive statistics, including measures such as the mean, standard deviation (SD), and range (minimum and maximum values). Nonetheless, the continuous variables that did not have a normal distribution were assessed using the median and interquartile range (IQR). Alternatively, the categorical variables were displayed using frequency and percentage.

Chi-square analysis was used to compare categorical variables, including sex and marital status. We compared the high- and low-traffic volume groups using an independent *t*-test for normally distributed continuous variables such as LTL. In contrast, a Mann-Whitney *U* test compared non-normally distributed continuous variables, such as age and BMI. The categories of body fat percentage were coded as low = 0, normal = 1, high = 2, and very high = 3, according to the manufacturer guidelines of the scale. Similarly, the categories of visceral fat percentage were coded as normal = 0, high = 1, and very high = 2. The full details of the categories of body fat percentage and visceral fat percentage are illustrated in **Tables S5** and **S6** (Supplementary Materials).

The outcome variable in our study was LTL and provided as the T/S ratio without transforming the latter to base pairs. Traffic volume was the predictor variable and consisted of two parameters: light, and heavy vehicles. The traffic volume data was obtained from the traffic counting stations of the Ministry of Works [13]. At these stations, vehicles were classified into six categories: cars and taxis, vans, medium lorries, heavy lorries, buses, and motorbikes. We categorized cars, taxis, motorcycles, vans and medium trucks as light vehicles, and heavy lorries with buses as heavy vehicles. According to the Road Traffic Department, a light vehicle is defined as having an unladen weight of less than 7500 kg, whilst a heavy vehicle is characterized by an unladen weight of 7500 kg or more [12]. The individual covariates included age, sex, BMI, body and visceral fat percentages. The LTL relationships with the traffic volume parameters and individual covariates were determined using simple as well as multiple linear regression analysis in the study groups.

3. Results and discussion

3.1. Sociodemographic features of participants

The total number of participants in the current study was 202, where the Kuala Lumpur (urban) and Hulu Langat (rural) groups were equally constituted of 101 Malay adults. Each group has 36 men and 65 women who live and work in the research area. Table 1 details the participants' sociodemographic characteristics. For the Kuala Lumpur group, the age range was 19–75, with a median of 55 years (IQR 36.5–64). The median age of the Hulu Langat group was 53 years (IQR 40.5–59), with a range of 18–74. In terms of body mass index, the median BMI of the urban participants was 25.4 kg m^{-2} (IQR 23.1–29.7), whereas the median BMI of the rural participants was 25.8 kg m^{-2} (IQR 22.9–28.9). The Mann-Whitney *U* tests indicated that all these differences between both study groups regarding body weight, height, and BMI were not statistically significant ($p > 0.05$).

The Kuala Lumpur and Hulu Langat groups had body fat percentages of 33.9 (IQR 26.05–43.3) and 34.2 (IQR 24.8–42.4), respectively. We conducted additional analysis of this data using the categories of body fat percentage. The very high category was the most prevalent in both study groups, followed by the normal and high categories. In particular, the Kuala Lumpur group was composed of 34 subjects (13 male and 21 female) with a normal body fat percentage, 21 subjects (6 male and 15 female) with a high body fat percentage, and 46 subjects (17 male and 29 female) with a very high body fat percentage. Additionally, the Hulu Langat group consists of 38 subjects (18 male vs. 20 female) with a normal body fat percentage, 19 subjects (6 male vs. 13 female) with a high body fat percentage, and 44 subjects (12 male vs. 32 female) with a very high body fat percentage.

The median visceral fat percentage was reported as normal in both study groups, including Kuala Lumpur participants and Hulu Langat women. Nevertheless, the visceral fat percentage of Hulu Langat males was exclusively high. In addition, the Kuala Lumpur group exhibited a normal visceral fat percentage in 23 males and 45 females, while 13 men and 20 women exhibited a high visceral fat percentage. Conversely, 18 males and 49 females in the Hulu Langat group were reported to have a normal visceral fat percentage. Furthermore, 18 men and 16 women in the Hulu Langat group exhibited a high percentage of visceral fat. The Mann-Whitney *U* tests showed that the differences between the two study groups regarding visceral and body fat percentages were not

Table 1
Individual parameters of all participants.

Parameters		KL group (n = 101)		HL group (n = 101)	
		%	Median (IQR)	%	Median (IQR)
Sex					
	Male	35.6	–	35.6	–
	Female	64.4	–	64.4	–
Age (y)					
	Whole	100	55 (36.5–64)	100	53 (40.5–59)
	Male	35.6	55.5 (41.5–65)	35.6	52.5 (42–58.5)
	Female	64.4	54 (33–63.5)	64.4	53 (39.5–60)
Body BMI [a]					
	Whole	100	25.4 (23.1–29.7)	100	25.8 (22.9–28.9)
	Male	35.6	24.2 (22.5–28.4)	35.6	25.5 (22.7–28.7)
	Female	64.4	26.2 (23.4–32.3)	64.4	26.1 (23.4–29.4)
Body fat (% bw) [b]					
	Whole	100	33.9 (26.05–43.3)	100	34.2 (24.8–42.4)
	Male	35.6	26 (21–33.8)	35.6	22.35 (16.77–28.42)
	Female	64.4	38.1 (33.7–45.4)	64.4	39.5 (31.2–43.65)
Visceral fat					
	Whole	100	8 (7–10)	100	8 (6–10)
	Male	35.6	8 (7–11.75)	35.6	9.5 (7–13)
	Female	64.4	8 (7–10)	64.4	8 (6–9)
Marital status					
	Single	19.8	–	19.3	–
	Married	61.4	–	65.8	–
	Widow/ Divorced	18.8	–	14.9	–
Education					
	No education	10.9	–	10.9	–
	Primary	7.9	–	9.9	–
	Secondary	59.4	–	60.4	–
	Pre-university	13.9	–	11.9	–
	Tertiary	7.9	–	6.9	–
Occupation					
	Outdoor	22	–	34	–
	Indoor	78	–	66	–
Grilled diet [c]					
	No	11	–	25	–
	Yes	89	–	75	–
Passive smoking [d]					
	No	68.31	–	51.48	–
	Yes	31.69	–	48.52	–
Indoor grilling/ frying [e]					
	No	18	–	18	–
	Yes	82	–	82	–

Abbreviation: KL: Kuala Lumpur HL: Hulu Langat IQR: Interquartile range a: Body mass index (kg m^{-2}) b: percentage per weight c: Intake of grilled meal once weekly d: Presence of at least one smoker living with the participant in the same household e: Indoor grilling or frying once weekly.

statistically significant ($p > 0.05$) (Table 1).

When asked about their marital status, most of the Kuala Lumpur participants were married 61.4 %. The rest were almost equally divided into either single 19.8 % or widowed/divorced 18.8 %. Similarly, most of the Hulu Langat participants were married 65.8 %, with the single and widow/divorced status comprising 19.3 % and 14.9 %, respectively. Furthermore, a major part of these participants was educated, 89 % in both sampling areas, with only 10.9 % who were illiterate. The main portion of these educated people completed secondary education (59.4 % vs. 60.4 %), followed by those with pre-university degrees (13.9 % vs. 11.9 %) in the Kuala Lumpur vs. Hulu Langat subjects, respectively. The percentage of primary and tertiary education among the Kuala Lumpur respondents was similar 7.9 %. However, the tertiary education 6.9 % was less than the primary education 9.9 % among the Hulu Langat respondents (Table 1).

Participants' occupations were classified as either outdoor or indoor work. Outdoor work was documented in merely one fifth and one third of the subjects from Kuala Lumpur and Hulu Langat ($p = 0.03$), respectively, whereas the majority of participants were engaged in indoor employment. Among the participants, 31.69 % of Kuala Lumpur and 48.52 % of Hulu Langat groups reported the presence of smokers in their households ($p = 0.04$). Furthermore, most participants from both sampling regions reported consuming grilled meals once a week ($p = 0.01$) and engaging in indoor grilling or frying ($p = 0.85$) (Table 1).

3.2. Leukocyte telomere length

The LTL data of participants from both sampling locations were normally distributed (Table 2). The LTL values in the Kuala Lumpur group varied from 0.08 to 1.29, but in the Hulu Langat group, it ranged from 0.06 to 1.94. The Kuala Lumpur group had a mean LTL of 0.77 ± 0.23, while the Hulu Langat group had a longer mean of 1.09 ± 0.07. The difference in LTL between both groups was statistically significant ($p < 0.001$). In both study groups, female participants had longer LTL than male participants. In the Kuala Lumpur group, women had a mean LTL of 0.78 ± 0.23, whereas men had 0.76 ± 0.23 ($p = 0.9$). In the Hulu Langat group, females had a mean LTL of 1.10 ± 0.25, whereas males had 1.08 ± 0.27 ($p = 0.86$), as shown in Fig. 2 and Table 2. None of these sex variations in each study group were statistically significant. However, statistically significant differences were found between men ($p < 0.001$) and women ($p < 0.001$) of both groups.

The participants in each group were then divided into two categories based on their ages: those who were less than 65 years old and those who were 65 years old or over. In the Kuala Lumpur group, the mean LTL was longer in the < 65 years old participants, 0.78 ± 0.23, than in the ≥ 65 years old participants, 0.76 ± 0.25, as illustrated in Fig. 2 and Table 2. This difference was statistically insignificant ($p = 0.60$). Similarly, the mean LTL of the Hulu Langat group was longer in the < 65 years old participants, 1.10 ± 0.25, than in the ≥ 65 years old participants, 1.05 ± 0.31. However, this difference was statistically not significant ($p = 0.56$). Interestingly, there were statistically significant differences between the < 65 years old participants of both study groups ($p < 0.001$) and the ≥ 65 years old participants of both study groups ($p = 0.002$).

We further studied these individual characteristics based on the four quartiles of the leukocyte telomere length, as illustrated in Table 3. The individuals with the shortest telomere length, as measured by the lowest quartile (with a mean of 0.57 and a range of 0.08–0.72), were found to be 11 years older than those with the longest telomere length, as measured by the highest quartile (with a mean of 1.32 and a range of 1.13–1.94) (Table 3). The highest quartile of telomere length exhibited

Table 2
Descriptive data of LTL among KL and HL groups.

		KL (n = 101) Mean (SD)	HL (n = 101) Mean (SD)	p-value
Whole		0.77 (± 0.23) (n = 101)	1.09 (± 0.07) (n = 101)	<0.001
Sex	Male	0.76 (± 0.23) (n = 36)	1.08 (± 0.27) (n = 36)	<0.001
	Female	0.78 (± 0.23) (n = 65)	1.10 (± 0.25) (n = 65)	<0.001
Age	<65y	0.78 (± 0.23) (n = 78)	1.10 (± 0.25) (n = 86)	<0.001
	≥65y	0.75 (± 0.25) (n = 23)	1.05 (± 0.31) (n = 15)	0.002

Abbreviation: KL: Kuala Lumpur HL: Hulu Langat SD: Standard deviation.

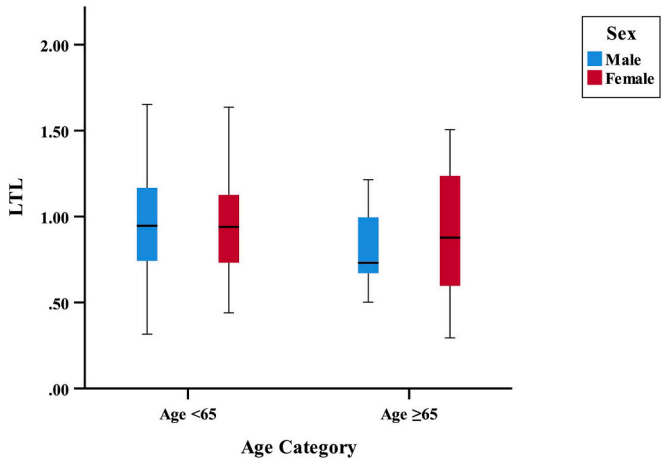

Fig. 2. Boxplot of LTL by age category and sex.

a notable increase in the proportion of women as well as a decrease in both BMI and body fat percentage compared to the lowest quartile. However, these differences were not statistically significant.

Various studies stated that men have on average, shorter telomeres than women [29–31]. Notable research by Lansdrop et al. [29] demonstrates that females have a longer LTL than males, a difference that is already evident at birth [32]. Some research has shed light on this phenomenon. An estrogen-responsive factor can activate telomerase, the enzyme responsible for adding telomere sequences to the ends of chromosomes [30]. Another possible explanation is that estrogen's effects upregulate the expression of antioxidant enzymes, which in turn counteract oxidative stress [33].

In addition, many researchers have stated that the shortening of leukocyte telomere length is associated with an increase in age [34–38]. Once differentiated into specific tissue or blood cells, the majority of cells, with the exception of certain stem cells, lose their telomerase activity [39]. Furthermore, the production of stem cells and other renewing cells decreases as the individual's age increases. Recently, Moix et al. [37] confirmed that telomeres shorten with age, with a greater reduction in males than females [40].

3.3. Relationship between the traffic volume parameters, individual covariates, and confounding variables with LTL

Our models examining the association between traffic volume and LTL were adjusted for individual covariates and the confounding variables. Multicollinearity was evaluated, revealing none of these variables featuring a variance inflation factor (VIF) of more than 5. The traffic volume consisted of two parameters: light and heavy vehicles. The simple linear regression (SLR) analysis demonstrated a statistically significant inverse association between LTL and all of these two parameters, as shown in **Table S7** (Supplementary Materials). Remarkably, we observed that these factors maintained a statistically significant inverse relationship with LTL even when we entirely integrated them into the multiple linear regression (MLR^1) model. These two variables collectively accounted for 31 % of the variance in LTL, with an LTL reduction of 0.38 units [(95 % CI: −0.42, 0.03), $p = 0.02$] for each increased light vehicle and an LTL loss of 0.19 units [(95 % CI: −0.25, 0.02) $p = 0.04$] for each increased heavy vehicle.

The SLR analysis revealed that the LTL of all subjects had negative relationships with all individual covariates (age, male sex, weight, height, BMI, body fat percentage, and visceral fat percentage). Nevertheless, none of these relationships were statistically significant. Similarly, some of the confounding variables, such as the outdoor occupation, regular intake of grilled meals, the presence of indoor passive smoking, and indoor grilling, were negatively associated with LTL. However, only the marital and educational status showed positive associations with LTL. Despite that, all these associations lacked any statistical significance, as illustrated in **Table S7** (Supplementary Materials). Subsequently, we implemented the MLR^1 analysis to evaluate the relationship of entire individual covariates and confounding variables with the LTL. None of these factors exhibit a significant relationship with the LTL. The R^2 of the model was 0.05, meaning that only 5 % of the LTL variance can be explained by these factors.

Next, to reduce the potential confounding variables, the adjusted MLR^2 analysis was carried out. We accounted for various individual covariates, including age, sex, BMI, body and visceral fat percentage, and confounding factors, such as marital and educational status, outdoor occupation, and dietary practices (e.g., frequent consumption of grilled foods and indoor grilling). The adjusted MLR^2 analysis enabled us to discern the effect of traffic volume on the LTL while controlling for these potential confounders. Incorporating these variables into the model allowed us to confirm that the statistically significant relationship between traffic volume and LTL was not influenced by any individual characteristics or activities that could affect telomere length, as shown in **Table S7** (Supplementary Materials). Collectively, these variables explained 35 % of the variance in LTL, with 31 % of that variance attributable to traffic volume alone. The LTL decreased by 0.38 units [(95 %CI: 0.26, 0.5), $p = 0.01$], and 0.16 units [(95 %CI: −0.16, 0.19), $p = 0.04$] for each increase in a single light and heavy vehicle, respectively.

Exposure to high amounts of traffic-related air pollutants has been linked to shorter telomeres in healthy people, according to some research [41–43]. Extended exposures being anticipated, traffic-related air pollutants may shorten telomere length after prolonged pro-oxidant exposures. The observed TL shortening could be involved in the biological pathways of short- and long-term particulate matter (PM) effects [41]. The findings of the current study align with our previous study [44], since the average concentration of $PM_{2.5}$ in Kuala Lumpur was 55.63 μg m^{-3}, which is twice as high as the $PM_{2.5}$ concentration in Hulu Langat, which was 25.51 μg m^{-3}. In Malaysia, motor vehicles are accountable for 64.72 % of Malaysia's ambient pollution [45], and are responsible for over 70 % of air pollution in urban areas [9]. Concurrently, our research revealed that the urban participants had substantially shorter telomeres than the rural participants, even after adjusting

Table 3
Individual parameters of all participants by the quartiles of LTL.

Parameters	N^1	1st Q^2	2nd Q^2	3rd Q^2	4th Q^2	P-value
		0.57 (0.08–0.72)	0.82 (0.73–0.92)	1.02 (0.93–1.12)	1.32 (1.13–1.94)	
N^1	202	47	54	51	50	–
Female sex, n (%)	130	29 (22.3)	34 (26.2)	32 (24.6)	35 (26.9)	0.92
Age, median	202	59	55	53	48	0.07
BMI, median	202	26.3	25.7	25.6	25.35	0.65
Body fat %, median	202	35.5	34.05	33.8	33.5	0.73
Visceral fat %, median	202	8	8	8	8	0.5

Abbreviation: N^1: Total number of subjects Q^2: Quartile of LTL with the mean and (range).

for individual characteristics and confounding variables.

Air pollution is a pervasive public health concern. The third and ninth risk factors for global mortality and morbidity, respectively, are indoor and outdoor air pollution [46]. In 2050, it is anticipated that 4.3 million urban residents will perish prematurely as a result of outdoor air pollution, which is responsible for 65 % of the global mortality rate [47]. Some studies were carried out on the traffic officers, showing that the mean LTL was shorter for those operating in high traffic intensity versus low traffic intensity [43,48]. Therefore, LTL is shorter in subjects exposed to traffic pollution, suggesting early biological aging and disease susceptibility [43,48,49].

Furthermore, several studies have demonstrated that individuals with significantly shortened LTL are at a higher risk of developing several cardiovascular diseases (CVD), such as myocardial infarction [11,50], coronary heart disease [51], heart failure [52], hypertension [53], and stroke [50]. The causal effect of shortened genetically determined LTL on CVD and cancer was demonstrated in a study that employed the Mendelian randomization design [54]. Telomere length has the potential to serve as a cardiovascular biomarker for biological aging. To date, inflammatory reactions and oxidative stress have been recognized as critical mechanisms that connect air pollution with associated morbidity and mortality [55].

The constituents of human telomeres comprise thousands of TTAGGG nucleotide repetitions and proteins [56]. The specific sequence TTAGGG yields telomeres possessing a high guanine concentration and renders them susceptible to oxidative stress [57]. Oxidative stress can accumulate and result in telomere degradation over the lifespan of a cell. The steady shortening of telomeres occurs after each DNA replication cycle, accelerating cellular aging. ROS are the principal substances associated with oxidative stress. In addition, ROS readily interact with nucleic acids, proteins, and lipids. Insufficient antioxidant capacity of the cells against these ROS will result in telomere degradation. On the other hand, chronic inflammation drastically affects telomere dynamics through several pathways, resulting in cellular aging and health impacts [58]. Chronic inflammation can impede telomerase activity via proinflammatory cytokines and expedite telomere attrition, resulting in premature cellular senescence [59]. Moreover, chronic inflammation elevates oxidative stress through the production of ROS, hence exacerbating telomeres shortening [60]. The latter affects mitochondrial activity, leading to increased ROS generation and subsequent oxidative damage. Thus, dysfunctional mitochondria accelerate cellular aging and enhance the inflammatory response [61].

3.4. Strengths and limitations

To the best of our knowledge, this is the first study in Malaysia to compare the LTL of adult populations living in high (urban) and low (rural) traffic volume areas. Additionally, we investigated the relationships between traffic volume and individual parameters with leukocyte telomere length in healthy, non-smoking, and non-alcoholic adult participants. Despite that, there are only a few constraints associated with the present study. The primary constraint is the disproportionate male-female ratio. Potentially, this disparity in numbers could result in inaccurate representations and distort the relationships between the sex of subjects and their telomere length. Another significant limitation is the cross-sectional design, which limits our ability to infer causality between air pollution exposure and telomere shortening. The last limitation is the 65 % unexplained variance in LTL, which indicates that a significant portion of the variation in telomere length remains inadequately comprehended and may be affected by factors not yet accounted for in the analysis. This highlights the necessity for more research to investigate other genetic, environmental, and lifestyle determinants that may affect telomere length, as well as to elucidate the complex interactions among these factors. Furthermore, it indicates that LTL is probably a multifaceted phenotype with numerous contributing factors, some of which are still unknown. Additionally, longitudinal studies are imperative to investigate the air pollution generated by the traffic volume in these urban and rural areas to provide additional evidence regarding the relationship between telomere length shortening and air pollution arising from traffic volume.

4. Conclusion

Our study highlights the differences in leukocyte telomere length (LTL) between adult populations living in high (urban) and low (rural) traffic volume areas in Malaysia. The mean LTL of the Kuala Lumpur group was significantly shorter (0.77 ± 0.23) than that of the Hulu Langat group (1.09 ± 0.07). Individual covariates, such as age, male sex, BMI, and body and visceral fat percentages, were negatively associated with LTL across all participants. Notably, we found a strong inverse relationship between heavy traffic exposure and LTL, suggesting that traffic-related pollution may play a significant role in telomere shortening. These findings underscore the importance of further investigating the environmental pollution caused by daily traffic emissions in both urban and rural regions. Understanding the detrimental effects of traffic-related air pollutants on telomere length, a key biomarker of biological aging, could provide valuable insights into the long-term health risks posed by air pollution and guide public health policies aimed at mitigating these risks.

CRediT authorship contribution statement

Samer Al-Battawi: Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yu Bin Ho:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Mohd Talib Latif:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Vivien How:** Writing – review & editing, Supervision, Funding acquisition, Formal analysis. **Haris Hafizal Bin Abd Hamid:** Writing – review & editing. **Sarah Hameed:** Writing – review & editing. **Karuppiah Thilakavathy:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests (e.g., any funding for the research project)/personal relationships (e.g., the author is an employee of a profitable company) which may be considered as potential competing interests:

Yu Bin Ho reports financial support was provided by Malaysia Ministry of Higher Education. Reports a relationship with that includes: Has patent pending to. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.enceco.2025.03.007.

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