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TELOMERE LENGTH VARIATIONS IN CORRELATIONS WITH AGE AND PHYSIOLOGICAL DISTRESS IN HYPERTENSIVE PATIENTS OF QUETTA CITY, BALOCHISTAN

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Abstract

The telomeric sequence, composed of repeated six-nucleotide units (TTAGGG), plays a crucial role in cellular ageing. Telomere length generally decreases with age but can also be influenced by factors such as gender, body mass index (BMI), medication, and lifestyle choices. In this study, DNA was extracted using the WizPrep™ gDNA Mini Kit (Blood) and amplified via quantitative polymerase chain reaction (qPCR) to determine cycle threshold values. These values were used to estimate the average telomere length for each DNA sample.

Among the hypertensive individuals analyzed (n=50), a negative correlation between age and telomere length was observed in the majority (68%). However, specific factors contributed to deviations from this pattern. Gender emerged as the most significant variable, with females exhibiting longer telomeres (average: 6,718 base pairs) compared to males (average: 5,914 base pairs), accounting for 16% of the observed deviations. BMI also influenced telomere length, as shorter telomeres were noted in obese individuals and longer telomeres in underweight individuals, affecting 12% of the samples. Lifestyle factors such as smoking (smokers: 5,816 base pairs; non-smokers: 6,596 base pairs) and physical activity (higher activity correlated with longer telomeres) were linked to telomere length variations, contributing to deviations in 2% of cases.

Despite these findings, the effects of other variables such as medication, diabetes, and dietary habits on telomere length remain unclear and warrant further research.

Keywords: *Aging, Hypertension, qPCR, Telomere length, T/S ratio WizPrep gDNA Mini Kit*

INTRODUCTION

Telomeres, comprising repetitive DNA sequences ("TTAGGG") and associated proteins, are specialized structures located at the ends of eukaryotic chromosomes. The term "telomere" is derived from the Greek words telos ("end") and meros ("part"), signifying their position as the "end parts" of chromosomes. These structures play a critical role in preserving chromosomal stability. In humans, telomeres range from 5 to 15 kilobase pairs in length and feature a guanine-rich single-stranded DNA overhang. This DNA is organized into protective loop structures that prevent chromosomal end-to-end fusions, rearrangements, and exonucleolytic degradation. The formation and stabilization of these loops are facilitated by the Shelterin complex, a specialized assembly of six proteins dedicated to telomere maintenance (1).

Telomeres naturally shorten with each cell division due to the "end-replication problem," a limitation of DNA polymerase in fully replicating the lagging strand during DNA replication. Approximately 50–200 base pairs are lost during each replication cycle, leading to a critical length known as Hayflick's limit. Functional telomeres are crucial as they prevent cellular DNA-repair mechanisms from mistakenly recognizing chromosome ends as DNA breaks (2).

Once telomeres reach a critically short length, they lose their protective function, triggering processes such as senescence (permanent cell cycle arrest) or apoptosis (programmed cell death). Consequently, normal somatic cells can undergo only a finite number of divisions before senescence ensues. The length of telomeres thus determines the replicative capacity of cells, often earning them the designation of a "biological clock." This gradual shortening contributes to cellular aging and the limited lifespan of cells.

In 1995, Allsopp *et al.* (3) conducted a survey, later reviewed by Okuda et al., 2002 (4) and examined age-related changes in telomere length (TL) using postmortem cerebral tissue samples. Their findings revealed telomere shortening rates ranging from 0 to 10 base pairs per year, with both positive and negative correlations observed among the samples. For instance, in the occipital lobe gray and white matter of 31 individuals aged 0–80 years, a significant negative correlation between TL and age (approximately 10 base pairs per year) was reported. Conversely, a separate cohort of 40 elderly individuals (aged 80–100 years) with longer telomeres exhibited a positive correlation (5).

Cawthon *et al.,* (2003) extended these observations, showing that individuals over 60 years with shorter telomeres had a higher mortality risk. Their findings suggest that longer telomeres might be associated with extended lifespans in older populations, influenced by specific selection criteria (6). These studies underscore the complex relationship between telomere length, aging, and mortality, emphasizing the need for further research into telomere dynamics.

The observations of Montpetit *et al.,* 2014 (7) highlighted a correlation between gender and telomere length, reporting that women typically have longer telomeres than men. This trend was observed across longitudinal and cross-sectional studies, with females showing a slower rate of telomere shortening than males. However, the strength of this correlation varied depending on the measurement methods used (8). Researchers proposed that the slower rate of telomere attrition in females contributes to their comparatively longer telomeres (9, 10).

Other studies have presented mixed findings and did not consistently observe longer telomeres in females (11, 12). One survey reported minimal gender-based differences in TL at birth (10), while another found that newborn females had longer telomeres than their male counterparts (13). Furthermore, no significant gender differences were observed in TL among younger participants (ages 19–37) in the Bogalusa Heart Study (12). However, in the Family Heart Study (ages 30–93), females exhibited longer telomeres than males, suggesting that the relationship between gender and TL may vary with age.

In the current study, males exhibited shorter telomeres (5,914 base pairs) compared to females (6,718 base pairs) on average. Although gender did not significantly affect TL in 32 individuals, it emerged as the primary factor influencing telomere variation in individuals aged 41, 47, 48, and 59 years when other variables such as BMI, medication, smoking, and blood sugar levels were held constant.

These findings suggest that gender may play a key role in determining telomere length, particularly in specific age groups. Males tend to have shorter telomeres than females, even when controlling for other confounding factors. However, the limitations of individual studies highlight the need for further investigation to confirm these results and explore the broader implications of gender differences in telomere biology.

The studies conducted by Olaussen *et al.,* 2006 (5) and Greider (1996) (14), have shown that body composition, specifically BMI (body mass index), can have an impact on telomere length. Females with a BMI greater than 30 were found to have reduced telomere length compared to those with a BMI below 20 (15).

In the Cardiovascular Health Study, a correlation between BMI and telomere length was observed only in overweight males with a BMI greater than 27, but not in females with a BMI greater than 25 (16).

In the present research, samples of individuals aged 44, 47, 48, 59, 62 and 65 years were categorized into obese, overweight and underweight groups, while keeping all other factors constant. The results indicated that individuals with obesity had the shortest telomeres, while those classified as underweight had longer telomeres. This suggests that BMI also affects telomere length in hypertensive patients.

As hypertension is commonly associated with diabetes mellitus, it is crucial to control high blood pressure to prevent damage to blood vessels and the heart. Research conducted by Nordfjäll *et al.*, 2008 (17), found evidence that certain medications used in the therapy of high blood pressure can influence telomerase and reverse transcriptase activity, which adds telomere repeats to chromosome ends, thereby extending telomeres.

The 2011 American College of Cardiology Foundation recommends initiating anti-high blood pressure medications at the minimum effective dose and gradually increasing the dosage based on the individual's blood pressure response until reaching the maximum dose (6). This approach aims to manage high blood pressure effectively while considering potential effects on telomere length and cellular health.

The American Diabetes Association recommends using medications that lower lipid content in type II diabetic patients over 40 years of age with multiple cardiovascular risk factors, including high blood pressure. Statins should be prescribed if low-density lipoprotein cholesterol levels are above 100mg/dl in patients with low risk. Metformin, a medication for glucose control, is associated with a reduced risk of diabetes-related complications in diabetic patients, and it is linked to lower weight gain and fewer hypoglycemic events compared to sulfonylureas and insulin (18). In the management of type II diabetes, metformin is considered the first-line pharmacological treatment.

In the current research, samples of individuals aged 41, 47, 48, 58, 62 and 65 years were taking medications, but there were variations in BMI and gender among the samples, making the effect of medication on telomere length unclear in this study. Additionally, some samples showed variations in diet, physical activity and smoking habits, which could also influence an individual's telomere length.

The study indicated that individuals who engaged in regular exercise and physical activities tended to have longer telomeres compared to those with less physical activity (as shown in Table II). On the other hand, smokers exhibited a higher rate of telomere attrition, on average, compared to non-smokers (as shown in Table III). These findings suggest that lifestyle factors, such as physical activity and smoking habits, may play a role in telomere length. However, the specific impact of medications on telomere length in this study remains unclear due to the presence of confounding factors such as BMI, gender, diet, physical activity and smoking habits.

MATERIALS AND METHODS

SAMPLING, SAMPLE SIZE AND TECHNIQUE

This study is an experimental research that involved collecting data through a questionnaire-based survey and conducting statistical data analysis and data evaluation. The researchers collected whole blood samples from 50 volunteer patients aged 40 and above. Each patient's blood was drawn using the venipuncture technique and 3mL of blood was stored in falcon tubes containing Ethylene diamine tetra acetic acid (EDTA) to prevent clotting. Genomic Deoxyribonucleic Acid (DNA) extraction was then performed directly from the blood samples using the WizPrep™ gDNA Mini Kit-Blood, Korea.

The DNA extraction steps followed the instructions provided in the manual of the WizPrep gDNA Mini Kit-Blood. To estimate the amount of DNA extracted, a Gel electrophoresis technique was used. The gel bands were visualized on a Trans illuminator under UV light.

After visualizing the gel bands, the quantity of DNA was determined using computer software called Image Java (Image J). The gel image was imported into the Image J program and adjusted to match the gel image captured on the Trans illuminator. Next, a "calibration lane" and "sample lanes" were selected by creating rectangular boxes and a graph was plotted to show the relationship between band intensity and pixels on the image. The area of each peak in the graph was calculated to find out the DNA quantity in each lane. The obtained data was recorded as the concentrations of stock DNA samples in spreadsheet format.

REAL-TIME qPCR

PREPARATION OF DILUTIONS

Before conducting Quantitative Polymerase Chain Reaction (qPCR), all the extracted Stock DNA samples were diluted using Polymerase Chain Reaction (PCR) grade water to achieve a concentration of 5 ng/µl. For the telomere reaction, telomere standards (as listed in Table 4) were serially diluted by a factor of 1.68, starting with the first dilution having a concentration of 6.1 ng/µl. For the Single Copy Gene (SCG) reaction, the telomere standards were also serially diluted, but the first dilution was made to have a concentration of 2 ng/µl (Table I).

Table I. PCR reaction mix, Telomeres and SCG serial dilutions

Syber Green	**PCR grade water**	**Diluted DNA**	**Forward Primer**	**Reverse Primer**	
	10ul	6ul	2ul	0.5ul	1.5ul
Slandered Dilution	**TS1**	**TS2**	**TS3**	**TS4**	**TS5**
Telomere	6.1ng/ul	3.63ng/ul	2.16ng/ul	1.29ng/ul	0.77ng/ul
Single copy Gene	1.80 ng/ul	1.07 ng/ul	0.64 ng/ul	0.38 ng/ul	0.23ng/ul

The PCR primers for the reaction were prepared by diluting the stock to a concentration of 100 pmol/µl. A Master Mix solution was then prepared, which included components for the negative template control (NTC), standards (which should be run in every PCR tray) and samples.

SETUP qPCR REACTION

To set up the qPCR reaction, the following steps were performed:

The PCR tray was prepared with each well containing the necessary components, including PCR-grade water, master mix, primers, standards and samples (as specified in Table 4). The prepared PCR tray was placed in the Real-time PCR machine and the lid was closed securely to ensure proper sealing. The program for the PCR run was set up using PCR running software. Sample identifiers were placed, including the negative template control (NTC), positive control and the quantities of standards used in the experiment. The PCR machine was turned on and the qPCR reaction was initiated according to the set program. The PCR reaction was allowed to run for duration of 2 hours, during which amplification and detection of the target DNA sequences occurred. During the PCR run, the cycle threshold (Ct value) for each sample was recorded for both the telomere primers and the single copy gene reactions.

After completing the PCR run, a standard curve was deduced using the data from the telomere standard dilutions to assess the PCR efficiency. The Ct values obtained from the qPCR reaction were used to generate the standard curve, which helps in determining the relative quantity of the target DNA in the samples. The standard curve and the Ct values are crucial for quantifying the amount of target DNA present in the samples accurately and for assessing the efficiency and reliability of the qPCR reaction.

CALCULATION OF TELOMERE LENGTH

The length of telomere for each sample was calculated by using the ratio between Ct value of telomere and single copy gene using formulae: Telomere length= 4270 X Ct value for telomere/Ct value of SCG (18). These calculated lengths of all samples were then analyzed statistically in concordance with high blood pressure ranges by using the IBM SPSS tool.

RESULTS AND DISCUSSION

The research included a total of 50 samples collected from various sources, such as BMC, Civil Hospital, Door to Door and SBK Women's University, Quetta, Balochistan from the patients suffering from hypertension. The genomic DNA was extracted using the WizPrep™ gDNA Mini Kit (Blood) and amplified using qPCR.

The Telomere Length (TL) qPCR assay results are presented in Fig. 1. Each sample was tested using two assays, one for Telomere and another for Single Copy Gene. During these qPCR assays of the 50 samples, different DNA copy numbers were generated until the fluorescence detection reached its exponential curve.

The Ct value represents the cycle number at which the fluorescence signal reaches a detectable level, indicating the presence of the target DNA.

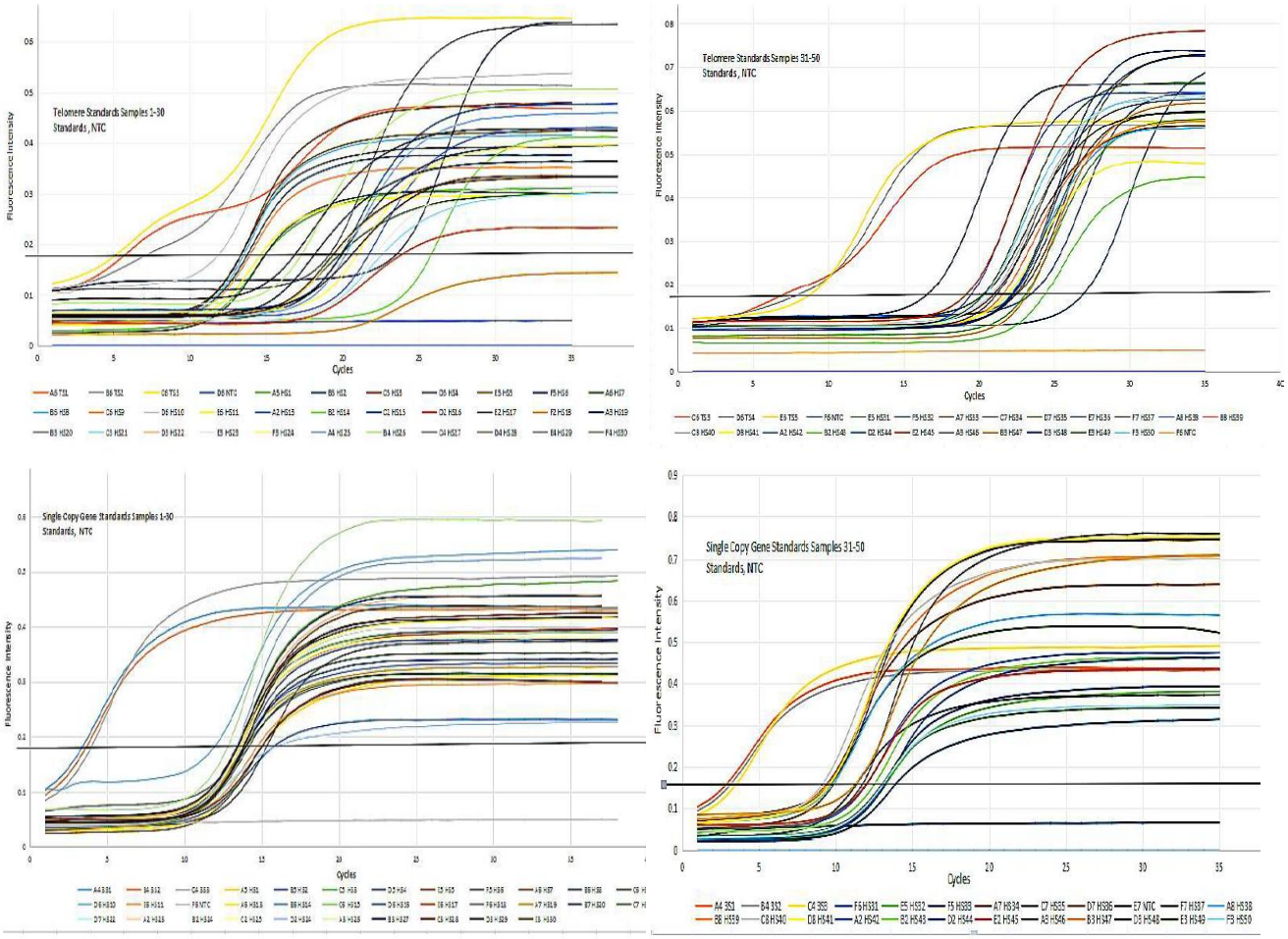

Fig. 1. (a) Amplification curve of Telomere standards with samples 1 to 30, **(b)** Amplification curve of Telomere standards with samples 31 to 50, **(c)** Amplification curve of single copy standard with samples 1 to 30, **(d)** Amplification curve of single copy standard with samples 31 to 50

Fig. 1a illustrates the Telomere assay, where the fluorescence amount reaches its exponential curve after 10 cycles. The figure includes three telomere standards, denoted by brown, grey and yellow colors, which serve as references for quantification. Additionally, 30 samples are represented by 30 different colors, each indicating the relative telomere length of the corresponding sample.

Fig. 1b illustrates the Telomere assay, where the amount of fluorescence reaches its exponential curve after 15 cycles. The figure includes three telomere standards, represented by brown, grey and yellow colors, which serve as reference points for quantification. Additionally, 20 samples are depicted in the figure, each represented by a distinct color, indicating the relative telomere length of the corresponding sample.

Fig. 1c illustrates the Single Copy Gene (SCG) assay, where the amount of fluorescence reaches its exponential curve after 10 cycles. The figure includes three SCG standards, represented by blue, brown and green curve lines, which serve as reference points for quantification. Additionally, 30 samples are depicted in the figure, each represented by a distinct color, indicating the relative abundance of the single copy gene in the corresponding sample.

Fig. 1d illustrates the Single Copy Gene (SCG) assay, where the amount of fluorescence reaches its exponential curve after 10 cycles. The figure includes three SCG standards, represented by brown, grey and yellow curve lines, which serve as reference points for quantification. Additionally, 20 samples are depicted in the figure, each represented by a distinct color, indicating the relative abundance of the single copy gene in the corresponding sample.

In the current research, after analyzing the DNA samples from 50 individuals affected by hypertension, the data and results indicated a negative correlation between aging and telomere length. The

findings showed that as individual's age, their telomeres tend to become shorter (Table II) i.e. with increasing age length of telomere decreases.

These results are also supported by the work of Kunieda *et al.*, 2006 (18), Nordfjäll *et al.*, *2006* (17), Valdes *et al.,* 2005 (20) and Hunt *et al.,* 2008 (8) suggesting that decreasing telomere can be used an indicator of growing age. All the samples were distributed gender-wise in 3 groups of "high blood pressure" ranges shown in Table I.

Table II. Mean telomere length in the following groups

		N	Mean	Std. Deviation	±SEM
Gender	Male	26	5913.9500	2461.0985	482.66
	Female	24	6718.0875	1506.1135	307.43
Physical Activity	Yes	17	6671.2471	6671.247	1787.45
	No	33	6108.6545	6108.654	2214.98
Smokers	Smokers	19	5816.189	2524.304	578.11
	Non Smokers	31	6596.426	1730.112	310.73

Table III. Telomeres and SCG serial dilutions

Slandered Dilution	TS1	TS2	TS3	TS4	TS5
Telomere	6.1ng/ul	3.63ng/ul	2.16ng/ul	1.29ng/ul	0.77ng/ul
Single copy Gene	1.80 ng/ul	1.07 ng/ul	0.64 ng/ul	0.38 ng/ul	0.23ng/ul

Negative values of correlation coefficient in males having more than 200 mm Hg systolic vessel pressure (Fig. 1b) and in females with 160-200 and >200 mmHg pressure (Fig. 1b and Fig. 1c) indicates a negative correlation between TL and age of individuals.

Table IV. Correlation coefficient males and females with different systolic blood pressure

Range of high systolic blood pressure	Male	Female
120-160 (mmHg)	r = 0.626	r = 0.483
161-200 (mmHg)	r = 0.829	r = 0.505
>201 (mmHg)	r = 0.424	r = 0.003

A positive value of correlation coefficient in males having 120-160 and 160-200 mm Hg systolic vessel pressure (Fig. 1c and Fig. 1d), and in females with 120-160 mmHg pressure indicates a positive correlation between TL and age of hypertensive individuals.

CONCLUSION

The results indicate that a majority of hypertensive individuals (32 out of 50, or 68%) exhibit a negative correlation between aging and telomere length, suggesting that telomeres tend to shorten as hypertensive individuals age. This telomere shortening aligns with the cellular aging process and is associated with various age-related conditions and diseases. However, a positive correlation between telomere length and aging was observed in a subset of hypertensive individuals. These deviations from the expected negative correlation are influenced by certain physical and environmental factors.

Gender emerged as the most significant factor, with females exhibiting longer telomeres than males (16% of the samples showed deviation from the negative correlation). Additionally, deviations were observed in 12% of samples due to variations in body mass index (BMI), with obese individuals showing the shortest telomeres and underweight individuals displaying longer telomeres. Smoking and levels of physical activity also contributed to the variability in telomere shortening, albeit in a smaller proportion of cases (2% of samples showed deviation).

The effects of other factors, such as medication, diabetes, diet, and additional lifestyle variables, remain unclear and require further investigation to better understand their impact on telomere dynamics.

Conflict of Interest:

Authors have no conflict of interest.

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