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Original Article

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A study on telomere length and 25-hydroxy vitamin D in association with glycaemic status in patients with diabetes mellitus

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Abstract

***Background & Aims*:** Telomere length is affected by vitamin D levels, and a relationship exists between telomere length and type 2 diabetes mellitus (T2DM), a condition associated with aging. This study aimed to investigate the connection between telomere length and 25(OH)D levels in diabetic individuals exhibiting various glycaemic profiles.

***Materials & Methods*:** Glycated hemoglobin (HbA1c) levels and vitamin D concentrations were measured in a cohort of 60 patients diagnosed with type 2 diabetes mellitus (T2DM), as well as in a control group of age-matched healthy individuals without diabetes. The analysis of telomere length was performed using quantitative real-time polymerase chain reaction (qPCR).

***Results*:** The findings revealed that the average telomere length (kb/genome) was significantly decreased in the diabetic group in comparison with the control group (diabetics: 4.21 ± 1.45 [3.29 - 4.65]; controls: 5.35 ± 1.85 [4.27-6.28]). Vitamin D levels were found to be notably lower in the diabetic cases (20.2 ± 8.5 vs. 51.52 ± 11.5, $P = 0.0001$). In the diabetic cohort, the Telomere length exhibited a significant negative correlation with HbA1c (rho = -0.97; $P = 0.0002$) and fasting blood sugar levels (rho = -0.95; $P = 0.0003$). Patients receiving insulin treatment demonstrated shorter telomere lengths compared to those on oral medication (4.32 ± 1.51 vs. 5.19 ± 1.71, $P = 0.07$). Insulin-treated individuals also had lower vitamin D levels than their orally treated counterparts (21.5 ± 9.5 vs. 50.8 ± 12.5, $P = 0.004$).

Discussion: The findings reveal an inverse relationship between HbA1c levels and telomere length, highlighting a possible connection between glycemic control and cellular aging in diabetic individuals. Furthermore, although there is an indication that vitamin D deficiency may influence telomere shortening, the observed correlation is rather weak.

***Conclusion*:** Vitamin D deficiency, as well as higher HbA1c levels shortens the Telomere length; therefore, Vitamin D supplementation with optimized glycaemic control may contribute to preserving the telomere integrity in diabetic individuals**.**

***Keywords*:** Aging**,** Glycaemic control, Telomere length, Type 2 diabetes mellitus, Vitamin D

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Introduction

Telomeres are unique DNA structures located at the ends of chromosomes, playing a crucial role in protecting the genome from interchromosomal fusion, recombination, repair processes, and nucleolytic damage. Research has established a connection between telomere length and age-related diseases, particularly type 2 diabetes mellitus (T2DM) (1-3). Evidence suggests that lower levels of vitamin D, combined with shorter telomeres, may elevate the risk of developing T2DM and its related complications. It has been observed that human telomere length diminishes at an estimated rate of 24.8 to 27.7 base pairs annually (4-6). Several factors, including lifestyle choices, genetic predispositions, and gender, significantly influence telomere length. Research suggests that cellular and tissue impairments associated with telomere dysfunction may originate from oxidative stress and persistent inflammatory processes (6, 7). Telomere shortening has been linked to various diseases, including viral infections, immune disorders, Alzheimer's disease, and cardiovascular conditions. The biologically active form of vitamin D, 1α, 25 dihydroxyvitamin D3 (calcitriol), affects telomere length. Calcitriol's anti-inflammatory and antiproliferative effects could lead to decreased cellular turnover, which might slow the progression of telomere shortening (8, 9). However, studies exploring the relationship between telomere length and circulating levels of 25(OH)D, the primary indicator of vitamin D status, are still limited (9-11). Variations in vitamin D levels can influence the mechanisms that regulate telomere length by reducing inflammation and cell proliferation, which in turn affects leukocyte counts and contributes to further telomere shortening (11, 12). Increased telomere length, along with elevated vitamin D levels, has been associated with a lower risk of chronic diseases, including type 2 diabetes and its progression (13, 14). A study was designed to investigate the relationship between telomere length and 25(OH)D concentrations in patients with T2DM, hypothesizing that a 25(OH)D concentration of less than 25 nmol/L would correlate with reduced telomere length in this population.

Materials & Methods

Inclusion of Sample

The research enrolled 60 diabetic patients alongside 60 age-matched healthy controls without diabetes, ensuring both groups were comparable in age and metabolic status. The research included non-diabetic adults aged 18 and older, as well as both inpatients and outpatients with type 2 diabetes mellitus (T2DM), regardless of the duration of their diagnosis, who were receiving treatment with either insulin or oral hypoglycemic agents. The study excluded individuals with documented histories of chronic inflammatory or infectious conditions, pancreatic disorders, diabetes secondary to other systemic diseases, or type 1 diabetes mellitus. It was ascertained that neither participant had been using vitamin D supplements. A thorough clinical examination was performed, encompassing a general physical and systemic assessment. Serum levels of 25-hydroxyvitamin D and telomere lengths of peripheral blood mononuclear cells were measured in both groups. Body mass index (BMI) was calculated using the formula weight (kg)/height (m^2), with obesity defined as a BMI exceeding 27 kg/m^2. Blood pressure was measured using a sphygmomanometer, with hypertension classified according to the Joint National Committee-7 guidelines as a systolic blood pressure (SBP) greater than 140 mmHg and a diastolic blood pressure (DBP) above 90 mmHg. Fasting blood sugar (FBS), postprandial blood sugar (PPBS), and HbA1c levels was conducted in accordance with the diagnostic criteria established by the American Diabetes Association in 2020. FBS and PPBS were determined through the use of a strip glucometer, while HbA1c levels were determined through high-performance liquid chromatography.

Circulating 25(OH)D Assay and Definition of Vitamin D Status

Blood samples of 5 mL were collected from the antecubital veins of participants prior to 7:00 a.m., following an overnight fasting period. Subsequent to venipuncture, 95% of the whole blood samples were transferred to the laboratory for analysis within 1 hour.

The serum levels of total 25-hydroxyvitamin D (25(OH)D) were assessed using a radio-immunoassay (RIA) kit. A diagnosis of vitamin D deficiency was established when the circulating concentration of 25(OH)D fell below 25 nmol/L.

Telomere Length

Blood samples underwent separation of mononuclear cells (MNCs) via HISTOPAQUE-based density gradient centrifugation. Following this, DNA was purified from the isolated peripheral blood MNCs using the QIAamp DNA Micro Kit (Qiagen, Germany), ensuring high-quality genomic material for analysis. The concentration of the isolated DNA was determined with a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA). To evaluate the absolute telomere length (TL) for each participant, quantitative real-time PCR (qPCR) was utilized. This method involved measuring telomere length in peripheral blood mononuclear cells by comparing the relative quantities of telomeric sequences to that of a single reference gene. All PCR reactions were conducted using a thermal cycler from Applied Biosystems (USA).

Serum 25-Hydroxyvitamin D

The serum was evaluated for several parameters, such as C-reactive protein (CRP), creatinine levels, lipid profile, and the concentration of 25-hydroxyvitamin D. The determination of 25-hydroxyvitamin D in the serum was performed using ultra-high-performance liquid chromatography (UHPLC; Thermo Scientific, USA).

Measurement of Telomere Length and Activity

Real-time quantitative polymerase chain reaction (RT-qPCR) was applied to measure relative telomere lengths in DNA samples derived from both human subjects and cultured cell lines. The evaluation of telomere length was performed using the Cawthon method. DNA was isolated from coronary artery intimal tissue using the DNeasy Tissue Kit (Qiagen, Germany), adhering strictly to the manufacturer's protocol. The analysis utilized the SYBR Green RT-qPCR Core Reagent Kit (Bio-Rad Laboratories, India). Reactions for the 36B4 gene and telomere were executed across two plates containing unknown samples. Each DNA sample, including control tubes from the Jurkat cell line, underwent three independent assays. Additionally, a no template control (NTC) tube, which contained only distilled water and PCR master mix, was included on each plate to monitor for primer dimer formation. In line with the Cawthon protocol, a standard curve was established on each plate using DNA from Jurkat cell lines, which included duplicates from five serially diluted wells, with final concentrations ranging from 12.6 to 100 ng. Telomere length was expressed as the ratio of telomere (T) to the single-copy 36B4 gene (S). A stock mix was prepared that contained 1x PCR buffer (Fermentas 10X PCR Hotstart buffer + KCl), 2 mM MgCl2, 0.2 mM dNTPs, 0.5 µM of each primer, 0.05 units/µL of Maxima Taq polymerase (Fermentas), and 0.2x SYBR Green I. The thermal cycling conditions for the 36B4 primer included a step at 58.0°C for 1 minute and 30 seconds during Stage 2 of Step 2. The PCR was designed to detect SYBR Green fluorescence during Stage 2, a phase in which SYBR Green binds to double-stranded DNA (dsDNA).

The primer sequences were as follows:
Tel1-
GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTG
AGGGT;
Tel2-
TCCCGACTATCCCTATCCCTATCCCTATCCCTA
TCCCTA;
36B4u-CAAGTGGGAAGGTGTAATCC;
36B4d- CCCATTCTATCATCAACGGGTACAA.

The amplification of samples was performed using a BioRad CFX96 thermocycler. To assess the relative lengths of telomeres, the difference in cycle thresholds (ΔCt) was determined by comparing the amplification of telomeres to that of a single gene copy. Genomic DNA extracted from the Jurkat control cell sample served as the reference for comparison with the sample cells.

Calculating T/S and Relative T/S Ratio

The telomere-to-single-copy gene (T/S) ratio, which reflects the average length of telomeres, serves as a standard for evaluating telomere lengths across different

samples. To ensure that the amplification of samples is normalized relative to that of a single gene, a gene with a known copy number is chosen. This method allows for the establishment of a comparative scale or value ratio by correlating the telomere repeat content of unknown samples with that of known single gene repeats. The 36B4 gene, located on chromosome 12 and encoding the acidic ribosomal phosphoprotein P0, also known as β-globin, is employed as a reference for measuring telomere length. For gene dosage analysis, the validated 36B4 gene is utilized. The ratio of the 36B4 gene copy number to the β-globin gene copy number in the experimental DNA is compared to that in the reference DNA. The T/S ratio, a critical metric for evaluating experimental samples, reference DNA, and the 35ng Jurkat control, was determined via Cawthon's formula.

[2Ct (telomeres)/2Ct(36B4)]-1 = 2-ΔCt

Cawthon proposes the following equation to calculate the relative T/S ratio in experimental samples.

2-(ΔCt1-ΔCt2) = 2-ΔΔ Ct

The same values can be derived through simpler equations. In this research, the T/S ratio was determined using the following formula:

ΔCt = Ct (telomere) – Ct (36B4)

The relative T/S ratio was subsequently computed using the following formula:

ΔΔCt = ΔCt (unknown) – ΔCt (control)

The relative T/S ratio can be calculated by inputting the T/S ratio values for unknown samples, controls, and standards into Microsoft Excel, accompanied by the necessary formulas. An increase in the relative T/S ratio corresponds to an increase in telomere length.

Lifestyle

The health questionnaire examined various lifestyle factors, including smoking habits, alcohol consumption, and levels of physical activity. To evaluate these elements within the population, a Physical Activity Questionnaire (PAQ) was utilized. Participants were classified into three distinct groups according to their PAQ scores: low (scores ranging from 0 to 1), moderate (scores between 2 and 6), and high (scores from 7 to 18). This categorization of physical activity levels was determined by both the frequency and intensity of weekly exercise. Regarding smoking behavior, individuals were categorized as non-smokers, occasional smokers, or regular smokers.

Statistical Analysis:

Categorical data were expressed as percentages. $\chi 2$ test was used for categorical variables. The classification of 25(OH)D concentrations followed the SACN guidelines for vitamin D, categorizing levels < 25 nmol/L as low. To maintain a balanced distribution of participants in groups, moderate levels were defined as 25-50 nmol/L, and high levels were identified > 50 nmol/L. These variables underwent a fitting process that involved fitting, discarding, and refitting to create the most effective and parsimonious model, with continuous evaluation of model fit statistics. Model 1 was unadjusted, while Model 2 included adjustments for alcohol and smoking use. In addition to the aforementioned adjustments, Model 3 was also modified to account for BMI and physical activity. Furthermore, Model 4 accounted for HbA1c levels. The models were analyzed separately according to gender. All results were presented as means ± SD. Group comparisons were conducted using the student's t-test or, when appropriate, ANOVA. *P-value* of < 0.05 was considered statistically significant. The statistical analysis was performed using a commercial statistical software package (IBM SPSS Statistics 20.0; Chicago, IL, USA).

Results

The study's participants had a mean age of 42.5 years, with a standard deviation of 7.3 years, and their ages varied from 32 to 68 years. Within this cohort, 10 individuals were classified as overweight, exhibiting an average body mass index (BMI) of 26.5 kg/m² and a standard deviation of 5.7. Furthermore, 20 participants reported being smokers. The participants were categorized into two age groups: those aged 32 to 55 years (group 1, n = 36) and those aged 56 to 68 years (group 2, n = 24). The mean ages of the groups were 39.5 (±7.5) years and 62.5 (±6.5) years, respectively,

demonstrating a statistically significant disparity ($P < 0.001$). However, no significant differences were found in terms of sex distribution, BMI, or blood pressure between the two age categories.

PCR Data Analysis

The relative T/S ratio, an indicator of telomere length, exhibited a decrease in the diabetic group relative to the normal lymphocytes in the control group. The diabetic cohort exhibited a notable decrease in telomere length, quantified in kilobases per genome, with an average of 4.21 ± 1.45 (range: 3.29–4.65) compared to 5.35 ± 1.85 (range: 4.27–6.28) observed in the control group (Table 1). Notably, the telomeres of individuals with diabetes were significantly shorter ($P = 0.04$). The mean telomere lengths for those with diabetes and those without were recorded as 4.21 ± 1.45 and 5.35 ± 1.85 kb/diploid genome, respectively.

Table 1. Basic characteristics and laboratory variables of diabetic cases and control

Demographics	**Controls (Mean ± SD)**	**Cases (Mean ± SD)**	***P-value***
Age (years)	49.5 ± 16.5	51.65 ± 17.5	0.07*
Gender			
Male	38	35	0.22[c]
Female	22	25	
BMI (kg/m^2)	24.04 ± 2.03	27.34 ± 1.11	0.0002*
HTN			
Yes	10	18	0.2
No	50	42	
Smoking			
Yes	15	20	0.56
No	45	40	
Physical activity			
Regular	17	42	0.0001
Sometimes	24	12	
Never	19	6	
Treatment of DM			
Oral	-	33 33	-
Insulin	-	26.67	-
Both	-	40	-
Duration of diabetes (years)	16.35 ± 10.08	-	-
SBP	115.5 ±7.5	116.5 ± 8.5	0.0001
DBP	77.8 ± 9.5	89.5 ± 10.9	0.0001
25-hydroxy vitamin D (ng/ml)	51.52 ± 11.5	20.2 ± 8.5	0.0001
Fasting blood sugar (mg/dl)	98.2 ± 10.5	125.8 ± 15.5	0.025
Total cholesterol (mg/dl)	185.5 ± 20.8	255.5 ± 50.5	0.0001
Triglycerides (mg/dl)	99.7 ± 19.5	240.31 ± 52.5	0.0001
HDL-C (mg/dl)	49.6 ± 8.5	42.18 ± 10.5	0.0001
LDL-C (mg/dl)	58.5 ± 12.5	131.5 ± 45.8	0.0001
VLDL-C (mg/dl)	21.3 ± 10.6	49.9 ± 11.2	0.0001
Serum creatinine (mg/dl)	0.85 ± 0.075	0.98 ± 0.08	0.08

Demographics	Controls (Mean ± SD)	Cases (Mean ± SD)	*P-value*
Telomere length (kb/genome)	5.35 ± 1.85	4.21 ± 1.45	0.04
HbA1c (%)	5.49 ± 0.6	8.5 ± 2.6	0.0002

*Welch t-test, ᶜChi square test

The relative T/S ratio, an indicator of telomere length, was found to be lower in diabetic individuals compared to normal lymphocytes in the control group. The average telomere length, expressed in kilobases per genome, was significantly reduced in the diabetic cohort, with measurements of 4.21 ± 1.45 (range: 3.29 - 4.65) for the diabetic cases and 5.35 ± 1.85 (range: 4.27 - 6.28) for the controls. Notably, diabetic patients exhibited considerably shorter telomeres ($P = 0.04$). The mean telomere lengths for diabetic and non-diabetic participants were recorded as 4.21 ± 1.45 and 5.35 ± 1.85 kb/diploid genome, respectively. In the diabetic population, a significant negative correlation was observed between telomere length and body mass index (BMI) (rho = -0.35; $P = 0.009$). Additionally, within this group, there were strong negative associations between telomere length and various metabolic parameters, including HbA1c (rho = -0.97; $P = 0.0002$), fasting blood sugar (FBS) (rho = -0.95; $P = 0.0003$), and postprandial blood sugar (PPBS) (rho = -0.96; $P = 0.0001$). Furthermore, vitamin D levels showed a negative correlation with BMI (r = -0.22; $P = 0.05$), duration of diabetes (rho = -0.25; $P = 0.02$), and serum creatinine levels (r = -0.27; $P = 0.04$). In the diabetic group, telomere length was positively correlated with vitamin D levels (rho = 0.12; $P = 0.5$) (Table 3). A one-tailed t-test indicated that diabetic patients receiving insulin therapy had significantly lower average vitamin D levels compared to those on oral medications ($P = 0.0056$, odds ratio 1.22 (95% CI: [1.07, 1.39]). Additionally, insulin-treated diabetics had shorter telomeres than those on oral medication (4.32 ± 1.51 vs 5.19 ± 1.71, $P = 0.004$).

Table 2. Correlations of Clinical Parameters with Telomere Length and Vitamin D Status in Diabetic Patients

Variable	Telomere length		Vitamin D	
	rho	P	r	P
Age	0.19	0.2	-0.04	0.7
BMI	-0.35	0.009	-0.22	0.05
Duration of T2DM	0.11	0.28	-0.25	0.02
HbA1c (%)	-0.97	0.0002	0.07	0.6
FBS (mg/dl)	-0.95	0.0003	0.05	0.85
PPBS (mg/dl)	-0.96	0.0002	0.045	0.65
Serum creatinine (mg/dl)	-0.05	0.8	-0.27	0.04
Vitamin D	0.15	0.5	-	-

Rho: Spearman rank correlation, r: Pearson correlation coefficient

Table 3. Telomere length in diabetic cases and controls classified by age group

Group	Age/sex	HbA1c < 6.5	HbA1c 6.5-7.5	HbA1c 7.5-8.5	HbA1c > 8.5
Cases	< 55 y	4.41 ± 1.40	4.33 ± 1.44	4.12 ± 1.49	4.0 ± 1.52
	> 55 y	4.35 ± 1.44	4.22 ± 1.51	4.0 ± 1.47	4.0 ± 1.56
Control	< 55 y	5.35 ± 1.95	5.31 ± 1.74	5.31 ± 1.9	5.29 ± 1.87
	> 55 y	5.29 ± 1.88	5.28 ± 1.78	5.32 ± 1.88	5.25 ± 1.75

Group	Age/sex	HbA1c < 6.5	HbA1c 6.5-7.5	HbA1c 7.5-8.5	HbA1c > 8.5
Cases	Male	4.45 ± 1.44	4.32 ± 1.55	4.22 ± 1.54	4.1 ± 1.44
	Female	4.41 ± 1.52	4.28 ± 1.49	4.1 ± 1.56	4.0 ± 1.58
Control	Male	5.37 ± 1.85	5.32 ± 1.77	5.33 ± 1.85	5.31 ± 1.79
	Female	5.34 ± 1.78	5.29 ± 1.72	5.31 ± 1.90	5.29 ± 1.88

Participants' Characteristics

Significant differences were observed between male and female participants in terms of BMI classification, levels of physical activity, telomere length, and HbA1c measurements when applying the three established cut-off points for 25(OH) vitamin D (low, moderate, and high). The study's sample primarily comprised female participants, who generally maintained a normal weight, participated in moderate physical activity, and abstained from alcohol consumption.

Circulating 25(OH) Vitamin-D Levels and Telomere Length by Sex

In the unadjusted analysis (95% CI = 2.2-465.5, B = 229.5 ± 105.5, *P* = 0.05), very-old men with vitamin D levels below 25 nmol/L demonstrated shorter telomere lengths compared to those with vitamin D levels between 25 and 50 nmol/L. This trend persisted in the adjusted model, which considered relevant confounding variables (95% CI = 10.5-495.6, B = 241.2 ± 112.2, *P* = 0.04). Conversely, very-old women with 25(OH) vitamin D levels exceeding 50 nmol/L exhibited longer telomere lengths than those with levels between 25 and 50 nmol/L in the unadjusted model (95% CI = 14.2-121.3, B = 75.1 ± 21.5, *P* = 0.014). The significance of this relationship was sustained even after adjusting for confounding variables, including smoking, alcohol intake, BMI, and physical activity (95% CI = 6.2-128.5, B = 71.1 ± 22.5, *P* = 0.05). However, upon further adjustment for HbA1c, the strength of the association diminished (95% CI = −0.8-129.2, B = 64.1 ± 22.9, *P* = 0.06) (Table 4).

Table 4. Association between 25(OH) vitamin-D cut-offs and telomere length by sex

Sex	Model	25(OH) vitamin-D	β Coefficient	Adj. R Square	95% CI	*P*
Men (n = 304)	Model 1 (unadjusted model)	Low	229.5	0.009	2.2, 465.5	0.05
		Moderate	(ref)		(ref)	(ref)
		High	55.2		−11.6, 119.2	0.09
	Model 2 (adjusted for smoking and alcohol)	Low	255.5	0.008	25.2, 480.5	0.03
		Moderate	(ref)		(ref)	(ref)
		High	62.5		−6.5, 114.6	0.09
	Model 3 (adjusted for BMI and physical activity)	Low	245.2	0.007	10.2, 483.6	0.05
		Moderate	(ref)		(ref)	(ref)
		High	61.2		−4.8, 132.5	0.06
	Model 4 (adjusted for HbA1c%)	Low	241.2	0.022	10.5, 495.6	0.04
		Moderate	(ref)		(ref)	(ref)
		High	65.4		−3.1, 135.2	0.05
Women (n = 471)	Model 1	Low	27.8	0.02	−159.1, 215.5	0.8
		Moderate	(ref)		(ref)	(ref)
		High	75.1		14.2, 121.3	0.02
	Model 2	Low	19.5	0.008	−175.2, 198.2	0.78

Sex	Model	25(OH) vitamin-D	β Coefficient	Adj. R Square	95% CI	*P*
		Moderate	(ref)		(ref)	(ref)
		High	70.5		17.2, 132.4	0.02
		Low	−5.6		−218.2, 203.6	0.9
	Model 3	Moderate	(ref)	0.02	(ref)	(ref)
		High	71.1		6.2, 128.5	0.05
		Low	−22.5		−218.5, 190.5	0.9
	Model 4	Moderate	(ref)	0.02	(ref)	(ref)
		High	64.1		−0.8, 129.2	0.06

Reference (Ref): control group. 25-Hydroxyvitamin D (25[OH]D) thresholds were defined as: <25 nmol/L (low), 25–50 nmol/L (moderate; reference category), and >50 nmol/L (high).

Predictors of Telomere Length

A lack of correlation was observed between telomere length and factors such as smoking, alcohol consumption, levels of physical activity, body mass index (BMI), use of supplements, number of health conditions, or HbA1c% across the entire study population. The sole notable correlation observed was between telomere length and sex (95% CI = 0.000-0.001, *P* = 0.007). When participants were divided by gender, no significant associations were found between telomere length and the covariates, except for a significant relationship with BMI among female participants (95% CI = 0.0034-0.044, *P* = 0.039).

Circulating 25(OH)D levels and Telomere Length in very-old aged subjects:

In older adults, a significant positive association was observed between 25(OH)D levels and telomere length. The initial analysis revealed that individuals with 25(OH)D concentrations exceeding 50 nmol/L exhibited longer telomeres compared to those with levels below this threshold (95% CI = 15.5-98.6, B = 79.2 ± 18.6, *P* = 0.006). This association remained statistically significant even after controlling for various confounding variables, including smoking status, alcohol intake, body mass index (BMI), physical activity, and HbA1c levels (95% CI = 10.5-99.3, B = 59.8 ± 15.6, *P* = 0.02) (Table 5).

Table 5. Relationship Between 25(OH)D Thresholds and Telomere Length

Model	25 (OH)D	β Coefficient	Adj. R Square	95% CI	*P-value*
Unadjusted model	Low	79.2	0.008	−66.2, 198.5	0.3
	Moderate	(ref)		(ref)	(ref)
	High	59.6		15.5, 98.6	0.006
Adjusted for smoking and alcohol	Low	79.8	0.008	−59.9, 199.5	0.3
	Moderate	(ref)		(ref)	(ref)
	High	66.8		19.2, 101.5	0.006
Adjusted for BMI and physical activity	Low	69.5	0.005	−69.0, 210.5	0.3
	Moderate	(ref)		(ref)	(ref)
	High	55.5		10.5, 109.2	0.02
Adjusted for HbA1c%.	Low	69.5	0.004	−75.3, 210.5	0.212
	Moderate	(ref)		(ref)	(ref)
	High	59.8		10.5, 99.3	0.02

Reference group (Ref): 25-Hydroxyvitamin D (25(OH)D) thresholds were defined as <25 nmol/L (low), 25–50 nmol/L (moderate; reference category), and >50 nmol/L (high).

Discussion

Telomeres are essential for protecting the genome by preventing chromosome fusion, facilitating repair processes, minimizing unnecessary recombination, and guarding against nucleolytic degradation. During DNA replication, telomeres progressively shorten, which can eventually lead to cellular death or senescence once they reach a critical length. Furthermore, inflammation is closely associated with various dietary factors, including excessive caloric intake and high-sugar diets. While making lifestyle changes can be difficult, vitamin D levels can be effectively managed through sun exposure or dietary supplements. In this context, the study aimed to explore the relationship between telomere length and the concentration of 25-hydroxyvitamin D (25(OH)D) (15,16).

In contrast to a prior study involving white males that found a lack of significant relationship between telomere length and vitamin D biomarkers (25(OH)D or 1,25(OH)D), recent research has identified a correlation between 25(OH)D levels and telomere length in men. Several biologically plausible hypotheses have been proposed regarding how sex may affect the relationship between 25(OH)D concentration and telomere length, including the possibility that men may possess shorter telomeres than women. Additionally, estrogen, recognized for its antioxidant properties and its role in regulating antioxidant genes, may enhance telomerase activity. Therefore, it is crucial to acknowledge that the relationship between vitamin D levels and telomere length may be influenced by differences between sexes (17).

Our investigation revealed a notable positive correlation between telomere length and serum levels of 25 (OH)D (surpassing 50 nmol/L) in fully adjusted models. This study indicates that telomere length is directly related to vitamin D concentrations, indicating that longer telomeres correspond to higher levels of vitamin D within the adequate range. Additionally, a relationship was identified between vitamin D and HbA1c levels, both of which influence telomere length (18). Elevated HbA1c levels and diminished vitamin D concentrations were found to accelerate telomere shortening. The research demonstrated that participants with normal to high vitamin D levels experienced a slower decline in telomere length compared to those with deficient levels, supporting the findings of Baltzis et al (19). Patients with diabetic foot complications exhibited decreased telomerase activity, increased waist circumference, and higher neuropathy impairment scores, reflecting a clinical profile akin to that of diabetics with ulcers. Moreover, the study underscored a significant difference in body mass index (BMI) between individuals with diabetes and those without diabetes (20). It was observed that a majority of the patients were classified as obese, establishing a clear connection between inadequate glycemic control in type 2 diabetes and obesity. In diabetic patients, an inverse relationship was noted between telomere length and BMI. Gurung et al. also found a negative correlation between telomere length and both BMI and waist-to-hip ratio (WHR), with statistical significance ($P < 0.05$) ($P < 0.05$) (21).

The investigation by Arsenis et al. demonstrated a negative relationship between telomere length and DNA damage markers in relation to exercise duration. Engaging in physical activity promotes fat mobilization and enhances detoxification processes by mitigating oxidative stress, thereby protecting telomeres and DNA (22). A significant disparity in average blood glucose and HbA1c levels was noted between diabetic individuals and those without diabetes. Piplani et al. reported that telomere shortening was markedly more severe in individuals with type 2 diabetes mellitus (T2DM), indicating substantial telomere loss in this population (23). Moreover, vitamin D levels were found to be considerably lower in individuals with diabetes compared to their non-diabetic peers, corroborating the findings of Athanassiou et al. (24). Regular physical activity and adequate vitamin D levels were associated with reduced insulin resistance, facilitating weight loss and aiding in the prevention of type 2 diabetes. Conversely, insufficient physical activity has been linked to lower vitamin D levels due to decreased

lipolysis (25). Diabetic individuals who participated in regular exercise demonstrated significantly higher average vitamin D levels than those who did not, with a P-value of less than 0.0001. Additionally, an inverse correlation was observed between vitamin D levels and the duration of type 2 diabetes among affected individuals. The research conducted by Alaidarous et al. (26) indicated that the duration of type 2 diabetes is an independent predictor of vitamin D deficiency. In diabetic patients, telomere length exhibited a significant negative correlation with HbA1c, fasting blood sugar (FBS), and postprandial blood sugar (PPBS)

Dudinskaya et al. conducted research that identified a negative correlation between telomere length and both fasting blood sugar (FBS) and HbA1c, with HbA1c showing a statistically significant association ($P = 0.03$) (27). Furthermore, patients receiving insulin therapy had notably lower levels of vitamin D compared to those on oral medications. This reduction in vitamin D among diabetic individuals is associated with impaired beta-cell function. Research by Mauss et al. revealed that elevated FBS ($\beta = 3.13$; 95% CI: 0.78, 5.47; $P \leq 0.01$) and HbA1c were linked to severe vitamin D deficiency, defined as levels falling below 10 ng/ml (28). The connection between telomere length and 25(OH)D levels can be elucidated through various mechanisms. One potential explanation is that the active form of vitamin D may reduce telomere shortening via anti-inflammatory and antiproliferative pathways. It has been shown to decrease systemic inflammatory mediators, such as interleukin-2 and tumor necrosis factor, which may contribute to a slower rate of telomere length loss. The processes of aging are significantly affected by oxidative stress and inflammation, and the interplay of these factors appears to regulate telomere length dynamics (29). In our previous study, we found that circulating coronary endothelial progenitor cells in the coronary arteries of patients with coronary artery disease (CAD) who had undergone coronary artery bypass grafting (CABG) exhibited signs of senescence and functional impairment (30).

Strengths and Limitations

The results may assist healthcare professionals in predicting outcomes for patients experiencing diabetic complications, such as coronary heart disease, diabetes mellitus, and cardiovascular disease. Consequently, physicians can adopt preventive strategies and monitor potential complications more effectively. However, a limitation of this research is its exclusive focus on individuals with type 2 diabetes. Future studies should explore whether similar associations exist in patients with type 1 diabetes, pancreatic disorders, diabetes resulting from other systemic conditions, or established cases of infectious or chronic inflammatory diseases.

Conclusion

The study revealed a positive correlation between telomere length and the levels of 25(OH)D within the participant group. Individuals with diabetes exhibited significantly lower vitamin D levels, which were associated with their higher body mass index (BMI) and sedentary lifestyle. Given that elevated vitamin D levels are linked to increased telomerase activity, a relationship between vitamin D concentrations and telomere length was established. Furthermore, a decline in telomere length was noted in conjunction with low vitamin D levels and elevated HbA1c levels, which were also found to be interconnected. In conclusion, increasing vitamin D levels may support the preservation of telomeres and mitigate the cellular aging process. Additionally, investigating the impact of vitamin D on telomere shortening could serve as a valuable metric for evaluating effective glycemic control in individuals with type 2 diabetes.

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Ethical statement

The research adhered to the stipulations outlined in the Declaration of Helsinki. Approval for the study was granted by the Institutional Ethics Committee (Reference No. NIMS/IEC/2024/RC-01).

Data availability

None declared.

Conflict of interest

There are no conflicts of interest.

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Author contributions

None declared.

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