ORIGINAL ARTICLE

Association of Telomere Length and Serum Vitamin D Levels with Type 2 Diabetes Mellitus and its Related Complications: A Possible Future Perspective

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ABSTRACT

Evidence show that shortened telomere length (TL) and low Vitamin D levels can increase the risk of type 2 diabetes mellitus (T2DM) and its associated complications. T2DM has been considered as an age-related disease, it may be associated with TL. The study aimed to evaluate the association of TL and Vitamin D levels with complications of T2DM and the impact of Vitamin D on TL in patients with T2DM. This 1-year cross-sectional study was conducted at a tertiary care hospital on 90 patients. Height, weight, body mass index, waist-hip ratio was calculated. Fasting blood sugars, postprandial blood sugar, and glycated hemoglobin (HbA1c) were analyzed. Absolute TL was obtained from quantitative real-time polymerase chain reaction (qPCR). Vitamin D estimation was done by chemiluminescent immunoassay. Descriptive analysis of the data was done using R i386 3.6.3. The study found a positive correlation between TL and Vitamin D levels ($r = 0.64$; $P < 0.0001$). The interaction with high HbA1c levels and lower levels of Vitamin D led to the shortening of TL ($P = 0.0001$). The median of TL and mean of Vitamin D levels were significantly less in the diabetic group ($P < 0.0001$). Vitamin D levels positively affected the TL and its levels had an inverse relation with the HbA1c levels. This association had a significant effect on the shortening of TL. Vitamin D also had a significant association with other diabetic complications that instigated the shortening of TL. Therefore, assessing the role of Vitamin D levels on the shortening of TL can prove to be crucial biomarkers in managing optimal glycemic levels in T2DM patients.

Key words: Anthropometry, glycated hemoglobin, telomere length, type 2 diabetes mellitus, Vitamin D

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Background

Telomeres present at the chromosomal ends are made of repetitive nucleotide sequences. They are often considered as biomarkers for biological ageing.[1] Telomere length (TL) varies due to genetic, environmental factors, cancer, and various age-related diseases like cardiovascular disease, type 2 diabetes mellitus (T2DM).[1,2] Among the chronic diseases, T2DM is one of the most prevalent diseases globally with a prediction that by the year 2030 around 483 million individuals might get affected.[1,3] Since T2DM has been considered an age-related disease, it may be associated with TL. Evidence have established that there is shortened TL in patients with T2DM, while others have shown a negative co-relation of TL and T2DM.[2,3] It has been reported that the levels of Vitamin D affect the mechanisms of insulin secretion and insulin sensitivity in T2DM patients. Besides this, it is considered an important marker for predicting

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glycemic levels.[4] Furthermore, the levels of Vitamin D could affect the TL mechanisms by decreasing the cell proliferation and inflammation that affect the total count of leukocytes and further attrition of leukocyte TL.[5-7] Vitamin D is involved in many metabolic processes such as cellular differentiation, apoptosis, cell proliferation, and many more, as a result of such multiple functions of Vitamin D, it can be predicted that it is highly associated with cellular ageing and telomere biology.[8] It has been reported that high Vitamin D levels and longer TL can reduce the risk of chronic diseases like T2DM and its progression. Another aspect is the increased levels of oxidative stress and inflammation may lead to reduction of telomerase activity and increase the telomere shortening which are the main feature in obese individuals.[8] According to a study, it was seen that those with the lowest quartile of omega-3 fatty acids undergo accelerated telomere shortening. In such cases, Vitamin D supplements play a vital role in correcting the TL activity in obese individuals. Since Vitamin D is associated with a range of vital cellular processes impacting cellular ageing and telomere biology its role needs to be determined.[6,9] Hyperglycemia-induced activation of polyol pathway exhausts NADPH, depleting the supply of reducing equivalents and synthesis of reduced glutathione.[10]

Hence, the present study aimed to evaluate the association of TL and Vitamin D levels in T2DM and its related complications and to find the impact of Vitamin D on TL as well. Further, the study also attempted to find its associations with parameters such as physical activity, body mass index (BMI), waist-hip ratio (WHR), diabetic complications, etc., in this subset of patients.

Methods

A 1 year cross-sectional study was conducted at a tertiary care hospital and medical research centre in South India. The study is approved by the Institutional ethics committee (No. MDC/DOME/50). Written informed consents were obtained from the participants. A total of 90 patients were included and subdivided into two groups based on symptoms, fasting blood sugars (FBS), postprandial blood sugar (PPBS), and glycated hemoglobin (HbA1c) as diabetic and nondiabetic (normal). Inpatient or outpatient T2DM with any duration of disease on treatment with oral hypoglycemic agents or insulin (n = 45) and nondiabetic (n = 45) patients above the age of 18 years were included in the study. None of the patients were on Vitamin D supplements. Patients with Type 1 DM, pancreatic disorders, or who had diabetes secondary to other systemic diseases or known cases of chronic inflammatory or infectious diseases based on good clinical history were excluded from the study. Demographic data and detailed history were recorded. A thorough physical examination included the recording of vital data (pulse rate, blood pressure, temperature, and respiratory rate), general physical and systemic examination were done. The anthropometric measurements such as height, weight, BMI, and WHR of each patient in the study were systematically measured, calculated, and documented.

The FBS, PPBS, and HbA1c levels were measured and documented as per the diagnostic criteria given by the American Diabetes Association, 2020.[11] FBS and PPBS were measured by using strip glucometer. HbA1c was estimated by high-performance liquid chromatography method. The absolute TL of each subject was obtained by qPCR. The procedure followed is explained below. For sample size calculation below formula was used,

$n = (z\_[1-\alpha/2] + z\_[1-\beta])\text{^}2\,([\tau+\gamma]\,\sigma\_1\text{^}2]/(\gamma\,[\mu\_1-\mu\_2]\text{^}2) + ([\tau\text{^}2+\gamma\text{^}3]\,z\_[1-\alpha/2]\text{^}2)/(2\,\gamma([[\tau+\gamma)]]\text{^}2)$

n = the sample size required for each group,

$\tau = (\sigma\_2\text{^}2)/(\sigma\_1\text{^}2)$,

$\gamma = 1$,

β = power of the test,

α = statistical significance level.

where $\sigma\_0\text{^}2$ and $\sigma\_1\text{^}2$ are variances of two groups.

In a study, it was found that mean TL in T2DM was 6.01 kb ± 0.2, and in controls, it was 9.11 ± 0.6.[12]

Hence, the α is taken to be 0.05 and power β =0.90. Here, $\tau = (\sigma\_2\text{^}2)/(\sigma\_1\text{^}2)$ =8.998089 ≈ 9. Therefore, n = (1.96 + 1.645)^2× ((9 + 1)[[1.78]]^2)/(1 [9.11 − 6.01]^2) + ([9^2 + 1^3][[1.96]]^2)/(2 × 1([[1 + 9)]]^2), n = 45. Hence, for each group at least 45 subjects are needed for the study. The above sample size is representative of the same.

DNA sample collection, extraction, amplification, and measurement

About 3 ml of venous blood sample was collected from the subjects participating in the study in an EDTA bulb,[13,14] and processed immediately for DNA isolation and for assessing the TL by Real-time polymerase chain reaction (PCR) as per the standard procedure. The Qiagen DNA Mini Kit (QIAGEN Inc., California, United States Lot no. 163032024 manufactured in 2019) was used to extract the DNA from the peripheral blood leukocytes.[15] After the DNA was isolated its TL was measured using quantitative RT-PCR as T/S ratio. Telomere standard curve was generated by plotting CT (cycle threshold) values against the amount of telomere sequence in kb per reaction [Figure 1]. Cycling conditions for both the Telomere and 36B4 amplicons were as follows: 10 min at 95°C followed by 40 cycles at 95°C for 15s and 60°C for 1 min, followed by a melt curve. The 36B4 is a Single-Copy Gene and serves as a reference gene in the conventional qPCR technique in the measurement of

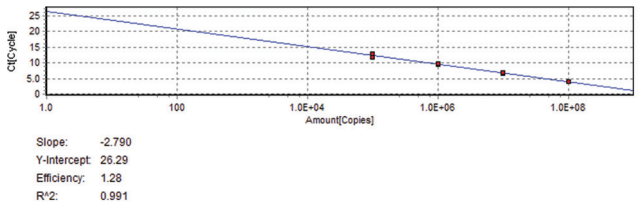

Figure 1: Standard curve for polymerase chain reaction cycles (generated by plotting cycle threshold values against the amount of telomere sequence in kb per reaction)

Telomeres [Figure 2].[14] All samples were analysed in the ABI Step One Plus RT-PCR System with SDS version Step One Plus software.[13]

Chemiluminescence immunoAssay for Vitamin D

DiaSorin LIAISON® 25 Hydroxy Vitamin D TOTAL Assay 100 kit (test code 310600), was used to assess the Vitamin D levels in our patients. The test was advanced chemiluminescence technology with magnetic microparticles. One hundred µL standard or sample was added to each well and then incubated for 90 min at 37°C. The liquid is then removed. Following which 100 µL Biotinylated detection Ab was added and then incubated for 1 h at 37°C. The fluid was aspirated and washed thrice. One hundred µL avidin-horseradish peroxidase Conjugate was added and incubated for 30 min at 37°C. Fluid is aspirated and washed 5 times. One hundred µL of substrate mixture solution was added and incubated for 5 min at 37°C. Readings were immediately noted after the substrate reaction time and results were calculated to obtain the Vitamin D levels.[16]

Statistical analysis

Descriptive analysis of the data was performed using R i386 3.6.3. Continuous variables were represented by mean ± standard deviation form and compared using *t*-test, Welch *t*-test, Mann–Whitney *U*-test, ANOVA, Kruskal–Wallis test. Categorical variables were represented by frequency tables and compared using the Chi-square test, Cochran Armitage test. $P < 0.05$ was considered as significant.

Results

A total of 90 patients were considered in the study, with a subdivision of 45 diabetic and 45 nondiabetic. The mean age of the participants was 58.29 ± 13.87 (in years). One-tailed Welch *t*-test shows that the mean BMI of diabetic subjects is significantly more than nondiabetic subjects ($P < 0.0001$), odds ratio [OR] 4.59 (95% confidence interval CI: [2.67, 9.64]). Using one-tailed Mann–Whitney *U*-test; the median of WHR was significantly more in diabetic subjects than nondiabetic patients ($P = 0.0006$), OR 7.47 (95% CI: [2.65,24.15]). The Cochran Armitage test showed a significant linear decreasing trend in the proportion of diabetes over physical activity. The chances of developing T2DM if the patient exercised regularly, exercised sometimes and who never exercised was 20.0%, 30.77%, and 69.39%, respectively [Figure 3 and Table 1].

Cerebrovascular disease (CVD) ($P = 0.0185$) and coronary artery disease (CAD) ($P < 0.0001$) are significantly associated with Diabetes. The odds ratio (OR) of CVD and CAD for diabetic patients is 14.96 (95% CI: [1.04, 214.57]), 9.80 (95% CI: [3.01,31.95]), respectively, times higher than nondiabetic patients [Figure 4 and Table 2].

TL is significantly less in diabetic group ($P < 0.0001$). The mean TL in diabetic and nondiabetic being 87.71 ± 76.07 and 548.58 ± 329.62 kb/diploid genome, respectively. Using one tailed *t*-test, it has been concluded that the mean of Vitamin D was significantly less in diabetic patients ($P < 0.0001$), odds 1.12 (95% CI: [1.06, 1.18]). Using Chi-square test, it is concluded that there is a significant association between elevated creatinine

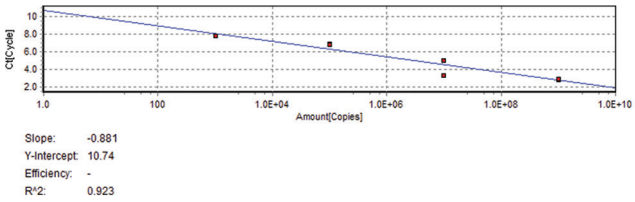

Figure 2: Single copy gene standard curve

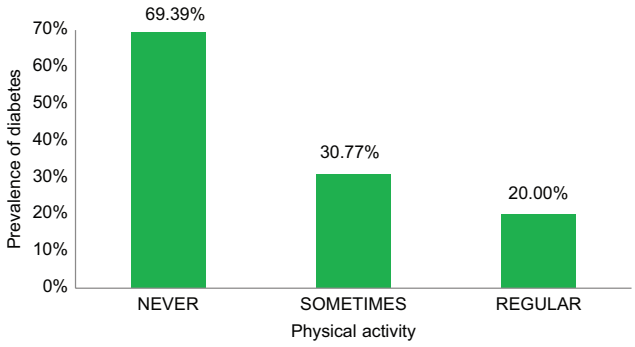

Figure 3: Prevalence of diabetes over physical activity

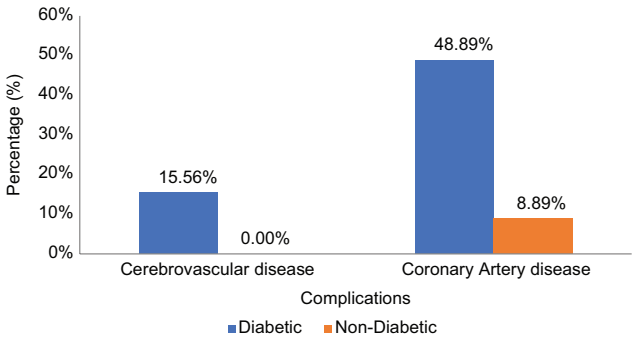

Figure 4: Comparison of complications (cerebrovascular disease, coronary artery disease) with diabetes status (in both diabetic and nondiabetic groups)

and diabetes. The odds of elevated creatinine is 10.75 (95% CI: [2.29, 50.51] times higher in diabetic patients [Table 3].

There is a significant interaction of HbA1c levels and Vitamin D levels on TL ($P < 0.0001$) [Table 4].

Using Spearman rank correlation, it has been concluded that there is a strong positive correlation between TL and Vitamin D (rho = 0.64; $P < 0.0001$) [Figure 5].

The mean Vitamin D in the diabetic patients who did physical activity regularly and occasionally was significantly different from diabetic patients who never did physical activity ($P < 0.0001$). The median of TL in the diabetic patients with CAD ($P = 0.0010$) was significantly less, odds 1.01 (95% CI: [1.01, 1.02]), and the mean Vitamin D level was significantly less for the diabetic subjects with CAD ($P < 0.0001$), odds 1.28 (95% CI: [1.16, 1.46]) than patients without CAD. The mean of TL and Vitamin D was significantly less in diabetic retinopathy (DR) patients among diabetic patients, than

Table 1: Demographic detail of the study participants

Factor	Diabetic (%)	Nondiabetic (%)	*P*	OR (95%CI)
Age (years)	60.89±9.29	55.69±17.00	0.0762[WT]	-
Gender				
Male	27 (60)	34 (75.56)	0.1144[C]	-
Female	18 (40)	11 (24.44)		
BMI	27.34±1.11	24.04±2.03	<0.0001[WT]	4.59 (2.67-9.64)
WHR	0.92±0.05	0.88±0.04	0.0006[W]	7.47 (2.65-24.15)
Educational level				
Illiterate	15 (33.33)	8 (17.78)	0.3816[C]	-
Primary	5 (11.11)	5 (11.11)		
High school	7 (15.56)	6 (13.33)		
PUC	13 (28.89)	16 (35.56)		
Graduate	5 (11.11)	10 (22.22)		
Occupation				
Retired	9 (20)	6 (13.33)	0.0325[MC]	6.55 (1.73-24.69)
Housewife	18 (40)	11 (24.44)		
Private employee	1 (2.22)	4 (8.89)		
Farmer	14 (31.11)	10 (22.22)		
Business	3 (6.67)	10 (22.22)		
Government employee	0	2 (4.44)		
Studying	0	2 (4.44)		
Marital status				
Married	43 (95.56)	36 (80)	0.0410[MC]	-
Widow	1 (2.22)	2 (4.44)		
Divorced	1 (2.22)	1 (2.22)		
Unmarried	0	6 (13.33)		
HTN				
Yes	8 (17.78)	3 (6.67)	0.1980	-
No	37 (82.22)	42 (93.33)		
Smoking				
Yes	6 (13.33)	6 (13.33)	>0.99[C]	-
No	39 (86.67)	39 (86.67)		
Alcohol status				
Yes	6 (13.33)	8 (17.78)	0.5608[C]	-
No	39 (86.67)	37 (82.22)		
Physical activity				
Sometimes	8 (17.78)	18 (40)	<0.0001[CA]	5.10 (1.82-14.40)
Never	34 (75.56)	15 (33.33)		
Regular	3 (6.67)	12 (26.67)		9.07 (2.22-36.89)
Diet				
Vegetarian	6 (13.33)	5 (11.11)	>0.99[MC]	
Both	39 (86.67)	40 (88.89)		
Duration of diabetes (years)	16.35±10.08	-	-	-
Onset age	44.07±7.60	-	-	-
Treatment of DM				
Oral	15 (33.33)	-	-	-
Insulin	12 (26.67)	-		
Both	18 (40)	-		
DR (yes)	12 (26.67)	-	-	-

[WT]Welch *t*-test, [MC]Chi square test with simulation, [CA]Cochran Armitage test, [M]Mann–Whitney U-test. CI: Confidence interval, OR: Odds ratio, BMI: Body mass index, HTN: Hypertension, PUC: Preuniversity course, DM: Diabetes mellitus, WHR: Waist-hip-ratio, DR: Diabetic retinopathy

those without DR ($P < 0.0001$), odds 1.03 (95% CI: [1.01, 1.06]) and ($P = 0.0030$), odds 1.19 (95% CI: [1.06, 1.38]), respectively [Table 5].

TL was negatively correlated with BMI (rho = −0.39; $P = 0.0083$) and WHR (rho = −0.28; $P = 0.0332$) in diabetic patients. There was a significant high negative correlation between TL and parameters

of HbA1c (rho = −0.99; $P < 0.0001$), FBS (rho = −0.97; $P < 0.0001$), PPBS (rho = −0.98; $P < 0.0001$) in diabetic group. Vitamin D levels was negatively correlated with BMI ($r = -0.25$; $P = 0.0470$), duration of diabetes mellitus (−0.32; $P = 0.0165$), and serum creatinine ($r = -0.31$;$P = 0.0381$) [Table 6].

The mean HbA1c for CAD subjects were significantly more than normals (without CAD) in both diabetic ($P = 0.01$) and nondiabetic group ($P = 0.02$). The median of HbA1c for subjects with DR in diabetic group was significantly more than patients without DR ($P = 0.0104$) [Figure 6]. Using Pearson correlation coefficient, it is concluded that BMI was significantly positively correlated with HbA1c in diabetic group ($r = 0.38$; $P = 0.0107$). There was a significant strong positive correlation of HbA1c with FBS ($r = 0.98$; $r = 0.58$), PPBS ($r = 0.98$; $r = 0.62$) in both groups ($P < 0.0001$) [Figure 7 and Table 7].

Using one-tailed *t*-test, it was concluded that the mean of Vitamin D was significantly less for patients on insulin treatment than oral treatment in the diabetic group ($P = 0.0044$), odds 1.20 (95% CI: [1.07, 1.41]) [Table 8].

Discussion

Diabetes is depicted by elevated blood glucose levels, whereas the TL is used as a biological marker of cell ageing. Clinical evidence shows a significant co-relation between T2DM and TL.[17] Hence, the current study aimed to find the association of TL and Vitamin D in T2DM and the impact of Vitamin D on the TL in patients withT2DM.

This study found that Vitamin D levels were proportional to the TL i.e. higher the Vitamin D levels (Vitamin D levels in the sufficiency range) longer the telomeres. The present study also found co-relation of Vitamin D and HbA1c levels and its effect on the TL. Higher HbA1c and lower Vitamin D levels lead to increased pace in TL shortening. This is the uniqueness of the study to find such associations in T2DM patients. According to this study, individuals with a normal to the high level of Vitamin D, the TL shortening was slower compared to those with low levels of Vitamin D.

Several metabolic factors such as obesity, insulin resistance or physical inactivity have been associated with shortened telomeres, which was also reported by Baltzis *et al.*;[18] after assessing telomerase activity in diabetic patients having or not, foot ulcer, on ($n = 58$) nonulcer group and ($n = 32$) ulcer group. Their study concluded that the patients with diabetic foot had significantly greater waist circumference and neuropathy disability scores along with exhibiting lower telomerase activity, indicating the possible existence of a common clinical profile among ulcer-bearing diabetic patients.

In our study, anthropometric variables like BMI and WHR showed a significant difference in the diabetic group than the nondiabetic group, which was in concordance to study by Vasanthakumar and Kambar. This indicated that the patients were obese that directly links with poor control of blood glucose levels among individuals with T2DM, while WHR is also a good predictor of visceral adipose tissue.[19] Further, TL was negatively correlated with BMI ($r = -0.39$; $P = 0.0083$) and

Table 2: Association of diabetes and complications

Complications	Diabetic (%)	Nondiabetic (%)	*P*	OR (95% CI)
CVD				
Yes	7 (15.56)	0	0.0185^{MC}	14.96 (1.04-214.57)
No	38 (84.44)	45 (100)		
CAD				
Yes	22 (48.89)	4 (8.89)	<0.0001	9.80 (3.01-31.95)
No	23 (51.11)	41 (91.11)		

P-value calculated using the Chi-square test with simulation. CI: Confidence interval, OR: Odds ratio, CVD: Cerebrovascular disease, CAD: Coronary artery disease

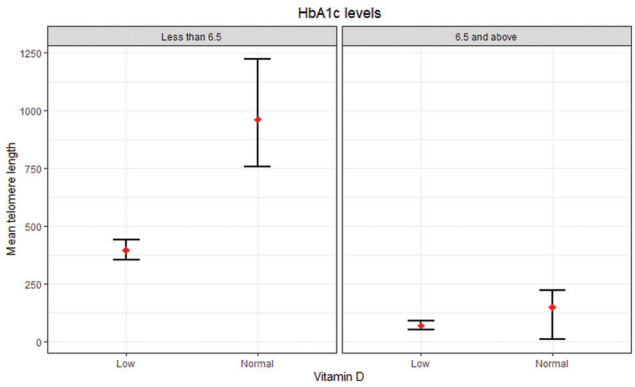

Figure 5: Relationship between glycated hemoglobin and Vitamin D with telomere length (depicting the impact of diabetes mellitus [represented by glycated hemoglobin] and Vitamin D levels on the telomere length)

Table 3: Comparison of clinical investigation between diabetic and nondiabetic subjects

Clinical investigation	Diabetic±SD	Nondiabetic±SD	*P*	OR (95% CI)
HbA1c (%)	8.72±2.19	5.56±0.4	<0.0001^{WT}	NA
FBS (mg/dl)	173.91±33.61	85.31±5.24	<0.0001^{WT}	NA
PPBS (mg/dl)	287.84±82.09	115.16±10.35	<0.0001^{M}	NA
TL (kb/diploid genome)	87.71±76.07	548.58±329.62	<0.0001^{M}	-
Serum creatinine (mg/dl)	1.61±1.58	0.88±0.19	0.0956^{M}	-
Elevated creatinine	15 (33.33%)	2 (4.44%)	0.0015	10.75 (2.29-50.51)
Vitamin D	14.53±9.45	25.43±10.69	<0.0001^{T}	1.12 (1.06-1.18)

^{M}Mann–Whitney U-test; ^{WT}Welch t-test; ^{T}*T*-test. Clinical investigations represented in mean±SD for diabetic and nondiabetic group SD: Standard deviation, CI: Confidence interval, OR: Odds ratio, HbA1c: Glycated hemoglobin, FBS: Fasting blood sugars, PPBS: Postprandial blood sugar, TL: Telomere length

WHR ($r = -0.28$; $P = 0.0332$) in diabetic subjects. This concurs with the exploration by Lee *et al.* where the BMI and WHR were inversely associated with TL ($P < 0.05$).[20]

Findings from another study showed a higher prevalence of diabetes in those who were less physically active ($P < 0.001$) similar to our findings where the majority (75.5%) of the patients who were never involved in physical activity were significantly higher in the diabetic group showing a significant corelation between the groups ($P < 0.0001$).[21] The study by Arsenis *et al.* demonstrated that exercise duration was inversely correlated with biomarkers of DNA damage and TL. Exercise mobilizes fat and enhances the detoxification process reducing oxidative stress, preserving DNA and telomeres.[22]

There was the significant mean difference between HbA1c and FBS between both diabetic and nondiabetic groups ($P < 0.0001$). A study by Dave *et al.*[23] demonstrated the significance of HbA1c and FBS among three different groups of diabetic patients ($P < 0.001$). This showed that level of HbA1c linearly associated with the abnormal glucose level. Values of PPBS was also significantly different between diabetics and nondiabetics ($P < 0.0001$). The TL in diabetics and nondiabetic individuals kb/diploid genome ($P < 0.0001$) showing gross shortening of telomere in T2DM in accordance with the study by Piplani *et al.*, where the TL shortening was significantly higher in the T2DM patients ($P = 0.00019$).[24] The creatinine levels were highly elevated in the diabetic group than the nondiabetics ($P = 0.0015$). This result is supported by a study where raised serum creatinine was observed more in the diabetic patients (15/100) than the controls (2/100).[25] Vitamin D levels were significantly lower in the diabetic patients than the nondiabetics ($P < 0.0001$) which corroborates with a study by Kostoglou-Athanassiou *et al.*[26] The mean Vitamin D of diabetic patients was significantly different in those who regularly and occasionally did physical activity from those diabetic patients who were never involved in the physical activity ($P < 0.0001$).

A study by Kavadar *et al.*[27] found statistically significant difference for Vitamin D levels and a low physical activity (PA) in the insulin-resistant patients ($P = 0.001$). The study has thrown light on the influence of physical activity and a normal Vitamin

Table 4: Relationship between glycated hemoglobin, Vitamin D with telomere length

	F	*P*
HbA1c level	66.01	<0.0001
Vitamin D level	88.38	<0.0001
HbA1c: Vitamin D	16.03	0.0001

HbA1c classified into<6.5 and≥6.5, Vitamin D≤30 is considered as low, Type III sum of the square is used. *P*-value calculated using two-way ANOVA. HbA1c: Glycated hemoglobin

Table 5: Comparison of telomere length and Vitamin D over different factors in diabetic subjects

Variable	Sub-variable	TL±SD	*P* OR (95% CI)	Vitamin D±SD	*P* OR (95% CI)
Gender	Male	79.61±74.26	0.317[M]	14.78±7.42	0.8321[T]
	Female	99.85±79.27		14.16±12.10	
Occupation	Retired	103.67±93.43	0.3066[KW]	14.34±7.62	0.9949[A]
	Housewife	99.85±79.27		14.16±12.10	
	Farmer	57.15±56.59		14.96±8.14	
	Others	104.10±75.78		15.16±5.87	
Marital status	Married	86.03±74.97	0.5838[M]	14.38±9.09	>0.99[M]
	Widow/divorced	123.76±125.71		17.93±20.75	
Smoking	Yes	60.5±80.87	0.2294[M]	11.51±4.99	0.4052[T]
	No	91.89±75.53		15±9.92	
Alcohol status	Yes	71.57±68.11	0.8072[M]	16.41±6.36	0.6073[T]
	No	90.19±77.73		14.25±9.87	
Physical activity	Sometimes	77.41±86.97	0.6184[KW]	24.4±3.40	<0.0001[A]
	Never	84.77±69.83		10.22±4.91	
	Regular	148.45±119.45		37.09±7.49	
Diet	Vegetarian	85.5±87.49	0.708[M]	13.8±9.50	0.9202[M]
	Both	88.05±75.45		14.65±9.56	
HTN	Yes	61.04±63.03	0.3723[M]	9.36±5.82	0.0874[T]
	No	93.47±78.15		15.65±9.76	
CVD	Yes	38.27±14.11	0.1394[M]	12.06±8.19	0.4568[T]
	No	96.81±79.38		14.99±9.69	
CAD	Yes	47.82±39.97	0.0010[M] 1.01 (1.01-1.02)	9.05±6.13	<0.0001[T] 1.28 (1.16-1.46)
	No	125.86±83.28		19.78±9.16	
DR	Yes	35.42±22.59	<0.0001[WT] 1.03 (1.01-1.06)	8.29±8.15	0.0030[T] 1.19 (1.06-1.38)
	No	106.72±79.89		16.80±8.94	

[M]Mann–Whitney U-test, [WT]Welch t-test, [T]*T*-test, [KW]Kruskal–Wallis test, [A]ANOVA. TL and Vitamin D are represented in mean±SD, for various variables. CI: Confidence interval, OR: Odds ratio, HTN: Hypertension, DR: Diabetic retinopathy, CVD: Cerebrovascular disease, CAD: Coronary artery disease, SD: Standard deviation, TL: Telomere length

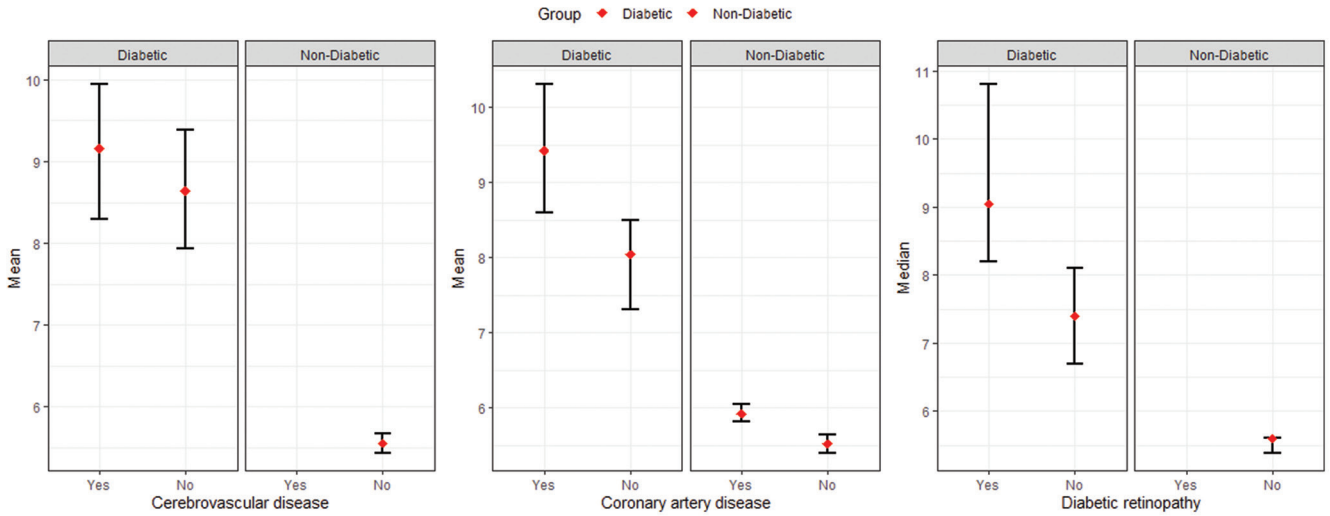

Figure 6: Relationship of glycated hemoglobin with complications such as cerebrovascular disease, coronary artery disease, and diabetic retinopathy in both diabetic and nondiabetic group

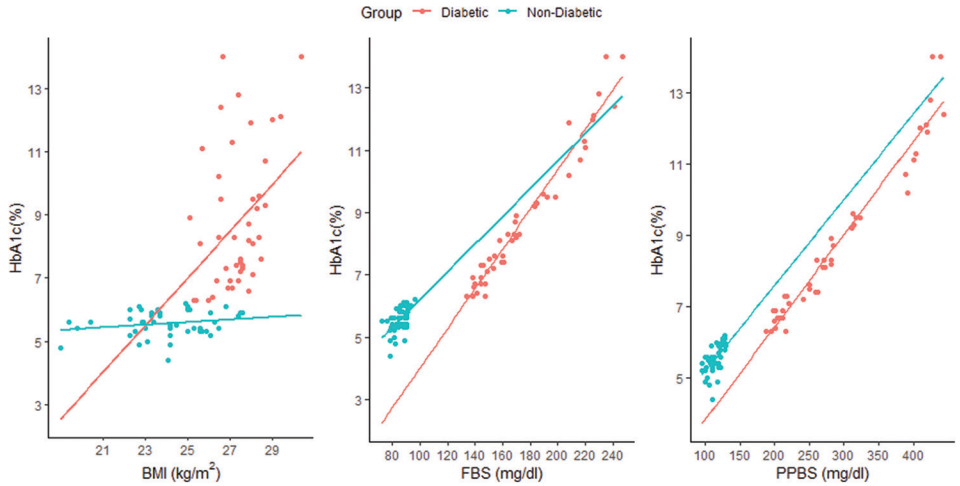

Figure 7: Relationship of glycated hemoglobin with body mass index, fasting blood sugars, and postprandial blood sugar in the diabetic and nondiabetic group

Table 6: Correlation of clinical investigations with telomere length and Vitamin D in diabetic subjects

Variable	TL		Vitamin D	
	rho	*P*	*r*	*P*
Age	0.20	0.1840	-0.05	0.718
BMI	-0.39	0.0083	-0.25	0.0470
Duration of T2DM	0.13	0.391	-0.32	0.0165
WHR	-0.28	0.0332	-0.02	0.9033
HbA1c (%)	-0.99	<0.0001	0.08	0.5897
FBS (mg/dl)	-0.97	<0.0001	0.06	0.7184
PPBS (mg/dl)	-0.98	<0.0001	0.05	0.7646
Serum creatinine (mg/dl)	-0.05	0.7316	-0.31	0.0381
Total leukocyte count	0.01	0.9472	-0.14	0.3299
Vitamin D	0.11	0.4594	-	-

Rho: Spearman rank correlation, *r*: Pearson correlation coefficient. BMI: Body mass index, WHR: Waist-hip-ratio, HbA1c: Glycated hemoglobin, FBS: Fasting blood sugars, PPBS: Postprandial blood sugar, T2DM: Type 2 diabetes mellitus, TL: Telomere length

D level on insulin levels by reducing its resistance, which can promote weight loss and prevent T2DM. Also, physical activity increases Vitamin D levels by increasing lipolysis.[27]

It was observed that the median of TL for diabetic subjects with CAD was significantly less than those without CAD ($P < 0.0010$) supporting the results of Xu *et al.* where the shorter TL was highly associated in the coronary heart disease patients than the control ($P < 0.0001$).[28] It was also found that the mean of TL was significantly shorter ($P < 0.0001$) in diabetic patients with DR, than those without it. These findings are in conformation with the study by Sharma *et al.* where the TL was shorter in different types of DR patients compared to the healthy controls ($P = 0.0001$).[29] The mean of Vitamin D was significantly less in DR patients in the diabetic patients ($P = 0.0030$). Similar observations were noticed in a study by Luo *et al.*, a meta-analysis with fifteen observational studies involving 17,664 subjects, showed that low serum

Table 7: Relationship of glycated hemoglobin with other factors in both groups

Variable	Diabetic±SD	*P*	Nondiabetic±SD	*P*
CVD				
Yes	9.16±1.16	0.5695[T]	-	NA
No	8.64±2.33		5.56±0.40	
CAD				
Yes	9.42±2.14	0.0165[T]	5.92±0.13	0.0273[T]
No	8.04±2.06		5.52±0.40	
DR*				
Yes	9.77±2.33	0.0104[M]	-	NA
No	8.33±2.04		-	
Physical activity				
Never	8.47±1.96	0.4003[A]	5.57±0.34	0.5057[A]
Sometimes	9.61±2.38		5.48±0.42	
Regular	9.13±4.21		5.66±0.44	
BMI				
r	0.38	0.0107	0.21	0.1645
WHR				
r	0.25	0.0938[Sp]	-0.05	0.7166
FBS				
r	0.98	<0.0001	0.58	<0.0001
PPBS				
r	0.98	<0.0001	0.62	<0.0001
Creatinine				
r	-0.14	0.3471	-0.09	0.5596

*Indicates significance, [T]*T*-test, [M]Mann–Whitney U-test, [Sp]Spearman rank correlation, [A]ANOVA. HbA1c in the diabetic and nondiabetic group expressed in mean±SD, for various variables or parameters. BMI: Body mass index, WHR: Waist-hip-ratio, HbA1c: Glycated hemoglobin, FBS: Fasting blood sugars, PPBS: Postprandial blood sugar, DR: Diabetic retinopathy, CVD: Cerebrovascular disease, CAD: Coronary artery disease, SD: Standard deviation, NA: Not applicable

Table 8: Comparison of glycated hemoglobin, telomere length, and Vitamin D with the treatment of diabetes mellitus

Factor	Treatment	Mean±SD	*P*	OR (95% CI)
HbA1C	Insulin	8.43±2.15	0.6050	-
	Oral	8.82±2.23		
TL	Insulin	73.77±61.73	0.9495[M]	-
	Oral	92.77±80.92		
Vitamin D	Insulin	8.08±4.79	0.0044	1.20 (1.07-1.41)
	Oral	16.88±9.67		

[M]Mann–Whitney U-test. CI: Confidence interval, OR: Odds ratio, HbA1c: Glycated hemoglobin, TL: Telomere length, SD: Standard deviation

Vitamin D levels are associated with an increased risk of DR among T2DM patients.[30]

The duration of T2DM was moderately significant and negatively correlated with Vitamin D levels ($r = -0.32$; $P = 0.0165$) among the diabetic patients. Similar results were obtained by Alaidarous *et al.*, who studied the impact of duration of T2DM on Vitamin D levels in 92 patients aged between 20 and 60 years. Their results concluded that T2DM duration was an independent predictor of Vitamin D deficiency.[31]

Parameters such as HbA1c ($r = -0.99$), FBS ($r = -0.97$), PPBS ($r = -0.98$) showed a significant negative co-relation with the TL among the diabetic patients ($P < 0.0001$). In a cross-sectional observational study by Dudinskaya *et al.*, the high-risk population from Moscow, Russia between 2012 and 2013 on 99 patients (33 males and 66 females) who previously underwent an outpatient examination in the FGBI National Research Centre for Preventive Medicine showed that the parameters of FBS and HbA1c were negatively co-related with TL; however, only HbA1c showed a significant co-relation ($P = 0.03$).[32] The level of serum creatinine level showed a significant negative co-relation with both TL (rho = −0.05; $P = 0.73$) and Vitamin D levels ($r = -0.31$; $P = 0.03$) but was significantly associated with the Vitamin D levels. Matching to the results of Kumarchandra *et al.*, where significant negative correlation between Vitamin D and serum creatinine was found ($r = -0.49$; $P = 0.013$).[33]

It was also observed that the mean HbA1c in the diabetic patients with CAD was significantly more than those diabetic patients without CAD and also with the nondiabetic patients ($P > 0.05$) in accordance with a study by Rezende *et al.* and Ul-Haque *et al.*[34,35] Further, the HbA1c level was positively co-related with BMI in diabetic patients ($r = 0.38$; $P = 0.0107$) in line with the findings by Babikr *et al.*[36] Moreover, correlation was similar and significance between HbA1c and FBS, PPBS between both the groups ($P < 0.0001$) was with a higher r-value for PPBS than the FBS in the nondiabetic group. A study showed better and stronger co-relation between PPBS and HbA1c than FBS and vice versa. However, it is strongly opined that PPBS can contribute in the overall control of hyperglycemia than FBS alone.[37]

Factors such as TL, Vitamin D levels, and HbA1c were compared with the diabetic treatment (oral and insulin). It was found that vivitamid levels were significantly less in the patients receiving insulin treatment than those receiving oral treatment ($P = 0.004$). It is evident that people with diabetes have low levels of Vitamin D due to a weaker beta-cell function. This is concurred by Mauss *et al.* with the results that severe Vitamin D deficiency (<10 ng/ml) was associated with increasing FBS (β 3.13; 95% CI: 0.78, 5.47; $P \leq 0.01$) and HbA1c.[38]

This study provides us strong evidence of the association between Vitamin D and TL with T2DM and its related complications, which is one of its kinds. The study design is new and unique where the relation of TL and Vitamin D levels in diabetes is sought, especially in Indian population where T2DM is an epidemic. Diabetes is progressing epidemically, so there is a need to discover newer mechanisms that govern it, to propose the latest modalities to prevent and treat diabetes. Based on anthropometric parameters obesity was related to TL. In addition, this is the first study that found an association between HbA1c levels and Vitamin D levels on TL. In future, the study can be extended to find the data about the association of TL and risk association of single nucleotide polymorphism rs2853669 with T2DM in the Indian population and finding the co-relation between total Vitamin D metabolites to predict the T2DM related complications.

Conclusion

The study justified its objective to find an association of TL and Vitamin D with T2DM. Vitamin D was significantly less in the diabetic group, which was supported by higher BMI, and low physical activity in the same group. Vitamin D levels were proportional to the TL, as increased telomerase activity is noticed in those with higher levels of Vitamin D. Vitamin D levels positively affected the TL and its levels had an inverse relation with the HbA1c levels. This interaction had a significant effect on the shortening of TL. Vitamin D also had a significant association with other diabetes-related problems that instigated shortening of TL. TL was significantly shorter in the diabetic subjects. Hastened TL shortening and very low Vitamin D levels were observed in those diabetic patients having CAD and DR. The anthropometric parameters such as BMI, WHR, and the parameter serum creatinine were negatively correlated with TL in diabetic patients. A higher HbA1c levels was associated with low levels of Vitamin D, which together was further responsible for the shortening of TL. Therefore, supplementation of Vitamin D or intake of Vitamin D-rich food, optimum physical activity can not only help in correcting the deficiency but can help in achieving better and optimum glycemic control. This will further have a positive impact on the telomerase activity, by controlling telomere attrition and slowing down the shortening of TL in this subset of the population. Overall, correcting the Vitamin D levels can positively improve the maintenance of telomere, which will slower cell ageing. Further, assessing the role of Vitamin D levels on the shortening of TL can prove to be crucial biomarkers in managing optimal glycemic levels in T2DM patients.

The strengths of this study are the findings, which will help clinicians to predict the prognosis in patients precipitating with complications of DR, CVD, and coronary heart disease. The clinicians can then monitor complications aggressively to prescribing preventive drugs. Our study findings if replicated in further studies having a larger sample size, possibly it could guide policymakers and researchers to focus on preventive aspects. Since the observations in this study are cross-sectional. Therefore, we are unable to justify whether shortened TL is a trait marker or shortening halts with treatment. We have included patients only with T2DM. If this association exists in patients with T1DM, pancreatic disorders, or who had diabetes secondary to other systemic diseases or known cases of chronic inflammatory or infectious diseases needs to be assessed.

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Ethical clearance

Ethical clearance obtained from the Institutional Ethics committee (No. MDC/DOME/50).

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Conflicts of interest

There are no conflicts of interest.

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