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Insulin treatment affects leukocyte telomere length in patients with type 2 diabetes: 6-year longitudinal study

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ABSTRACT

Objective: Many studies demonstrated a close relationship between type 2 diabetes mellitus (T2DM) and leukocyte telomere length (LTL). However, how the LTL changes in T2DM and what are the potential causal factors in it, particularly in patients during a long period treatment, have not been studied. Here we performed a longitudinal observation of LTL in trained T2DM patients during a 6-year follow-up and evaluated the possible risk factors that were associated with LTL alteration.

Methods: Seventy-six patients with T2DM were enrolled in this 6-year longitudinal study. The enrolled patients had no severe complication and had never received insulin therapy by the time. Patients were scheduled to visit once every one or two months and their medication changes were recorded. The LTL at the time when patients were enrolled was used as baseline, which was compared with the LTL at 6 year. Multivariable linear regression and exact logistic regression model were adopted to identify independent predictors of telomere length change and telomere length shortening, respectively.

Results: Sixty-four patients were successfully followed up. Although mean LTL decreased after 6 years, 30% (19/64) of patients demonstrated LTL lengthening and 70% (45/64) of patients demonstrated LTL shortening. Among them, 18 Patients received insulin treatment during the 6 years. Of these 18 patients, 16 patients showed decreased LTL and only two showed increased LTL. Linear regression analysis demonstrated that change in telomere length during the 6 years was associated inversely with insulin use (β-coefficients: −0.587, 95% CI: −0.198, −0.085, $P < 0.001$). Exact logistic regression analysis showed insulin use (OR: 17.355, 95% CI: 2.659, 35.627, $P = 0.013$) and LDL-C(OR: 3.493, 95% CI: 1.559, 10.063, $P = 0.007$)were independent predicts of telomere length shortening.

Conclusions: LTL may increase as well as decrease in T2DM who received antidiabetic treatment. Insulin use may accelerate telomere attrition. Insulin use and LDL-C can predict telomere shortening.

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1. Background

Telomeres are TTAGGG tandem repeat structures at the end of chromosomes, which maintain the stability of chromosomes, prevent the end fusion of chromosomes and protect chromosome structure. During cell division, telomere length is shortened due to the inability of DNA polymerase to fully replicate the 3′ end of linear DNA. When telomeres are shortened beyond the critical threshold, the cell becomes senescent or die.[1]

Shortened leukocyte telomere length (LTL) is hypothesized to be a novel biomarker for age and age-related diseases, such as cardiovascular disease (CVD), stroke, Alzheimer's disease and type 2 diabetes (T2DM).[2–6] T2DM is a disorder clearly related to age and a reduced life span. A number of studies including meta-analysis study show that telomere attrition is positively correlated with diabetes and diabetic complications.[7–9] In addition, two studies indicate that short LTL was associated with the incidence of T2DM.[10,11] Some studies demonstrate LTL can provide additive prognostic information on mortality risk in T2DM patients.[12]

Insulin use is a double-edged sword in diabetic treatment: it is necessary to treat diabetes, but, elevated levels of insulin are associated with weight gain and cardiovascular events.[13,14] One study showed that all concentrations of insulin under normal glucose or high

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concentrations of insulin under high glucose could promote glucose-induced endothelial senescence.[15]

Until now, limited research has investigated change in LTL in T2DM patients after long-term follow-up, and it is uncertain if insulin use is responsible. The present study aimed to investigate change in LTL and possible risk factors including insulin use underlying the change during a 6-year follow-up of T2DM patients.

2. Materials and methods

2.1. Patients

Seventy-six patients with T2DM visiting the outpatient department in Peking Union Medical College Hospital between April 2005 and April 2006 were recruited. Selection criteria: (1) age 18–70 years; (2) proven diagnosis of type 2 diabetes (WHO diagnostic criteria 1999), without severe complication; (3) without insulin treatment; (4) no severe hypertension (diastolic blood pressure ≥ 100, systolic blood pressure ≥ 200); (5) no cancer diagnosed; (6) no smoke and alcohol abuse; (7) no pregnancy plan or without being pregnant within a year for women. Participation in the long-term follow-up was on a voluntary basis.

All subjects were fully informed of the research design and the follow-up period. During 6 years period, regular visit once every one or two months was scheduled and the medication change was recorded. Treatment plan to the patients whose HbA1c was higher than 7.5% was adjusted as followings: 1. Lifestyle guidance, 2. Add one or two oral antidiabetic drugs to monotherapy or two-drug combination therapy, 3. If patients had taken three oral drugs, add basal insulin, premixed insulin or a four-injection per day regimen to oral antidiabetic drugs.

From April 2011 to March 2012, all participants were subjected to the same clinical assessment and blood sample collection as 6 years ago. A total of 64 patients were successfully followed up, and 18 patients accepted insulin therapy.

This study was approved by the Ethic Committee of Peking Union Medical College Hospital, Beijing, China. Written informed consent was obtained from all participants.

2.2. Clinical information and laboratory tests

The general clinic information includes age, sex, height, weight, body mass index (BMI), waist circumference, systolic blood pressure (SBP), diastolic blood pressure (DBP), duration of diabetes, and a complete record of oral hypoglycemic drugs. BMI was calculated as weight (kg)/height $(m)^2$. Blood samples were collected after 12 h overnight fasting for routine blood laboratory tests including fasting blood glucose (FBG), fasting insulin, triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL—C), low density lipoprotein cholesterol (LDL-C) and glycosylated hemoglobin (HbA1c). Blood samples were also used to obtain peripheral blood mononuclear cells (PBMCs) for genomic DNA extraction. HOMA-IR was used to measure insulin resistance; HOMA-IR = FPG*FINS/22.5.

2.3. Measurement of relative LTL

We measured relative telomere length in DNA from leukocytes in peripheral blood with fluorescent real-time quantitative PCR (qPCR) as reported previously in the telomere length studies of Cawthon et al.[16]. Briefly, qPCR was performed using the ABI Prism 7500 System (Applied Biosystems). β-globin was used as the internal reference gene. Ratio for repeated copy number of telomere (T) to single copy gene (S) (T/S ratio) were calculated from cycle threshold (Ct) for telomere (Cttel) and β-globin (Ctglo). T/S ratio was positively correlated with DNA telomere length and could be used to reflect the telomere length.[16]

qPCR reaction for DNA from each participant was triplicated to obtain Cttel and Ctglo. Inter-assay coefficient of variation (CV)was 0 to 9.5% for Cttel and 0 to 2.1% for Ctglo. Intra-assay CV was 0 to 4.9% for Cttel and 0 to 2.7% for Ctglo.

Primers were synthesized by Shanghai Sangon Biotechnology Co, Ltd. as followings.

Primer sequence (5′-3′): Telomere gene(16), forward:
5′-ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT-3′;
Reverse: 5′-TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA-3′.

β-globin gene, Forward:
5′-CGGCGGCGGGCGGCGCGGGTGGGCGGCTTCATCCACGTTCACCTTG-3′.
Reverse: 5′-GCCCGGCCCGCCGCGCCCGTCCCGCCGGAGGAGAAGTCGCCGTT-3′.

2.4. Statistical analysis

We used SAS 9.4 for exact logistic regression and SPSS 17.0 for the other statistical processing. A two-tailed P value <0.05 was considered statistically significant.

Baseline and follow-up telomere lengths were normally distributed. We categorized patients into two groups: LTL shortened and LTL lengthened. Change of telomere length was calculated by subtracting telomere length at baseline from telomere length at 6 year.

For variables with normal distribution, student's *t*-test was used to evaluate the differences between LTL-shortened and LTL-lengthened groups. Chi-square test was used to compare the constituent ratio. Variables with skewed distribution which were determined by one-sample Kolmogorov-Smirnov test, were examined by Kruskal-Wallis test appropriated transformed before analysis.

We used multivariable linear regression with enter method to identify independent predictors of LTL change. Due to the small sample, the independent variables were pre-screened. We set $P < 0.2$ as the standard to bring variables in Table 1 in the regression analysis. Insulin treatment, HOMA-IR, BMI, waist circumference, LDL-C and baseline telomere length met the criterion and were brought in the regression analysis.

We used exact logistic regression with enter method to detect the possible independent risk factors of LTL shortening. Independent variables were screened same as stated above. $P > 0.1$ as the criteria to remove variables from the model.

3. Results

3.1. LTL, clinical information and laboratory test results at baseline and 6 years

We initially recruited 76 participants from which 12 patients were excluded because of follow-up failure. Clinical information and results of laboratory tests from 64 successfully followed up subjects are shown in the (Table 1).

Table 1
General clinical information on T2DM patients before and after 6 years follow-up (n = 64).

	Before 6 years	After 6 years	P
Age (year)	57.7 ± 7.3	63.6 ± 6.9	
Male/female	24/40	24/40	
Duration of T2DM (year)	6.56 ± 7.17	12.86 ± 6.36	
Insulin use, n	0	18	
BMI (kg/m^2)	25.78 ± 3.18	26.01 ± 3.67	0.680
Abdominal circumference (cm)	89.85 ± 8.46	91.99 ± 11.80	0.634
Systolic blood pressure (mmHg)	120.31 ± 19.44	137.97 ± 16.77	0.009
Diastolic blood pressure (mmHg)	72.79 ± 10.08	73.31 ± 11.32	0.836
HbA1C%	6.67 ± 1.42	6.83 ± 1.21	0.007
LTL	1.07 ± 0.14	1.00 ± 0.15	0.001

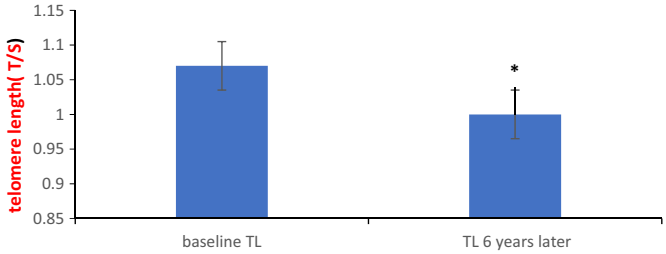

Fig. 1. Leukocyte telomere length at baseline and 6 year. T/S = telomere to single-copy gene ratio units. *: baseline vs 6 years later, P < 0.05.

All patients received oral hypoglycemic drugs at baseline. 18 patients had to start receiving insulin therapy at certain time points during the 6 years due to the failure of glucose control. 10 patients received oral hypoglycemic drugs combined with basal insulin therapy, 2 patients received basal-bolus insulin combined with oral hypoglycemic drugs, and 6 patients received premixed insulin combined with oral hypoglycemic drugs. The mean time of insulin use was 4.57 ± 2.01 years.

During the 6 years, the blood glucose of this cohort of patients was well-controlled with HbA1c 6.67 ± 1.42 at endpoint comparing with 6.83 ± 1.21 at baseline (P = 0.127). No difference was observed in BMI, waist circumference and DBP between baseline and 6 years later. However, SBP had significantly increased 6 year later (baseline: 120.31 ± 19.44 mmHg; 6 years later: 137.97 ± 16.77 mmHg, P = 0.009). The mean LTL after 6 years was shorter than that at baseline (1.07 ± 0.14 at baseline; 1.00 ± 0.15 6 years later, P = 0.001) (Fig. 1).

3.2. Clinical information and laboratory results in LTL shortened and lengthened patients

With the mean LTL shortened in 64 patients, we observed telomere shortening in 70% (45/64) patients and telomere lengthening in 30% (19/64) of patients (Fig. 2). Patients whose telomere shortened had greater baseline BMI, higher HOMA-IR at 6 year and more insulin use during 6 years compared with those with lengthened LTL. There were no significant differences in age, male sex, blood pressure, diabetic duration, LDL-C at 6 year (Table 2).

Six variables in Table 2 (insulin use, HOMA-IR, BMI, waist circumference, LDL-C, baseline TL, P < 0.2) was brought in the regression analysis.

3.3. Risk factors analysis of change in telomere length in type 2 diabetes

Multivariable regression analysis (enter method)showed that only insulin use (β-coefficients: −0.587, 95% CI: −0.198, −0.085, P < 0.001) was the independent predictor of change in telomere length (Table 3).

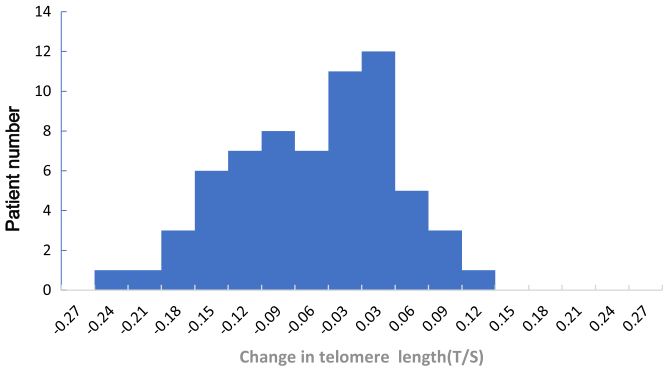

Fig. 2. Change in telomere length in 6 years. Six years change in LTL was yielded by subtracting baseline LTL from LTL 6 years later. T/S = telomere over single-copy gene ratio. N = 64.

Table 2
General clinical information on LTL decreased and increased T2DM patients.

	LTL shortened N = 45	LTL lengthened N = 19	P
Age at baseline	56.51 ± 2.67	57.35 ± 6.21	0.624
Male(%), n	15	9	0.289
Diabetes duration	6.16 ± 7.21	5.87 ± 6.25	0.952
Hypertension(%), n	23	11	0.619
Baseline BMI	25.33 ± 3.01	22.34 ± 2.28	0.009
Baseline Waistline	89.04 ± 8.57	83.65 ± 7.85	0.099
Baseline SBP	120.45 ± 19.63	118.89 ± 18.83	0.839
Baseline DBP	77.63 ± 10.77	77.56 ± 12.24	0.986
Baseline LDL-C	3.12 ± 1.18	3.06 ± 0.75	0.097
Baseline HbA1c	7.01 ± 1.40	6.92 ± 0.89	0.514
Baseline HOMA-IR	2.49 ± 2.36	1.25 ± 0.65	0.019
BaselineMetformin use(%), n	21	9	0.959
Insulin use between 6 years(%), n	16	2	0.042
Baseline statin use, n(%) n	17	8	0.746
Baseline telomere length	1.07 ± 0.17	1.01 ± 0.06	0.130

3.4. Risk factors analysis of telomere shortening in type 2 diabetes

Exact logistic regression (enter method) showed that LDL-C, and insulin use was the independent predictor of telomere shortening. Baseline LTL for this analysis was modified by multiplying 10 for an ideal OR value. But only insulin use (OR: 17.355; 95% CI:2.659, 35.627, P = 0.013) and LDL-C(OR: 3.493; 95% CI: 1.599, 10.063, P = 0.007)were significant (Table 4).

4. Discussion

This is the first longitudinal study to observe LTL change in diagnosed T2DM. The mean LTL over 6 years shortened, but we also observed telomere elongation in around one third of our patients. Usually, LTL was considered shortened with age. However, several longitudinal studies had indicated LTL might lengthen under some conditions. In a Finnish Diabetes Prevention Study, leukocyte LTL increased about two thirds in the individuals during follow-up of 4.5 years.[17] One study on the effect of stress on LTL in the survivors of cervical cancer showed that reduction in distress was associated with increased relative LTL after 4 months.[18] Another study investigated long-term effects of pronounced weight loss induced by bariatric surgery on telomere length and found that telomere length increased significantly by 0.024 ± 0.14 (P = 0.047) in 142 bariatric patients within 10 years after surgery.[19] Furthermore, two studies in patients with coronary heart disease over 5 or 2.5 years also found an increase in leukocyte telomere length in some individuals.[20,21] Unlike chronological age, change of telomere length is not linear. Instead, it correlates nonlinearly with observation period. 15% to 44% of people tend to show average lengthening when examined over a short period of 2 to 6 years. However, when observed over a decade or more, it is less likely to see lengthening.[22] Our finding of telomere change in type 2 diabetes is

Table 3
Risk factors of LTL change during a 6-year follow-up (multivariable regression analysis with enter method to analysis candidates in Table 2 retained at P<0.2).

Variables	N	β-coefficients	95%cCI		P
			Lower limit	Upper limit	
Insulin use	64	−0.587	−0.198	−0.085	<0.001
Baseline BMI	64	−0.040	−0.010	0.007	0.723
Baseline waistline	64	0.099	−0.002	0.004	0.442
Baseline LDL-C	64	−0.198	−0.049	0.003	0.078
Baseline HOMA-IR	64	−0.011	−0.002	0.002	0.931
Baseline LTL	64	0.160	−0.059	0.306	0.182

Analysis in 64 patients with type 2 diabetes. Negative regression coefficients denote faster telomere length loss.

Table 4
Risk factors of LTL shortening (exact logistic regression analysis with enter method to analysis candidates in Table 2 retained at P<0.2).

Variables	N	OR	95% CI		P
			Lower limit	Upper limit	
Insulin use	64	17.355	2.659	35.627	0.013
Baseline BMI	64	1.189	0.832-	1.578	0.157
Baseline waistline	64	4.010	1.494	10.398	0.127
Baseline LDL-C	64	3.493	1.559	10.063	0.007
Baseline HOMA-IR	64	1.043	0.971	2.310	0.389
Baseline LTL	64	3.109	0.511	1.108	0.089

Analysis in 64 patients with type 2 diabetes. OR value are for a 1 unit increase in LDL-C, BMI, waistline and HOMA-IR and a 0.1 unit increase in Baseline LTL.

similar to findings in other illness or conditions. The reason underlying the telomere lengthening are as follows: firstly, the telomerase protein, transcriptase component, and end-repair activities, together lengthen and stabilize telomeres.[22] Secondly, good blood glucose control might alleviate oxidative stress and then cause telomere lengthening. One study in T2DM indicated LTL is inversely correlated with glucose levels.[23] In our study, the blood glucose control was accomplished and glucose levels were lower after 6 years (HbA1c 6.67 ± 1.42 at endpoint vs 6.83 ± 1.21 at baseline, P = 0.127). Therefore, the telomere elongation evident in some of our patients could be due to the effect of lower glucose level.

We found for the first time a strong association between insulin use and LTL shortening in T2DM. However, the potential mechanisms are unclear. One study investigated the dose-dependent modulatory effects of insulin on glucose-induced endothelia senescence. They found all concentrations of insulin under normal glucose or high concentrations of insulin under high glucose promoted cellular senescence through NO-dependent and telomere-related mechanism.[15] Telomere attrition is a key feature of cell senescence. This study suggests that insulin is a contributing factor for telomere attrition. Another possibility is that insulin use reduced insulin resistance. Insulin treatment can lead to weight gain, both in type 1 diabetes and in T2DM.[13] With increasing weight, insulin can become less effective to control glycemia, resulting in higher insulin doses and hence more weight gain. Therefore, weight gain can aggravate insulin resistance in T2DM. Insulin resistance promotes reactive oxygen species (ROS) formation and favors a pro-inflammatory milieu, both these conditions playing a critical role in the telomere shortening.[24,25] Another study investigated the impact of caloric restriction (CR) on cardiac telomere biology in diabetes rats. They found decreased insulin resistance in diabetic rats fed with CR. Although the LTL had not changed, telomerase activity in diabetic rats was significantly increased after caloric restriction.[26]

We also found LDL-C is a risk factor of LTL shortening. A study in type 2 diabetes found reduced TL among South Asian T2DM men, which correlated with triglycerides and total cholesterol.[27] Another study investigating the association between LTL and metabolic syndrome found triglycerides were negatively associated with LTL in female participants.[28] It is well known that dyslipidemia can cause oxidative stress, resulting in telomere attrition.

For LTL measurement, efforts were made to avoid sampling bias. The assay was performed in the same laboratory by staff unaware of patients' clinical conditions, and the reaction of qPCR for each sample was triplicated. Inter assay CV was <9.5% and intra assay CV was <4.9%. Nevertheless, implications of this study are limited by the small sample size. Thus, here we solely report our longitudinal observation on the association of LTL with treatment in T2DM patients over 6 years and the possible risk factor involving in LTL shortening. Further investigation in larger and different populations with age-matched normal control will be necessary to clarify the relationship between change in LTL and T2DM treatment and to identify more risk factors.

Taken together, LTL may increase as well as decrease in T2DM who received antidiabetic treatment. Our study highlights the effect of insulin use in telomere shortening in type 2 diabetes. Insulin treatment might accelerate telomere shortening and LDL-C is a risk factor of LTL shortening in treatment of T2DM.

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