Original Article

Shorter leucocyte telomere length as a potential biomarker for nonalcoholic fatty liver disease-related advanced fibrosis in T2DM patients

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Background: Telomere length has been linked to hepatic fibrosis. Type 2 diabetes mellitus (T2DM) is considered as a particular risk for the development of hepatic fibrosis. This study is to explore the association of leucocyte telomere length (LTL) and nonalcoholic fatty liver disease (NAFLD)-related advanced fibrosis in T2DM patients.

Methods: A total of 442 patients with T2DM were enrolled from Tongji Hospital, Wuhan, China. Clinical features were collected and LTL was measured by Southern blot-based terminal restriction fragment length. Hepatic advanced fibrosis was determined by both the NAFLD fibrosis score (NFS) and fibrosis-4 score (FIB-4). Explanatory factors for advanced fibrosis in T2DM patients were identified using multiple logistic regressions.

Results: T2DM patients with advanced fibrosis had significant shorter LTL than the no-advanced group. Additionally, LTL, age, male and aminotransferase (ALT) were significantly associated with advanced fibrosis status in T2DM patients. Longer diabetes duration was found to have a strong association with advanced fibrosis in elder T2DM patients.

Conclusions: Shorter LTL was significantly associated with advanced fibrosis in T2DM patients. Longer diabetes duration was an independent risk factor for advanced fibrosis in old T2DM patients. Shorter LTL may be used as a biomarker for advanced fibrosis in T2DM patients.

Keywords: Leucocytes telomere length; hepatic advanced fibrosis; type 2 diabetes mellitus (T2DM)

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Introduction

The prevalence of nonalcoholic fatty liver disease (NAFLD) is rising dramatically worldwide, with approximately 1 billion individuals afflicted globally (1). NAFLD-related advanced fibrosis is considered to be the main risk factor among various disease spectrum of NAFLD (2,3) for all-cause mortality (4), especially for cardiovascular events (5). Patients with type 2 diabetes mellitus (T2DM) have extremely higher risk of NAFLD, which is around 49% to 62% (6,7). T2DM increases the risk of liver-related deaths, as well as overall mortality in NAFLD patients (6), meanwhile increases the risk of cardiovascular disease and accelerates the progression of macro and microvascular complications (8). In addition, patients with NAFLD presenting hepatocellular carcinoma in cirrhotic liver were more likely to be obese or have type 2 diabetes (9).

The prevalence of diabetes around the world, especially in China, is getting more challenging in recent years (10). Therefore, screening for NAFLD-related advanced fibrosis should be considered in T2DM patients.

The conventional methods used as diagnostic for liver fibrosis have some limitation. Liver biopsy is the golden standard for liver fibrosis. But liver biopsy is an invasive procedure, along with some complications and sampling error. Therefore, it is essential to identify the biomarker for screening NAFLD-related advanced fibrosis.

Telomere is duplicate sequences of (TTAGGG) that relates to shelterin proteins. Telomere is considered to protect chromosome from degradation (11). Oxidative stress-induced reactive oxygen species and chronic inflammation are major reasons for telomere shortening (12). Therefore telomeres are reported to be closely associated with metabolism disorder, such as obesity, diabetes, NAFLD, hypertension and dyslipidemia (13-16). Previous studies suggested that shorter telomere was observed in diabetes and NAFLD, similar as insulin resistance. Recently Kim *et al.* identified that shorter leucocyte telomeres were associated with advanced fibrosis among USA subjects (17). Considering that the association between telomere and NAFLD-related disease may be variable in different ethnicities (18,19), together with the higher prevalence of NAFLD-relevant fibrosis in diabetic patients, our study aims to investigate whether shorter leucocyte telomeres were linked to hepatic advanced fibrosis in Chinese diabetic patients.

Methods

Study population

A total of 483 participants were T2DM patients treated at Tongji Hospital (Wuhan, China) between Jan 2012 and May 2018, 41 were excluded according to exclusion criteria. Finally, 442 Han Chinese patients, ranging from 20 to 84 years old, were admitted under the following exclusion criteria: (I) over 210 gram of alcohol drinking every week for males, (II) over 140 gram of alcohol drink every week for females, (III) coexistent liver disease, (IV) treatment with systemic corticosteroids, (V) pregnancy, (VI) secondary diabetes. This study was approved by the ethics committee of Tongji Hospital. All the procedures complied with the provisions of the Declaration of Helsinki. Informed consents were obtained from all the patients.

Definition of hepatic advanced fibrosis

Hepatic advanced fibrosis was evaluated by NFS and FIB-4 score. NFS distinguishes whether patients have or have not advanced hepatic fibrosis, using the formula: NFS = −1.675 + 0.037 × age (year) + 0.094 × body mass index (BMI; kg/m^2) + 1.13 × impaired fasting glycaemia or diabetes (yes =1, no =0) + 0.99 × aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio − 0.013 × platelet (×10^9/L) − 0.66 × albumin (g/dL). Participants were stratified into three groups by tertiles of NFS. All the subjects were classified as one of the following three groups: advanced fibrosis (NFS >0.676), indeterminateness (NFS 0.676 to −1.455) and no-advanced fibrosis (NFS <−1.455) (20). FIB-4 score evaluates the degree of fibrosis with the following formula: FIB-4 = [age (year) × AST (U/L)]/[platelet (10^9/L) × ALT(U/L)$^{1/2}$]. Subjects were split into 3 groups, including those with non-advanced fibrosis (FIB-4 <1.30), indeterminateness (FIB-4 1.30 to 2.67), and advanced fibrosis (FIB-4 >2.67) (21).

Measurement of terminal restriction fragment length

AxyPrep Blood Genomic DNA Miniprep kit (Axygen, Corning, Inc., NY, USA) was used for Blood Genomic DNA extraction. The concentration of DNA samples was measured by Nanodrop. Genomic DNA were digested by Hinf I (R0155L, New England Bio Labs, Beverly, MA, USA) and RsaI (R0167L, New England BioLabs) at 37 ℃ overnight. Then, agarose gel electrophoresis was applied to separate the digested DNA at 70 volts for 18 hours. After that, denatured the gel in a pyrex container. Neutralize the gel for 30 min after drying the gel. Then the gel was transferred to a cylindrical hybridization tube. The gel was prehybridized with 10 mL prehybridization solution for 2 hours at 37 ℃. A ^{32}P-labeled telomeric probe was used to detect telomeres. The hot probe was made with 10 μL probe system (Deionized water 3.0 μL, T4PNK buffer 1.0 μL, ^{32}P-labeled telomeric probe 5'-(CCCTAA)3-3' [(10 pmol) 1.0 μL, [γ32-P] ATP (370MB q/mL) 3.0 μL, T4PNK (8 U/μL) 2.0 μL], then incubated at 37 ℃ for 1 hour and T4PNK was inactivated by heating at 68 ℃ for 10 min. After discarding the hybridization solution, the hot probe was added to the hybridization solution and let it hybridize overnight at 37 ℃. After the gel was washed, it was then exposed to a phosphor imager and scanned with a Typhoon system (Typhoon, GE Healthcare, Wisconsin, USA) separately, and the results were visualized with Image

Table 1 Anthropometric and biochemical characteristics of participants

Variables	No-advanced fibrosis (NFS <−1.455)	Indeterminate (−1.455 to 0.676)	Advanced fibrosis (>0.676)	P
Total N=442	136	233	73	–
Male/Female	65/71	135/98	47/26	0.000
Age (years)	48.03±13.49	54.95±12.48	60.23±13.812	0.000
Diabetes duration†	2.50 (0.08, 7.50)	5.00 (1.00, 10.50)	7.00 (1.00, 10.00)	0.002
BMI (kg/m^2)	23.25±4.36	24.80±3.33	25.54±4.30	0.001
Abdominal circumference (cm)	86.71±11.73	90.57±9.24	92.96±12.50	0.002
SBP (mmHg)	130.72±20.46	132.21±20.54	133.68±20.85	0.594
DBP (mmHg)	79.97±12.52	79.23±11.48	77.78±14.89	0.478
ALT (IU/L)†	17.00 (11.00, 26.75)	20.00 (14.00, 31.00)	26.00 (16.00, 36.00)	0.000
AST (IU/L)†	18.00 (14.00, 26.00)	19.00 (15.00, 24.50)	22.00 (16.00, 34.50)	0.000
GGT (IU/L)†	24.00 (17.00, 51.00)	26.00 (18.00, 52.00)	44.00 (25.00, 80.00)	0.285
TC (mM)	4.83±1.65	4.65±1.33	4.71±1.44	0.577
TG (mM)†	1.51 (1.00, 2.83)	1.85 (1.22, 3.28)	1.73 (1.07, 2.63)	0.124
HDL-C (mM)	1.04±0.40	1.01±0.28	1.05±0.39	0.681
LDL-C (mM)	2.73 ±0.97	2.60±0.90	2.83±1.08	0.262
FPG (mM)	8.58±3.49	8.49±3.25	8.50±2.85	0.971
2hPG (mM)	17.37±6.78	17.04±5.61	16.71±9.84	0.827
FC-P	2.37±1.63	2.56±1.73	2.60±1.21	0.648
2hC-P	6.20±4.44	6.35 ±3.80	6.30±3.37	0.959
HbA1c (%)	9.23±2.77	8.94±2.41	8.77±2.43	0.433

Data are means ± SD or median (interquartile range). †, Log transformed before analysis. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, aminotransferase activity; GGT, c-glutamyltransferase; TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FPG, fasting plasma glucose concentration; 2hPG, 2-h plasma glucose concentration; FC-P, fasting plasma C-peptide; 2hC-P, postprandial 2h C-peptide; HbA1c, hemoglobin A1c.

Quant software (Molecular Dynamics, Sunnyvale, CA). The weighted mean telomere length was calculated.

Statistical analysis

All data analysis was performed using SPSS (version 22.0). A value of P<0.05 was considered statistically significant. Student's *t*-test was used to test the difference between means of normally distributed data. Distribution of the continuous variables was carried out by Kolmogorov-Smirnov Test. Mann-Whitney test was applied to analysis Non-normally distributed data. χ^2 test was applied to examine categorical data. Pearson's correlations and Spearman's correlation were performed to examine the relationship between leucocyte telomere length (LTL) and other parameters. Multiple logistic regressions were performed to identify the risk factors for advanced fibrosis. The ANOVA trend analysis with polynomial contrast was used to estimate the association between hepatic fibrosis and eGFR. The accuracy of LTL as a biomarker for advanced fibrosis in T2DM patients was calculated by area under the receiver operating characteristic (ROC) curve.

Results

Characteristics of subjects

General characteristics of the subjects were summarized in *Table 1*. Among all the participants, 16.5% had advanced fibrosis, 30.8% had no-advanced fibrosis, and

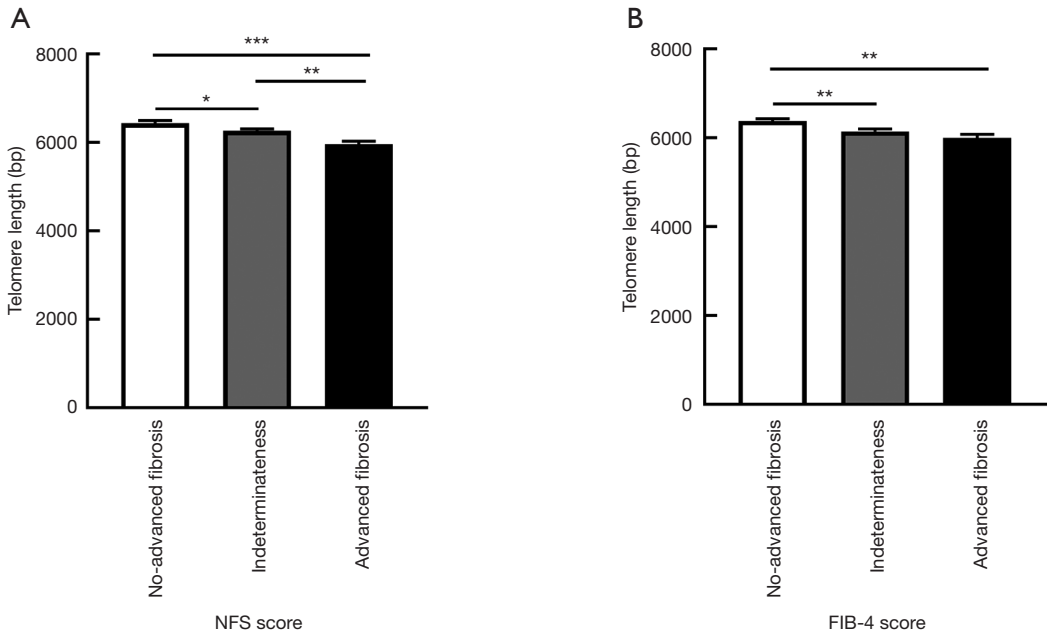

Figure 1 Telomere length in T2DM patients when stratified into three subgroups by the degree of hepatic fibrosis. Comparison of telomere length in three subgroups, hepatic fibrosis was accessed by NFS (A) and FIB-4 score (B). Telomere length is presented as the mean ± SE. *, P<0.05; **, P<0.01; ***, P<0.001.

remaining 52.7% were indeterminate. T2DM patients with hepatic advanced fibrosis were older (P<0.001), and had longer diabetes duration (P=0.002). BMI, abdominal circumference, ALT and AST were also significantly higher in diabetic patients with advanced fibrosis (all P<0.005). Since telomere was related to metabolic indexes with gender difference (13), characteristics of the male and female subjects were displayed in *Tables S1*,*S2*, respectively. In addition, complications are main concerns in diabetes. We analyzed the change of diabetes-related complication in T2DM patients with different degree of hepatic fibrosis. With hepatic fibrosis worsened, renal function [assessed by glomerular filtration rate (eGFR)] also gradually decreased in our study (*Figure S1A*). Consistent with previous report, patients with advanced fibrosis had a higher prevalence of diabetic complications, including diabetic retinopathy and diabetic peripheral neuropathy (*Figure S1B*). Meanwhile, the rate of major cardiovascular events (MACE) were also increased in patients with advanced fibrosis (*Figure S1C*).

LTL in T2DM subjects with advance fibrosis

To access the association of LTL and hepatic fibrosis, we divided the participants into three groups based on their NFS or FIB-4 scores. LTL ranged from 4,909 to 8,951 bp in total. When classified by NFS score, LTL were shorter (P<0.001) along with the increased severity of hepatic fibrosis in T2DM patients. Diabetic patients with advanced fibrosis had shorter telomere length (5,959.88±62.36 bp) compared to those without advanced fibrosis (6,430.95±64.41 bp) (*Figure 1A*). Those with advanced fibrosis still had significant shorter LTL even after being adjusted by age (data not shown). In addition, T2DM patients with advanced fibrosis, when stratified by FIB-4 score, still had significant shorter LTL compared to those without advanced fibrosis (*Figure 1B*). When taking the gender into consideration, shorter LTL was observed in diabetic patients with advanced fibrosis in both male and female (*Figure S2A*,*B*). Meanwhile, LTL showed no difference between male and female (*Figure S2C*).

Association of LTL with age and diabetes duration

We next investigated the correlation between LTL and a cluster of anthropometric and biochemical parameters (*Table 2*). The analysis indicated a significant negative association of LTL with age (r=–0.273, P<0.001), diabetes duration (r=–0.107, P=0.038) and triglyceride (TG) (r=0.153, P=0.006). But the association of LTL with diabetes duration and TG was no longer significant after

Table 2 Correlations of LTL with anthropometric parameters and biochemical indexes in all subjects

Variables	LTL			
	Before adjusted age		After adjusted age	
	r	P	r	P
Age (years)	−0.273	0.000		
Diabetes duration (years)	−0.107	0.038	−0.016	0.782
BMI (kg/m^2)	0.030	0.596		
Abdominal circumference (cm)	−0.004	0.942		
ALT (IU/L)	−0.035	0.497		
AST (IU/L)	−0.031	0.538		
GGT (IU/L)	−0.029	0.602		
TC (mM)	0.095	0.083		
TG (mM)	0.153	0.006	0.023	0.683
HDL-C (mM)	−0.071	0.213		
LDL-C (mM)	0.035	0.544		
FPG (mM)	0.040	0.474		
2hPG (mM)	0.040	0.474		
FC-P	−0.011	0.866		
2hC-P	0.025	0.703		
HbA1c (%)	−0.013	0.812		

BMI, body mass index; ALT, aminotransferase activity; GGT, c-glutamyltransferase; TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FPG, fasting plasma glucose concentration; 2hPG, 2-h plasma glucose concentration; FC-P, fasting plasma C-peptide; 2hC-P, postprandial 2h C-peptide; HbA1c, hemoglobin A1c.

Table 3 Multiple logistic regression to examine the risk factors for advance fibrosis

Factor	P value	OR	95% CI
Age	0.000	1.239	1.129 to 1.359
Gender	0.028	0.217	0.056 to 0.850
ALT	0.000	1.133	1.061 to 1.210
LTL	0.002	0.997	0.996 to 0.999

Table 4 Multiple logistic regression to examine the risk factors for advance fibrosis in the patients over 60 years old

Factor	P value	OR	95% CI
Age	0.006	1.220	1.060 to 1.404
Gender	0.017	0.021	0.001 to 0.496
Diabetes duration	0.024	1.557	1.059 to 2.291
LTL	0.049	0.997	0.995 to 1.000
BMI	0.007	1.576	1.135 to 2.189

adjustment for age.

LTL as an independent factor of advanced fibrosis in T2DM patients

Explanatory factors of NAFLD-related advanced fibrosis in T2DM patients were evaluated by multiple logistic regressions, including gender, age, diabetes duration, BMI, abdominal circumference, ALT and AST, since these variables were significantly different between T2DM patients with and without advanced fibrosis. LTL was significantly associated with advanced fibrosis in T2DM patients (OR: 0.997, 95% CI: 0.996–0.999; P=0.002), together with age (OR: 1.239, 95% CI: 1.129–1.359; P<0.001), gender (OR: 0.217, 95% CI: 0.056–0.850; P=0.028) and ALT (OR: 1.133, 95% CI: 1.016–1.210; P<0.001) (*Table 3*). We also included all the parameters in *Table 1* to perform logistic regressions, LTL was still significantly associated with advanced fibrosis in T2DM patients (OR: 0.996, 95% CI: 0.992–0.999; P=0.014).To further explore the effect of diabetes duration on advanced fibrosis, participants were stratified by age (20–39, 40–59, >60 years). Diabetes duration was only significantly associated with advanced fibrosis in T2DM patients over 60 years old (OR: 1.557, 95% CI: 1.059–2.291; P=0.024) (*Table 4*).

Since gender had significant effect on advanced fibrosis, we performed logistic regressions to identify independent risk factors in male and female separately. LTL was still significantly associated with advanced fibrosis in male T2DM patients (OR: 0.999, 95% CI: 0.997–1.000; P=0.022), together with age (OR: 1.122, 95% CI: 1.051–1.198; P=0.001) and with ALT (OR: 1.028, 95% CI: 1.007–1.049; P=0.008) (*Table S3*). Interestingly, only LTL (OR: 0.998, 95% CI: 0.997–1.000; P=0.043) and age (OR: 1.115, 95% CI: 1.033–1.204; P=0.005) were significantly related to

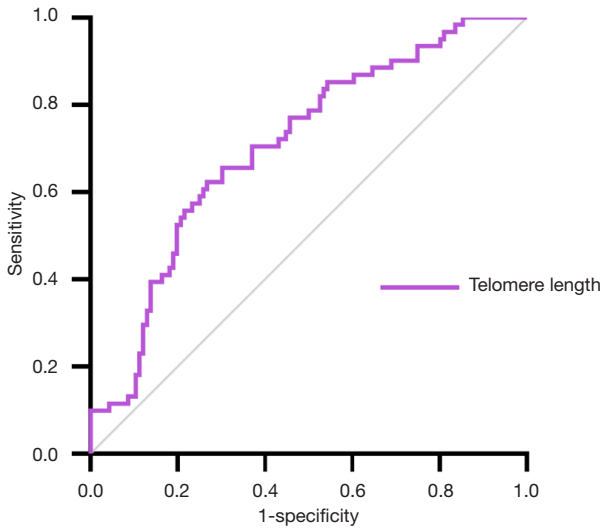

Figure 2 ROC curves for diagnosis of advanced fibrosis.

advanced fibrosis in female T2DM patients (*Table S4*).

ROC curves for advanced fibrosis

Based on the above results, LTL may be a potential biomarker for NAFLD-related advanced fibrosis in T2DM patients. The ROC curve shown in *Figure 2* represented the diagnostic accuracy of LTL. The area under curve was 0.707 (95% CI: 0.628–0.785). Setting cut-off value at 6,028.50bp as calculated by Youden index, sensitivity of LTL as biomarker for NAFLD-related advanced fibrosis in T2DM patients was estimated to be 62.3% and specificity to be 73.3%.

Discussion

This present cross-sectional study indicated that T2DM patients with advanced fibrosis had significantly shorter LTL in Chinese population, and LTL was negatively related to age and diabetes duration. LTL was significantly associated with the advanced fibrosis status in T2DM patients. In addition, diabetes duration only had a significant association with hepatic fibrosis when T2DM patients were over 60 years old.

The severity of hepatic fibrosis was reported to reversely relate to eGFR (22). Our study also suggested that diabetic patients with advanced fibrosis had lower eGFR. Meanwhile higher prevalence rate of MACE (4) and diabetes complications were observed in advanced fibrosis in current study, which was consistent with previous studies (23). Due to the strong relation between diversified hazard and advanced fibrosis, it's necessary to identify the biomarker for advanced fibrosis in T2DM patients.

Our finding suggested that advanced fibrosis had significantly shorter LTL in T2DM patients, no matter when advanced fibrosis was estimated by NFS and FIB-4 score system. In fact, shorter telomeres were also reported to link to other chronic liver diseases, such as alcoholic liver disease and viral hepatitis (24,25). Furthermore, patients with cirrhosis had shorter telomere than age-matched controls, and the association of short telomere with hepatic cirrhosis could be explained by the *TERE* gene variants which were components of telomerase (26). Recently, Kim et.al found that short LTL was reversely correlated with liver fibrosis, and this correlation was more obvious in elder subjects as well as among non-Hispanic Whites. Diabetes and obesity could not further promote the risk of advanced fibrosis in the continuous National Health and Nutrition Examination Survey (NHANES) in USA. Considering that the correlation between LTL and NAFLD-related disease is largely affected by ethnicity (18,19,27), it is still necessary to explore the relationship between LTL and liver fibrosis in Chinese diabetic patients. In accordance with previous study (17), this study identified that shorter LTL was closely related to advanced fibrosis in Chinese diabetic patients for the first time. And this correlation was confirmed, even after adjusting for age and gender.

Our observations were in line with previous finding that LTL was negatively correlated to age and diabetes duration (27). But the correlation between LTL and diabetes duration lost statistical significance after adjustment for age. No significant association between LTL and metabolic indexes were identified in our study. It's worth noting that the interactions between short LTL and metabolic indexes were still controversial. Nordfjall *et al.* found that LTL was only related to BMI in women. Meanwhile, LTL was negatively associated with high density lipoprotein (HDL) and 2hPG in man (13). And Nordfjall *et al.* also summarized the studies which focused on the relationship between LTL and metabolic indexes (BMI, hypertension, insulin, glucose, HDL, LDL, triglycerides, and cholesterol). Surprisingly, none of the metabolic indexes mentioned above were consistently associated with LTL.

Our results also suggested that short telomere length was a significant risk factor for advanced fibrosis status in addition to age, male and ALT in T2DM patients. One recent cross-sectional study determined several risk factors

associated with biopsy proven advanced fibrosis in T2DM patients (19), such as age and ALT which was in line with our study. Male was another risk factor in our study, which was in line with identified in previous study (28). Shorter telomere length was shown to be a biomarker of NAFLD-related advance fibrosis, which was independent of age, ALT and gender. Insulin resistance causes hyperinsulinemia (29) and inflammatory cytokines (30), which may lead to liver fibrosis and hepatocellular carcinoma by promoting proliferation and reducing apoptosis within the liver. In our study, even though diabetic patients with advanced fibrosis had more serious insulin resistance (estimated by HOMA-IR) than those without advanced fibrosis (0.87±0.07 versus 0.99±0.10; P=0.537), but the difference was not significant. And logistic regressions also suggested that HOMA-IR (OR: 1.012, 95% CI: 0.987–1.038; P=0.357) was not an independent risk factor for advanced fibrosis in diabetic patients. The explanation may be that subjects in our study were diabetic patients who had severe insulin resistance, and the difference between the diabetic patients with and without advanced fibrosis is not obvious.

The specific mechanism between short telomere and fibrosis was still remains obscure so far. Telomeres are regarded to protect chromosomes tips from degradation, end-to-end fusion and compensate for the DNA loss (31). Telomeres become shorter gradually during each cell division by DNA replication, so senescence or apoptosis may trigger cellular signaling cascades which may lead to shortened telomeres (32). Chronic liver injury induced by NAFLD lead to a series of pathophysiological changes, which include hepatic regeneration, increased cellular turnover and dysfunctional telomere repairs induced progressive telomere shortening (33,34). These pathophysiological changes may finally cause liver fibrosis. And oxidative stress is supposed to lead to reactive oxygen species and chronic inflammation, which may induce telomere shortening. Based on cross-sectional and prospective studies (35,36), chronic hepatic injury was reported to be significantly associated with the T2DM, In turn, T2DM leading to oxidative stress (37) and elevation of liver enzymes, induces progressive hepatic damage and regeneration. Therefore, T2DM may further damage fatty liver, and induce progressive telomere shortening.

Longer diabetes duration was reported to have a link with liver stiffness measurement (38), and our previous study identified that the shortening rate of LTL in T2DM patients with NAFLD was higher than those without NAFLD with the extension of diabetes. But the significant association of diabetes duration with hepatic fibrosis was not observed in our study population. In order to deeply estimate the role of diabetes duration in advanced fibrosis, diabetic patients were stratified by age. Interestingly, diabetes duration as a risk factor for advanced fibrosis was identified only in T2DM patients over 60 years old. One possible explanation for no correlation between diabetes duration and advanced fibrosis in other age-subgroups possibly was due to that liver fibrosis is more obvious in the older in the older population.

The strengths and limitations were discussed as follow. First, our study uncovered that shorter LTL was associated with NAFLD-related advanced fibrosis in T2DM patients. Therefore, it may provide a clinically safe, feasible and effective method to detect advanced fibrosis by measurement of LTL. Second, the southern blot-based method, terminal restriction fragment analysis, was used in our study to measure LTL, which is considered as the gold standard. This method is more accurate and repeatable compared to quantitative PCR in other studies. There were several limitations of our study. First, our study did not apply more accurate advanced fibrosis diagnosis methods such as liver biopsy. Second, we did not apply more accurate NAFLD diagnosis methods, such as CT, MRI, or biopsy-proven steatosis. Third, our study was cross-sectional, and the sample size was relatively small. In the future, we plan to perform longitudinal studies, which will be essential to evaluate the role of telomere length as a potential predictor to assess the pathogenesis of advanced fibrosis in T2DM patients.

Conclusions

In summary, our findings may have established a critical role for short telomere length as a biomarker for NAFLD-related fibrosis in diabetic patients. In addition, our results suggest that diabetes duration can be considered as a risk factor in the detection of advanced fibrosis in old T2DM patients. Further studies will be conducted to estimate the role of short telomere length in diagnosing NAFLD-advanced fibrosis identified by liver biopsy in T2DM patients.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by the ethics committee of Tongji Hospital. Informed consents were obtained from all the patients.

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Supplementary

Table S1 Anthropometric and biochemical characteristics of participants in male

Variables	Non-advance fibrosis	Advance fibrosis	P
Age (years)	45.48±13.47	58.89±14.27	0.000
Diabetes duration†	2.00 (0.07, 5.75)	5.50 (0.96, 12.00)	0.044
BMI (kg/m^2)	24.06±4.83	25.38±4.30	0.210
Abdominal circumference (cm)	88.21±12.62	92.27±12.86	0.175
SBP (mmHg)	129.23±18.87	132.64±21.50	0.376
DBP (mmHg)	82.51±12.35	77.30±15.00	0.047
ALT (IU/L)†	22.00 (13.00, 41.00)	28.50 (17.00, 47.75)	0.047
AST (IU/L)†	19.00 (17.00, 42.00)	27.00 (15.00, 40.50)	0.152
GGT (IU/L)†	40.00 (20.00, 72.00)	44.50 (25.75, 90.00)	0.424
TC (mM)	4.31±1.38	4.50±1.46	0.574
TG (mM)†	1.51 (0.97, 2.85)	1.62 (1.03, 2.26)	0.379
HDL-C (mM)	0.92±0.31	0.99±0.43	0.387
LDL-C (mM)	2.53±0.90	2.65±1.08	0.602
FPG (mM)	8.79±4.10	8.12±2.28	0.372
2hPG (mM)	17.10±6.86	17.40±11.72	0.881
FC-P	2.54±1.84	2.57±1.29	0.928
2hC-P	6.61±4.99	6.53±3.84	0.945
HbA1c (%)	9.21±3.05	8.86±2.55	0.530

Data are means± SD or median (interquartile range). †, Log transformed before analysis. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, aminotransferase activity; GGT, c-glutamyltransferase; TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FPG, fasting plasma glucose concentration; 2hPG, 2-h plasma glucose concentration; FC-P, Fasting plasma C-peptide; 2hC-P, postprandial 2h C-peptide; HbA1c, hemoglobin A1c.

Table S2 Anthropometric and biochemical characteristics of participants in female

Variables	Non-advance fibrosis	Advance fibrosis	P
Age (years)	50.37±13.17	62.65±12.85	0.000
Diabetes duration†	1.50 (0.08, 7.00)	5.00 (0.50, 9.00)	0.044
BMI (kg/m^2)	22.61±3.90	25.85±4.38	0.003
Abdominal circumference (cm)	85.44±10.92	94.27±11.95	0.005
SBP (mmHg)	132.08±21.85	135.58±19.91	0.477
DBP (mmHg)	77.65±12.30	78.65±14.95	0.737
ALT (IU/L)†	13.50 (10.00, 21.50)	26.00 (14.00, 57.00)	0.097
AST (IU/L)†	16.00 (13.00, 20.75)	27.00 (16.00, 37.00)	0.041
GGT (IU/L)	20.00 (15.25, 26.00)	52.00 (18.00, 80.00)	0.050
TC (mM)	5.24±1.74	5.03±1.39	0.617
TG (mM)†	1.24 (0.98, 1.89)	1.52 (1.05, 2.09)	0.942
HDL-C (mM)	1.16±0.44	1.15±0.29	0.951
LDL-C (mM)	2.92 ±1.00	3.18±1.05	0.386
FPG (mM)	8.41±2.90	9.14±3.58	0.338
2hPG (mM)	17.59±6.76	15.53±5.43	0.219
FC-P	2.25±1.46	2.69±0.97	0.419
2hC-P	5.90±4.02	5.68 ±1.45	0.881
HbA1c (%)	9.24±2.51	8.60±2.24	0.275

Data are means ± SD or median (interquartile range). †, Log transformed before analysis. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, aminotransferase activity; GGT, c-glutamyltransferase; TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FPG, fasting plasma glucose concentration; 2hPG, 2-h plasma glucose concentration; FC-P, fasting plasma C-peptide; 2hC-P, postprandial 2h C-peptide; HbA1c, hemoglobin A1c.

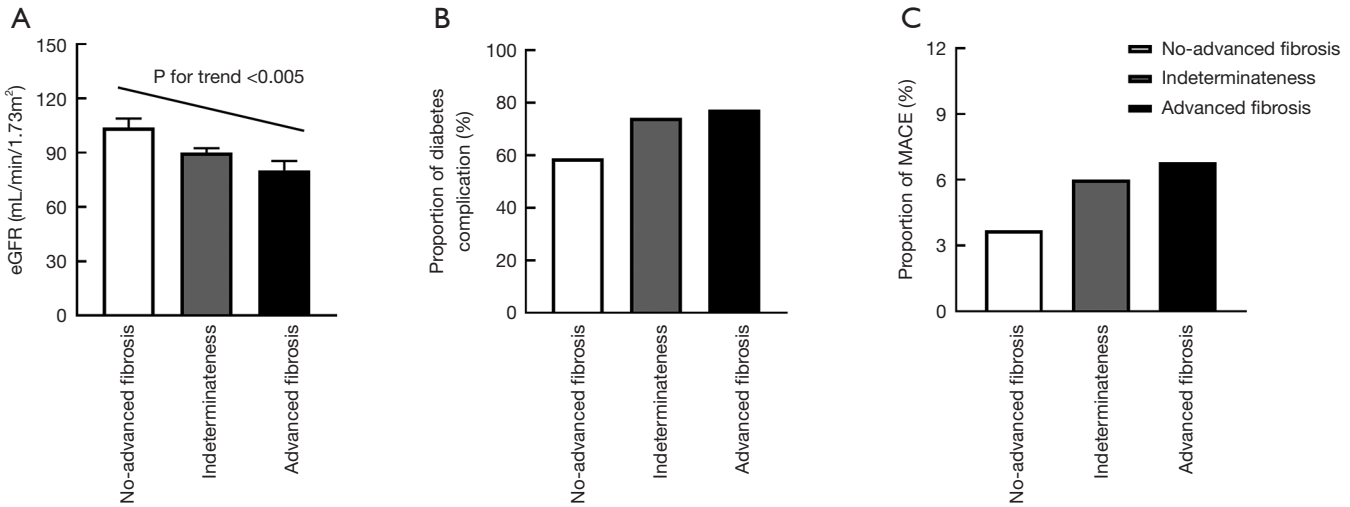

Figure S1 Complications in T2DM subjects according to different degree of hepatic fibrosis. (A) Renal function, (B) proportion of diabetes-related complications and (C) proportion of MACE in term of the different degrees of hepatic fibrosis.

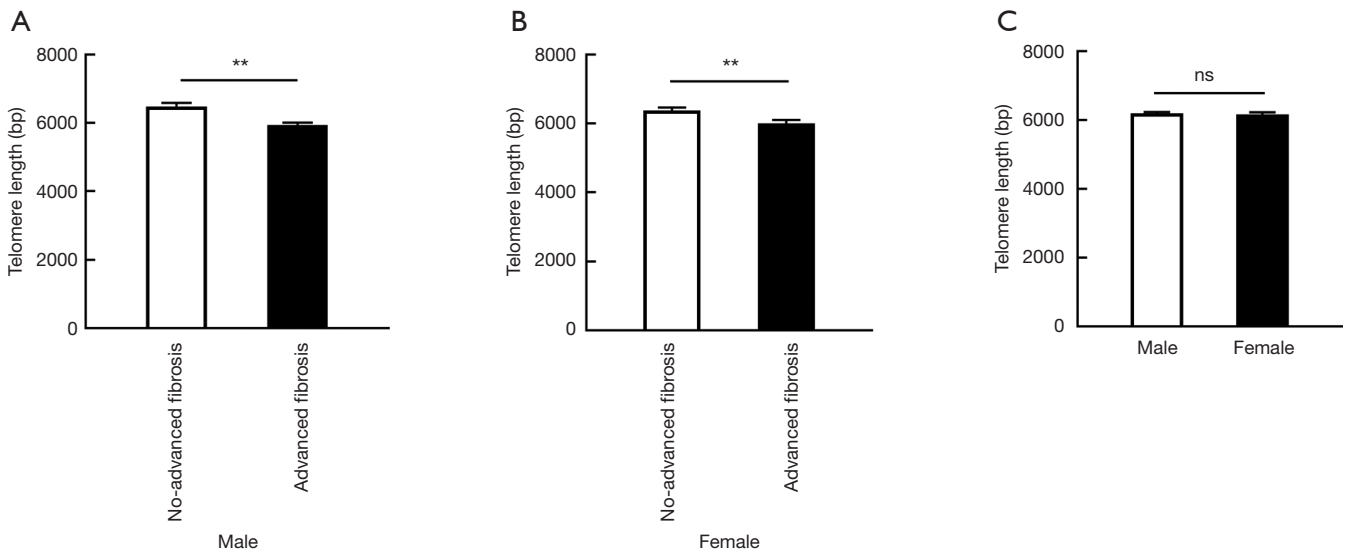

Figure S2 Telomere length between advanced fibrosis and non-advanced fibrosis according to gender. (A) Comparison of telomere length between advanced fibrosis and non-advanced fibrosis groups in male; (B) comparison of telomere length between advanced fibrosis and non-advanced fibrosis groups in female; (C) comparison of telomere length between male and female. Telomere length is presented as the mean ± SE. ns, P>0.05; **, P<0.01.

Table S3 Multiple logistic regression to examine the risk factors for advance fibrosis in male

Factor	P value	OR	95% CI
Age	0.001	1.122	1.051 to 1.198
ALT	0.008	1.028	1.007 to 1.049
LTL	0.022	0.999	0.997 to 1.000

Table S4 Multiple logistic regression to examine the risk factors for advance fibrosis in female

Factor	P value	OR	95% CI
Age	0.005	1.115	1.033 to 1.204
LTL	0.043	0.998	0.997 to 1.000