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Original Article

Correlation of the telomere length with type 2 diabetes mellitus in patients with ischemic heart disease

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ABSTRACT

Objective: The study aimed to explore the relationship of the telomere length with type 2 diabetes mellitus (DM) among patients with ischemic heart disease (IHD).
Method: This 2-year cross-sectional study included 130 male patients diagnosed with IHD through echocardiography and coronary angiography, wherein consecutive IHD patients with type 2 DM (65) and without type 2 DM (65) were selected. Baseline characteristics including age, gender, body mass index, and blood pressure were recorded. Laboratory investigations such as random blood sugar (RBS), fasting lipid profile, serum creatinine, and serum urea levels were measured. Quantitative real-time polymerase chain reaction was used for the measurement of the telomere length. The logistic regression analysis was used to predict the relationship of the telomere length with age and type 2 DM among patients with IHD.
Results: All the patients in the study were men, and most of them (diabetics = 22; nondiabetics = 20) were aged between 56 and 65 years. Age ($p = 0.003$), telomere length ($p < 0.001$), RBS ($p < 0.001$), serum creatinine ($p < 0013$), and serum urea ($p < 0.04$) were significantly higher in the diabetic subset than in the nondiabetic subset. No significant relationship was observed between age and the telomere length ($p = 0.813$); however, the mean telomere length was significantly high among the patients with type 2 DM than those without type 2 DM ($p = 0.005$). The logistic regression analysis showed that the telomere shortening ($p = 0.00019$) and RBS ($p < 0.0001$) were the significant risk factors for type 2 DM in patients with IHD.
Conclusion: The telomere shortening was significantly correlated with type 2 DM among the patients with IHD. However, multicentric studies with larger samples are required to validate the current observation.

1. Introduction

Worldwide, ischemic heart disease (IHD) is the major cause of death and acquired disability. Globally, it accounts for approximately 8.1 million deaths every year.[1] Along with traditional risk factors such as systemic hypertension, dyslipidemia, and body mass index (BMI), hyperglycemia is also an independent risk factor for the development of IHD.[2] Chronic hyperglycemia in patients with type 2 diabetes mellitus (DM) increases the oxidative stress, interferes with telomerase function, and results in telomere attrition or shortening.[3] Furthermore, it was reported that the telomere length was shorter in the diabetic patients than in the healthy individuals.[4]

Telomeres, found at the end of chromosomes, shorten with each cell cycle and reflect the aging of the organisms. Therefore, the telomere length is identified as the biological marker for cellular aging.[5] Male gender, smoking, adiposity, inflammation, obesity, physical inactivity, insulin resistance, oxidative stress, and

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ultraviolet irradiation along with advancing age are the attributed risk factors for the shortening of telomeres.[5–7] Multiple techniques have emerged to determine the telomere length[8]; however, quantitative polymerase chain reaction (qPCR) is considered as an amenable technique even in the analysis of small amounts of the DNA sample.[9] The qPCR technique expresses the telomere DNA content as the ratio of telomeric product (T)/single-copy gene (S) product (T/S ratio).[10]

Shortening of the telomere is related to an increased risk of age-related diseases such as peripheral vascular disease, IHD, myocardial infarction (MI), and cancer.[5] Shortening of telomeres is independently associated with the progression of metabolic syndromes via affecting the cellular metabolic rate.[11,12] Furthermore, some studies have reported a heritable shorter telomere as a risk factor in the development of type 2 DM.[13,14] The telomere shortening results in premature beta (β)-cell senescence, reduced β-cell mass, impaired insulin secretion, and glucose intolerance.[3] Several studies have reported association between the telomere length and essential hypertension, obesity, vascular dementia, and mortality due to heart disease.[15] However, there is a scarcity of literature exploring the relationship between the telomere length and type 2 DM in patients with IHD. Hence, this interesting observation compelled us to explore the relationship of the telomere length with type 2 DM among patients with IHD.

2. Methods

2.1. Study design

This 1-year cross-sectional study was conducted at the Department of General Medicine at Jawaharlal Nehru Medical College, KAHER. The ethical approval was obtained from Institutional Ethical and Research Committee, and the trial was registered under Clinical Trials Registry of India no. CTRI/2018/02/011663. Informed written consent was also obtained from the patients before their participation in the study. A total of 130 male patients with IHD, diagnosed through echocardiography and coronary angiography, were included in the study, wherein consecutive IHD patients with type 2 DM (65) and without type 2 DM (65) were selected. While female patients, patients with history of smoking, chronic alcoholism, and systemic illnesses were excluded from the study.

2.2. Data collection

Demographic data including age and gender of the patients were noted. Height and weight of the patients were measured for the calculation of BMI using a standard protocol.[16] Blood samples (5 ml) drawn from an antecubital vein were stored in a vacutainer containing a small amount of ethylenediaminetetraacetic acid (EDTA). Laboratory investigations such as random blood sugar (RBS), fasting lipid profile, serum creatinine, and serum urea levels of the patients were measured. Blood pressure (BP) was recorded using a sphygmomanometer. The ejection fraction was assessed using echocardiography.

2.3. DNA extraction, amplification, and measurement

DNA was extracted from the peripheral blood leukocytes using Qiagen DNA Mini Kit (QIAGEN Inc., California, United States). The telomere length of the isolated DNA was measured using quantitative real-time PCR (RT-PCR) (Applied Biosystems, California, United States) as T/S ratio.[17] Cycling conditions for both the telomere and 36B4 amplicons are as follows: 10 min at 95 °C, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min, followed by a melt curve with little modifications as described previously.[17] The 36B4 is a single-copy gene and serves as a reference gene in the conventional qPCR technique in the measurement of telomeres. All samples were analyzed in the ABI StepOnePlus RT-PCR System with SDS version StepOnePlus software (Applied Biosystems, California, United States).

2.4. Statistical analysis

Data were entered and coded in Microsoft excel spreadsheet. R-3.4.1 was used to analyze the data. Categorical data were represented in the numeric form, and continuous data were presented as mean ± standard deviation. Independent *t*-test was used to compare the mean difference between the two groups. Multivariate regression analysis was performed to determine the variables affecting the telomere length. Logistic regression analysis was used to predict the relationship of the telomere length, age, BMI, RBS, serum urea, and serum creatinine with the diabetic status among patients with IHD.

3. Results

A total of 130 IHD patients with or without type 2 DM were assessed during the study period. Most of the patients in both the diabetic (22) and nondiabetic (20) subsets were aged between 56 and 65 years. However, in the diabetic subset, many patients (61) were aged > 46 years (Table 1). All the patients in both the subsets were men; owing to the direct influence of female gender on the telomere length, women were excluded from our study.

Independent *t*-test was used to compare the baseline characteristics in the diabetic and nondiabetic subsets (Table 2). IHD patients with type 2 DM were older, had shorter telomere length ($p < 0.001$), and had higher BMI, systolic and diastolic BP, serum creatinine, serum urea, and triglyceride levels, whereas total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol were low compared with nondiabetic patients. Raised enzyme levels and pain, two-vessel coronary artery disease and drug history (aspirin + clopidogrel + atorvastatin + metoprolol + pantocid) were high in patients with type 2 diabetes. None of the diabetic patients had prior history of MI and coronary artery revascularization. However, a significant difference was observed between the diabetic and nondiabetic subset in terms of age ($p = 0.003$), telomere length ($p < 0.001$), RBS level ($p < 0.001$), serum creatinine level ($p < 0.013$), and serum urea level ($p < 0.04$).

Relationship of the telomere length with chronological age among IHD patients with and without diabetes is shown in Fig. 1 (panel A). Both in diabetic and nondiabetic patients, as the age increases, the telomere length slowly decreased. However, the decrease in the telomere length in nondiabetic patients was less compared with diabetic patients ($p = 0.813$). While comparing the diabetic and nondiabetic subset with the telomere length, the telomere length was significantly higher in the diabetic subset than in the nondiabetic subset ($p = 0.005$; Panel B, Fig. 1).

Table 1
Age distribution of diabetic and nondiabetic patients with ischemic heart disease.

Age (years)	Ischemic heart disease	
	Diabetic ($n = 65$)	Nondiabetic ($n = 65$)
25–35	0	4
36–45	4	16
46–55	18	10
56–65	22	20
> 65	21	15

Table 2
Baseline characteristics of diabetic and nondiabetic patients with ischemic heart disease.

Variable	Diabetic	Nondiabetic	*p* value
Age (years)	61.26 ± 10.44	54.92 ± 13.04	0.003*
Telomere length	1.32 ± 0.36	2.00 ± 0.52	<0.001*
BMI (kg/m^2)	24.64 ± 2.52	23.66 ± 3.19	0.054
RBS (mg/dL)	229 ± 71.09	136.29 ± 50.25	<0.001*
Blood pressure (mm Hg)			
Systolic	122.68 ± 21.74	122.43 ± 18.83	0.945
Diastolic	79.2 ± 10.39	78.37 ± 11.98	0.674
Renal functions(mg/dL)			
Serum creatinine	1.12 ± 0.54	0.93 ± 0.28	0.013*
Serum urea	38.09 ± 27.83	29.72 ± 16.88	0.04*
Lipids profile (mg/dL)			
Total cholesterol	179.08 ± 28.12	181.77 ± 38.42	0.649
LDL cholesterol	123.34 ± 25.02	127.32 ± 31.42	0.425
HDL cholesterol	37.74 ± 7.12	39.02 ± 7.02	0.305
Triglycerides	135.72 ± 28.12	126.77 ± 41.13	0.150
Signs and symptoms			
Elevated enzyme	13(20)	13(20)	0.6128
Pain	32(49.23)	27(41.54)	
Both	20(30.77)	25(38.46)	
Prior myocardial infarction			
Absent	65(100)	63(96.92)	0.4961
Present	0(0)	2(3.08)	
Coronary artery vessel involved			
1 vessel	1(1.54)	2(3.08)	0.4066
2 vessels	34(52.31)	26(40)	
3 vessels	30(46.15)	37(56.92)	
Drug history			
1,3,4,5,6	11(16.92)	10(15.38)	0.4167
1,2,3,6	5(7.69)	10(15.38)	
1,2,3,4,5,6	41(63.08)	34(52.31)	
1,2,4,5,6	8(12.31)	11(16.92)	

BMI, body mass index; RBS, random blood sugar; LDL, low-density lipoprotein; HDL, high-density lipoprotein; SD, standard deviation; 1, aspirin; 2, clopidogrel, 3, atorvastatin; 4, metoprolol; 5, pantocid.
Data were expressed in mean ± SD and n (%).
*Significant.

The risk factors influencing the telomere length were shown in Table 3. Of all, the diabetes status was found to be significantly correlated with the telomere length ($p < 0.0001$). From the logistic regression analysis, odds ratio showed that the telomere shortening ($p = 0.00019$), RBS ($p < 0.0001$) and serum urea were the significant risk factors for type 2 DM in patients with IHD. However, comparatively, the telomere shortening was 4.3 times higher in patients with type 2 DM (Table 4).

4. Discussion

The telomere shortening is hypothesized as the biomarker for age and age-related diseases.[18] Diabetes, complicated by cardiovascular diseases, increases mortality and results in higher levels of oxidative stress and inflammation, which in turn accelerate the telomere shortening.[11] Similarly, in our study, the telomere length in IHD patients with type 2 DM was significantly low compared with IHD patients without type 2 DM. Based on these findings, we can hypothesize that shorter telomeres promote the onset of type 2 DM in patients with IHD. Hence, the measurement of the telomere length may signify prognostic information for clinicians in the management of IHD patients with type 2 DM. Similarly, several studies[19,20] in different regions demonstrated that shorter telomeres, independently and significantly, predict the increased risk of type 2 DM. A literature-based meta-analysis also reported that the telomere shortening is an independent risk factor associated in the incidence of type 2 DM.[21] In contrast, a study by Aviv et al[22] reported that the telomere shortening in patients with type 2 DM could potentially be explained by the genetic regulation.

The telomere shortening indicates an important molecular mechanism for biological/vascular aging.[23] Although, in our study, increased age influenced the telomere length, it was insignificant. In contrast, studies conducted by Masi et al[24] ($r = -0.150$; $p = 0.002$) and Eguchi et al[25] ($r = -0.194$; $p < 0.001$) reported a negative correlation between the telomere length and age. Men have shorter telomere length owing to the higher prevalence of degenerative diseases compared with women.[26] Therefore, female patients were excluded from the study. However, there is a lack of literature to comment these findings. A similar study conducted by Tentolouris et al[23] in men reported that the telomere length in type 2 DM patients with microalbuminuria was shorter (6.64 ± 0.74 vs. 7.23 ± 1.01 kb, respectively, $p = 0.004$) than those without microalbuminuria.

In our study, the RBS, serum creatinine, and urea levels were significantly higher in IHD patients with type 2 DM. However, serum creatinine and urea levels were not found significant in IHD patients with type 2 DM. A study conducted by Gurung et al[27] reported that increased oxidative stress leads to telomere shortening and predicts the renal impairment in patients with type 2 DM.

Overall, the present study implicates that IHD patients with type 2 DM have low telomere length when compared with IHD patients without type 2 DM; the influence of age on the telomere length was statistically significant. The present study has few potential

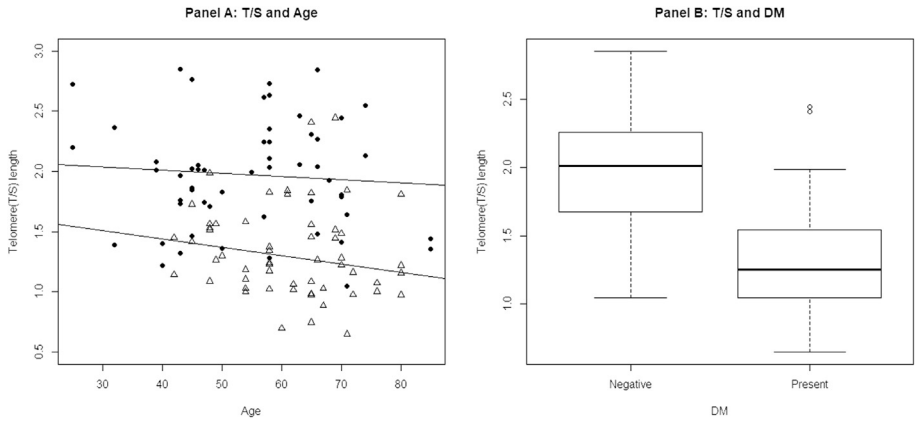

Fig. 1. Relationship of age and the diabetic status with the telomere length. Panel A: relationship between age and the telomere length; as the age increases, the telomere length slowly decreases in diabetic patients (lower line) and nondiabetic patients (upper line). Panel B: relationship between type 2 diabetes mellitus and the telomere length; the telomere length was high in the nondiabetic (absent) subset, whereas it was low in the diabetic (present) group. DM, diabetes mellitus; T/S, telomeric product/single-copy gene product.

Table 3
Multivariate regression analysis for the variables influencing the telomere length.

Variables	Estimate (95% CI)	*P*-value
Age	−0.0014 (−0.0053–0.0028)	0.5302
Diabetic patients	−0.4528 (−0.5640–−0.3000)	<0.0001
Dyslipidemia positive	−0.1014 (−0.2563–0.0452)	0.2027
BP	−0.0003 (−0.0033–0.0032)	0.8748
BMI	0.0001 (−0.0160–0.0191)	0.9880
RBS	0.0001 (−0.0009–0.0007)	0.9473
Serum urea	0.0008 (−0.0020–0.0036)	0.5652
Serum creatinine	0.0344 (−0.1081–0.1583)	0.6215
Sign (pain)	−0.0617 (−0.1701–0.0543)	0.3068
Sign (both enzymes elevated, pain)	−0.1095 (−0.2487–0.0052)	0.1157
Prior myocardial infarction present	−0.2176 (−0.5960–0.1082)	0.2194
coronary artery, two vessels involved	−0.1922 (−0.3917–0.0490)	0.1016
coronary artery, three vessels involved	−0.1894 (−0.3900–0.0515)	0.1079
Drug history	−0.0725 (−0.2212–0.3234)	0.3169

CI, confidence interval; BP, blood pressure; BMI, body mass index; RBS, random blood sugar.

Table 4
Logistic regression for the diabetic and nondiabetic groups.

	Estimate	Odds ratio (95% CI)	*p*-value
Diabetic			
Age	0.05	1.0607 (0.9956–1.1413)	0.0821
Telomere length	−4.343	0.0139 (0.0013–0.0854)	0.00019*
BMI	0.10	1.0427 (1.0255–1.0673)	0.45
RBS	0.046	0.9576 (0.9144–0.9981)	<0.0001*
Serum urea	−0.057	3.3682 (0.5078–35.1792)	0.0205*
Serum creatinine	0.867	1.0756 (0.8434–1.3977)	0.3799

CI, confidence interval; BMI, body mass index; RBS, random blood sugar.
*Significant.

limitations such as the small sample size enrolled in the study, which is a major limitation. Owing to the cross-sectional design of the study, large prospective multicentric studies are required to validate the exact relationship involved in the shortening of the telomere length in type 2 DM patients with IHD. However, this is the first study of its kind reporting a relationship between the telomere length and type 2 DM in patients with IHD.

Overall, the telomere shortening was significantly correlated with type 2 DM among patients with IHD. However, multicentric studies with larger samples are required to validate the current observations. Moreover, this study will be a great reflection of upcoming genetics in Indian population.

What is already known?

Shortening of telomere is associated with an increased risk of age-related diseases such as peripheral vascular disease, IHD, MI, and cancer. Previous studies have evidenced a heritable shorter telomere as a risk factor in the development of type 2 DM.

What this study adds?

The present study explored the relationship between the telomere length and type 2 DM among patients with IHD.

Source of funding

Nil.

Conflict of interest

All authors have none to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ihj.2018.09.007.

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