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Short telomere length is associated with arterial aging in patients with type 2 diabetes mellitus

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Abstract:

It is known that glucose disturbances contribute to micro- and macro vascular complications and vascular aging. Telomere length is considered to be a cellular aging biomarker. It is important to determine the telomere length role in vascular structural and functional changes in patients with diabetes mellitus. The cross-sectional observational study was conducted in a high-risk population from Moscow, Russia. 50 patients with diabetes and without clinical cardiovascular disease and 49 control group participants were included in the study. Glucose metabolism assessment tests, measuring intima–media complex thickness and determining the presence of atherosclerotic plaques, pulse wave velocity measurement, and telomere length measurement were administered to all participants. Vascular changes were more dramatic in patients with diabetes than in control group. And the telomeres were shorter in patients with diabetes. There were significant differences in the vascular wall condition among diabetes patients and no substantial differences in the arterial structure between groups with 'long' telomeres, however, there were statistically significant differences in the vascular wall condition between groups with 'short' telomeres. Vascular ageing signs were more prominent in patients with diabetes. However, despite diabetes, vascular changes in patients with 'long' telomeres were very modest and were similar to the vascular walls in healthy individuals. Thus, 'long' lymphocyte telomeres may have a protective effect on the vascular wall and may prevent vascular wall deterioration caused by glucose metabolism disorders.

Keywords: telomere length; vascular aging; diabetes mellitus; insulin resistance, arterial stiffness

Diabetes mellitus (DM) is a chronic noncommunicable disease that has reached epidemic proportions. Type 2 DM (T2DM) inevitably leads to microvascular and macrovascular complications that aggravate the course and prognosis of cardiovascular diseases (CVD).

A vast body of evidence indicates that age-related endothelial dysfunction and the vascular wall thickening and stiffening set up a metabolically and enzymatically active environment that contributes to the manifestation and progression of CVD (1). There are some processes contributing the vascular wall changes: an increased collagen content accompanied by the formation of strong cross-links between fibers; elastin content fragmentation and reduction; accumulation of advanced glycation end products (AGE) (2); intima–media complex (IMT) thickening due to accumulation of extracellular matrix proteins and smooth muscle cells; upregulation of adhesion molecules: and increased monocyte adhesion to the endothelial surface (3).

Experimental data show that glucose metabolism disorders induce vascular changes. For instance, Facchini et al. (2001) showed that hyperinsulinaemia regardless of hyperglycaemia may promote oxidative stress and thereby accelerate vascular ageing and the development of age-related diseases (4). In addition, insulin resistance (IR) is considered to be a predictor of atherosclerosis and CVD independently of other risk factors, such as blood lipid levels. And hyperglycaemia results in ageing, endothelial dysfunction, and arterial stiffness (5). Recently published studies demonstrate that arterial stiffness occurs in the initial stages of the glucose metabolism disorders, when IR is not yet accompanied by increased glucose level (6, 7).

Differences in aging rates in patients with T2DM may result from different ‘genetic protection’ levels. Some findings in vessel biology revealed the molecular mechanisms of ageing and methods of preventing or slowing down the arterial ageing process.

One of the most widely discussed genetic ageing markers is the peripheral blood lymphocyte telomere length. Lymphocyte telomeres are the ends of linear DNA that have the

repetitive nucleotide sequence TTAGGG. Telomeres protect linear chromosome ends from degradation and fusion and maintain genome stability. Due to incomplete replication of the DNA ends in somatic cell, telomere is truncated in every mitosis cycle. As soon as telomere DNA becomes forbiddingly short, the cell loses the ability to maintain genomic integrity, to repair DNA damages, to maintain the metabolic activity, and to divide (8, 9). Some authors describe the telomere as a 'molecular clock' that defines the cell's lifespan (10).

Experimental and clinical evidence indicates that the lymphocyte telomere length corresponds to the telomere length of the stem cells and endothelial progenitor cells. Thus, the lymphocyte telomere length could be used as a biomarker of vessel ageing. Therefore, the telomere length measuring in easily accessible tissues such as blood may be a substitute parameter for determining the telomere length in other tissues (11). The length and rate of telomere shortening are genetically determined; however, they are also influenced by external factors (12).

New data show an accelerated telomeres shortening in patients with T2DM and impaired glucose tolerance (13, 14), and it could be related to the IR. Telomere length is considered to be a marker of T2DM and diabetes complications (15). Compared with healthy individuals, the presence of short telomeres has been demonstrated in patients with IR, however, even shorter telomeres were observed in patients with T2DM (16). Some data allow us to make a link between shortened lymphocyte telomeres and the development of T2DM, CVD, and vascular ageing processes (17). These observations suggest that T2DM plays an important role in the processes of replicative senescence.

However, despite obvious scientific achievements in the field of vascular ageing, there are many unresolved issues, e.g. an insufficient number of ageing clinical studies in patients with T2DM, most published scientific studies had been mainly conducted in animals and cell cultures.

Studying the relationship between the vascular structure and function changes and replicative senescence in patients with T2DM is of particular interest.

Studying the pathogenesis of changes in the cardiovascular system in patients with T2DM is highly relevant due to the steady ageing of the population, T2DM and CVD prevalence in elderly, the lack of understanding of the ageing process in patients with diabetes, and the absence of effective methods to influence these processes.

Aim

to investigate an association between the artery structural and functional changes and peripheral blood lymphocyte telomere length in T2DM.

Materials and methods

The cross-sectional observational study was conducted in a high-risk population from Moscow, Russia. Patients who previously underwent an outpatient examination in the FGBI National Research Centre for Preventive Medicine (NRCPM) during 2012–2013 were selected for this cross-sectional study. The main study group included patients with T2DM with a disease duration of no longer than 12 months after diagnosis, glycated haemoglobin (HbA1c) levels of 6.5%–9.0%, and age of 45–75 years. The control group consisted of patients without T2DM and with no clinical CVD. "These patients with diabetes and controls were selected from a larger population-based study (Stazhesko I, et al, submitted manuscript), they were conducted similar analyses without focusing on the importance of type 2 diabetes in relation to the studied variables.

Criteria for exclusion from the study were as follows: type 1 DM and other specific types of diabetes; stage 3 arterial hypertension, blood pressure > 180/100 mm Hg; regular use of anti-hypertensive drugs; regular use of anti-diabetic drugs; severe diabetic microangiopathy (pre-

proliferative and proliferative diabetic retinopathy and stage 3b, 4 or 5 chronic kidney disease); and clinical CVD with New York Heart Association (NYHA) classification class II–IV chronic heart failure, presence of valvular heart disease; chronic liver and/or kidney failure; cancer; pregnancy; lactation; or refusal to participate in the study.

All the patients signed a legal informed consent form to participate in the study. The local ethics committee (LEC) FGBI NRCPM Ministry of Healthcare, Russian Federation; minutes of the LEC, meeting № 8, 29 November 2011, approved this study protocol.

All the patients underwent a standard clinical assessment during screening. The assessment included medical history; physical examination, height and weight measurements to calculate body mass index (BMI), systolic (SBP) and diastolic blood pressure (DBP) measured using a calibrated instrument with shoulder cuff (HEM-7200 M3, Omron Healthcare, Kyoto, Japan). Blood pressure was measured on the right arm after a 10-min rest in the sitting position three times with 2-min intervals, and the average of the three measurements was used for analysis. Arterial hypertension was defined as blood pressure ≥ 140/90 mm Hg. Blood samples were taken for clinical and biochemical laboratory tests. Rest and stress electrocardiograms (ECG) were recorded (treadmill test protocol BRUCE, Intertrack, Schiller, Miami, FL, USA). Patients with disorders, according to the data of blood tests, heart rhythm and/or cardiac conduction on ECG, and a positive stress test were excluded from the study.

158 patients were screened, 99 were included in the study. In all participants additional tests were conducted, including a glucose metabolism assessment; duplex scanning of the carotid arteries to measure IMT and determine the presence of atherosclerotic plaques; measurement of carotid–femoral pulse wave velocity (PWV); and telomere length measurement.

Glucose metabolism assessment

The glucose concentration was measured to assess the glucose metabolism using the glucose oxidase method on a SAPPHIRE-400 analyser using DiaSys diagnostic kits. The HbA1c

level was measured by liquid chromatography on a Sapphire 400 (Niigata Mechatronics, Tokyo, Japan) analyser according to the manufacturer's standard procedure.

Telomere length measurements

The relative length of peripheral blood lymphocyte DNA telomere was measured. The technique was based on a Cawthon study with some modifications (18). The telomere DNA in the genome was estimated by real-time polymerase chain reaction (PCR). The genomic single-copy DNA was measured by parallel real-time PCR. It was assumed that the ratio of telomere and single-copy matrices was proportional to the lymphocyte telomeres length.

Arterial stiffness measurements

To evaluate the vascular wall condition, the carotid–femoral PWV was measured by applanation tonometry (SphygmoCor system, AtCor Medical, West Ryde, NSW, Australia). A high precision applanation tonometry was superimposed on the proximal (carotid) and distal (femoral) artery after a short time interval to record pulse waves. Central blood pressure, SBP, DBP and ECG were recorded simultaneously with PWV. The distance travelled by the pulse wave between registration points was divided by the time needed, as determined by the time between the origin of pulsation and the R-wave position on ECG to calculate PWV. A PWV value > 10 m/s was considered to be an increased PWV.

Evaluation of IMT and subclinical atherosclerosis

The Q-LAB special application program (Philips, Eindhoven, The Netherlands) was used for duplex scanning of extracranial brachycephalic arteries in B-mode with parallel ECG recording. IMT was measured on the back wall of the common carotid artery (CCA). The sensor was located on the anterior and posterior margins of the m. Sternoclaidomastoideus. Scanning was performed in three planes: two longitudinal planes and the transverse plane. CCA IMT was measured 1.5–2 cm proximal to bifurcation on the artery wall most remote from the sensor. Internal and external carotid arteries were evaluated at the point of the visual maximum

thickening of diagnostic IMT scanning of CCA. The structural evaluation of IMT included echogenicity analysis and assessment of the preserved layer structure. Echogenicity of the surrounding tissue was considered baseline when determining echogenicity of the intima. Echogenicity of the vessel lumen was used for the media. The standards proposed by the experts of the European Society of Hypertension and the European Society of Cardiology (2003) were used to assess CCA IMT. IMT < 0.9 mm was considered as normal; increased thickness was 0.9–1.3 mm and diffuse IMT thickening > 1.3 mm was considered as atherosclerosis. The presence of atherosclerotic plaques was defined as an increase in IMT > 1.3 mm in CCA, a local increase in IMT of 0.5 mm or a 50% increase in nearby IMT. Local IMT thickening > 1.3 mm, which caused stenosis of the lumen but did not affect its internal anatomy, was considered as a plaque.

Statistical analysis

SAS 9.1 statistical software (SAS Institute, Cary, NC, USA) was used for analysis. All data were entered into a spreadsheet, and exploratory analysis was performed to identify data entry errors and missing values. Tests of skewness and kurtosis were performed for quantitative parameters and revealed a normal distribution for the majority of the quantitative parameters. Quantitative data are presented as mean and mean-square deviation. Comparative analysis of independent samples was conducted. Mean values of the clinical parameters were compared between the two groups using one-way analysis of variance for continuous variables and the χ-square test for categorical variables. A modified Student's t-test was used with Fisher arcsine transformation for the frequencies of the qualitative parameters. Pearson's linear correlation analysis was used to detect correlations between parameters. Multivariate regression equations and multiple linear regression analyses were used to identify, which correlations between parameters were independent. The null hypothesis was rejected at $p < 0.05$.

Results

In total, 99 (33 males and 66 females) patients were included in the study. The mean age was 52.4 ± 12.3 years. The patients were divided into two groups: those with T2DM (n = 50) and those without T2DM (n = 49). The patient groups were comparable in age and sex. No significant differences in the number of males/females were observed between the groups. T2DM duration was 0.9 ± 0.089 years. BMI in the T2DM group was significantly higher than that in the group of healthy individuals. Blood pressure did not differ significantly between the groups. Telomere length was significantly shorter in the T2DM group than in the control group. Fasting blood glucose (FBG) and HbA1c levels in patients with T2DM were significantly higher than those in the control group.

A significantly higher PWV and thickened IMT were observed in patients with T2DM than in the control group. The number of atherosclerotic plaques tended to be higher in the T2DM group than in the control group ($p = 0.08$). The main patient characteristics are shown in Table 1.

All the patients were divided into two groups according to the relative peripheral telomere length. The median telomere length was 9.75. All the patients with a telomere length less than the median were assigned to the 'short' telomere group, and patients with a telomere length equal to or above the median were assigned to the 'long' telomere group.

The vascular wall status and glucose metabolism parameters were compared with the telomere length in all the patients.

The severity of subclinical atherosclerosis and vascular stiffness were higher in patients with 'short' telomeres than in those with 'long' telomeres in both groups. In contrast, in patients with T2DM and 'long' telomeres, indicators of vascular ageing were significantly less frequent than in those with T2DM and 'short' telomeres: PWV and IMT in the 'long' telomere group were significantly lower ($p < 0.01$ for both parameters) and the number of atherosclerotic plaques was significantly fewer ($p = 0.03$) (Table 2).

The HbA1c level in patients with T2DM and ‘short’ telomeres was significantly higher than that in patients with T2DM and ‘long’ telomeres. Patients with T2DM and ‘short’ telomeres had higher rates of PWV ($p < 0.01$) and IMT ($p = 0.03$) and a greater number of atherosclerotic plaques ($p = 0.04$) than subjects without diabetes and ‘short’ telomeres. The vascular stiffness and subclinical atherosclerosis indicators did not differ in the T2DM group with ‘long’ telomeres and the control group: PWV and IMT were comparable in participants with and without T2DM ($p = 0.91$ and $p = 0\ 12$, respectively) and the number of atherosclerotic plaques did not differ significantly ($p = 0.97$) (Table 2).

Table 3 shows correlation analysis results for PWV and IMT with other parameters in participants with and without T2DM. PWV was significantly correlated with SBP, IMT and HbA1c. A significant negative correlation was detected between PWV and the telomere length in the T2DM group. IMT was significantly correlated with SBP and negatively with the telomere length. Also such correlation was observed between PWV and age, SBP and IMT, while a negative correlation was detected between PWV and telomere length in the control group. IMT was significantly correlated with age and SBP.

Analysis of the telomere length in the T2DM group demonstrated an inverse correlation between the telomere length, HbA1c and PWV (Table 4).

Multiple linear regression analysis with the telomere length as the dependent variable and age, PWV, FBG and HbA1c as independent variables showed that only PWV (inverse relationship) and HbA1c (direct relationship) were correlated with the telomere length (Table 5).

Discussion

We revealed that the vascular wall condition in patients with T2DM was significantly different from that in healthy individuals. Our results are consistent with those of other studies and have a pathophysiological basis (19). One of the possible explanations for increased vascular

wall rigidity in patients with T2DM is an AGE accumulation (20) that leads to the formation of cross-links between collagen molecules in the middle layer of the vascular wall, resulting in increased collagen rigidity and vascular wall stiffness. The presence of chronic hyperglycaemia in patients with T2DM amplifies protein glycation and AGE accumulation and results in significantly increased vascular stiffness and therefore in accelerated vascular wall ageing (19, 20). We demonstrated a correlation between arterial stiffness and HbA1c, which is the main indicator of glucose metabolism.

Our results demonstrated that the peripheral lymphocyte telomere length was shorter in patients with T2DM than in healthy individuals. A similar correlation was demonstrated in the study conducted by Hovatta et al. (16). However, Sampson et al. found no correlation between lymphocyte telomere shortening and glucose metabolism indicators in a European study, possibly because of the small number of patients in that study (21). Besides the significant differences in the HbA1c level between T2DM patients with ‘long’ and ‘short’ telomeres, this study shows also a negative correlation between the telomere length and HbA1c. These data suggest the damaging effect of hyperglycaemia on replicative ageing indicators. However, this finding needs additional larger scale research.

The most important results of this study are the finding of the independent negative relationship between the telomere length and PWV and independent positive association between the telomere length and HbA1c in T2DM patients. In other words, shorter telomeres in patients with T2DM are associated with stiffer vessels and poor diabetes control. The main cause of lymphocyte telomere shortening during the lifetime is an oxidative stress and the patterns associated with oxidative stress (obesity, IR, and chronic stress). Inflammation is associated with enhanced cell proliferation that leads to rapid telomere length shortening, and oxidative stress causes the single-stranded DNA breaks in telomere repeats promoting the accelerated telomeres shortening during the repeated divisions.

In DM patients the telomere length shortening is more prominent due to damaging effects of chronic hyperglycemia, AGE accumulation etc. One of AGE agents is HbA1c. It is known that the level of HbA1c determines the vascular changes rate and that HbA1c is known to be predictive of future cardiovascular diseases independent of FBG. It has been proved by some clinical studies that showed an association between the rates of the telomere shortening and the presence of T2DM (6, 22). It is also known that IR in T2DM is accompanied by endothelial dysfunction. The deficit in nitric oxide is considered to be the link between these processes. In cultured cells it has also been shown that IR may induce the smooth muscle cell proliferation and migration leading to vascular stiffness (3). Perhaps it is the telomere shortening that in association of T2DM results in the vascular aging and the T2DM related CVD development, although, this hypothesis requires further investigation and clarification.

Another important finding of this study is the evidence that the vascular walls in patients with T2DM and 'long' lymphocyte telomeres were not significantly different from vascular walls in healthy individuals without T2DM. This finding indicates that genetically long telomeres may prevent accelerated vessel ageing in patients with type 2 diabetes of short duration (patients with established diabetes duration < 1 year were included). Also It was not results of influence of any drug, as all these diabetes patients were free of drug treatment.

In contrast, patients with T2DM and 'short' lymphocyte telomeres demonstrated a higher vascular stiffness and subclinical atherosclerosis severity despite the relatively short diabetes duration. Notably, participants with and without T2DM were comparable in age and SPB/DBP readings and ratio. Therefore, the influence of age and blood pressure on the telomere length was comparable. Thus, 'short' telomeres were associated with rigid blood vessels and 'long' telomeres were associated with a better-preserved vascular wall.

One possible explanation is that lymphocytes are used to determine the telomere length in clinical practice, which essentially reflects the telomere length in stem and progenitor cells.

These cells are involved in damaged tissue repairing process and tissue differentiation and play an important role in maintaining the tissue homeostasis and ensuring the preservation of endothelial function. However, blood vessel stiffness is mainly determined by the condition of the extracellular matrix; thus, cells may be present in the matrix, and the replicative activity of these cells affects vascular stiffness. Slow telomere shortening is likely to affect the matrix by some other mechanism, but not by the replicative activity.

Increasing evidence suggests that lymphocyte telomeres shortening is a key component diminishing stem cell facilities and age-related tissue degeneration including vascular rigidity (20). However, the explanation is still lacking.

Conclusion

In summary, our observational cross-sectional study in a high CVD risk population showed the correlations between T2DM, cellular ageing processes, and the severity of subclinical morpho-functional changes in the vascular wall explained the higher incidence of CVD in patients with T2DM. Prevention of such changes may help prevent CVD in patients with T2DM, particularly in patients with 'short' peripheral lymphocyte telomeres.

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Table 1. Main clinical characteristics, results of duplex scanning of the carotid arteries and applanation tonometry and telomere length

Characteristic	T2DM+ (n = 50)	T2DM- (n = 49)	p
Age, years	56 ± 12.1	53.47 ± 11.91	0.15
Male, number/%	15/30	17/34	0.77
BMI, kg/m2	31.1±1.08	26.6±0.53	0.002
SBP, mm Hg	129.6±3.2	123.3±1.5	0.06
DBP, mm Hg	79.06±1.8	77.2±0.9	0.37
T2DM duration, years	0.9±0.089		
HbA1c, %	7.2±0.6	5.09±0.05	<0.001
FBG, mmol/l	8.1±0.333	5.3±0.051	<0.001
PWV, m/s	13.07±0.6	10.67±0.23	<0.001
IMT, mm	0.88±0.02	0.74±0.01	<0.001
Number of atherosclerotic Plaques	1.3±0.2	0.84±0.1	0.08
Relative telomere Length	9.53±0.1	9.86±0.1	0.02

Abbreviations: SBP-systolic blood pressure; DBP-diastolic blood pressure; BMI-body mass index; HbA1c-glycated haemoglobin; FBG-fasting blood glucose; PWV-carotid-femoral pulse wave velocity; IMT-intima-media thickness.

Table 2. Parameters for vascular wall status depending on the presence of T2DM and telomere length

Parameter	'Long' telomeres		'Short' telomeres		p
	T2DM+ (n = 29)	T2DM- (n = 27)	T2DM+ (n = 21)	T2DM- (n = 22)	
PWV, m/s	10.50±0.1	10.51±0.51	15.08±1.31	10.7±0.52	p^1=0.91 p^2<0.01 p^3<0.01 p^4=0.025
IMT, mm	0.80±0.09	0.73±0.03	0.87±0.05	0.78±0.13	p^1=0.12 p^2=0.03 p^3<0.01 p^4=0.04
Number of atherosclerotic plaques	0.76±0.04	0.78±0.02	1.02±0.29	0.89±0.22	p^1=0.97 p^2=0.04 p^3=0.03 p^4=0.03

p^1 – vascular wall characteristics differences in diabetic and nondiabetic patients with «long» telomeres

P^2 - vascular wall characteristics differences in diabetic and nondiabetic patients with «short» telomeres

p^3 - vascular wall characteristics differences in diabetic patients with «long» and with «short» telomeres

P^4 - vascular wall characteristics differences in nondiabetic patients with «long» and with «short» telomeres

Abbreviations: PWV-carotid-femoral pulse wave velocity; IMT-intima-media thickness

Table 3. Pearson's correlation analysis between pulse wave velocity (PWV) and intima–media thickness (IMT) and other parameters

Parameter	T2DM+ (n = 50)		T2DM- (n = 49)	
	PWV, m/s	IMT, mm	PWV, m/s	IMT, mm
Age, years	0.1953 p = 0.17	0.3564 p = 0.1501	0.3213 p = 0.001	0.3644 p = 0.0001
SBP, mm Hg	0.2717 p = 0.003	0.3231 p = 0.007	0.3784 p = 0.0021	0.3214 p = 0,0214
DBP, mm Hg	0.0983 p = 0.27502	0.2196 p = 0.133	0.01024 p = 0.2765	0.0538 p = 0.4245
BMI, kg/m2	0.3127 p = 0.001	0.1731 p = 0.142	0.0054 p = 0.8594	0.02985 p = 0.4211
FBG, mmol/l	0.3621 p = 0.301	0.2258 p = 0.0674	0.1738 p = 0.1422	0.1732 p = 0.1421
HbA1c, %	0.3526 p = 0.002	0.1571 p = 0.0699	0.1528 p = 0.152	0.1635 p = 0.0672
Relative telomere Length	-0.3564 p = 0.019	-0.3184 p = 0.0278	-0.3623 p = 0.0014	0.1673 p = 0.0711

Abbreviations: SBP-systolic blood pressure; DBP-diastolic blood pressure; BMI-body mass index; FBG-fasting blood glucose; HbA1c-glycated haemoglobin.

Table 4. Correlation analysis of relative telomere length and other parameters in patients with T2DM.

Parameter	Telomere length	
	r	p
Age, years	0.025	0.87
SBP, mm Hg	-0.03	0.84
DBP, mm Hg	0.12	0.5
BMI, kg/m2	-0.02	0.85
FBG, mmol/l	-0.31	0.52
HbA1c,%	-0.31	0.03
PWV, m/s	-0.35	<0.01
IMT, mm	-0.11	0.41
Number of atherosclerotic plaques	-0.13	0.14

Abbreviations: SBP-systolic blood pressure; DBP-diastolic blood pressure; BMI-body mass index; FBG-fasting blood glucose; HbA1c-glycated haemoglobin; PWV-carotid-femoral pulse wave velocity; IMT-intima-media thickness.

Table 5. Multiple linear regression analysis of telomere length based on age, FBG, HbA1c and PWV as independent variables in the T2DM

Parameter	β	Standard error	P
Age, years	0.029	0.530	0.85
PWV, m/s	-0.15	2.721	0.037
FBG, mmol/l	-0.02	0.537	0.98
HbA1c, %	0.067	0.841	0.036

Abbreviations: HbA1c-glycated haemoglobin; FBG-fasting blood glucose, PWV-carotid-femoral pulse wave velocity.