Длина теломер и состояние сосудистой стенки у пациентов с сахарным диабетом 2 типа

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Цель. Изучить изменения структуры и функции артерий в зависимости от длины теломер из периферических лимфоцитов и наличия сахарного диабета 2 типа (СД2).

Материалы и методы. В исследование включено 50 пациентов с СД2 без клинических проявлений сердечно-сосудистых заболеваний (ССЗ) и 49 человек контрольной группы. Всем участникам выполнена оценка углеводного обмена, дуплексное сканирование сонных артерий с определением толщины комплекса интима-медиа (ТКИМ), наличие атеросклеротических бляшек, измерение каротидно-феморальной скорости распространений пульсовой волны (СРПВ), измерение длины лимфоцитарных теломер.

Результаты. Изменения сосудов были более выражены у пациентов с СД2. У пациентов с СД2 длина лимфоцитарных теломер оказалась короче, чем у лиц без диабета (9,53±0,1 и 9,86±0,1, р=0,033). Все пациенты были разделены по длине лимфоцитарных теломер. В группе СД2 выявлены достоверные различия в состоянии сосудистой стенки: СРПВ у лиц с «длинными» теломерами – 10,58±0,1 м/с, с «короткими» теломерами – 15,08±1,3 м/с (р=0,012), ТКИМ у лиц с «длинными» теломерами – 0,80±0,09 мм, с «короткими теломерами» – 0,87±0,05 мм (р=0,024). При сравнении групп с «длинными» теломерами не выявлено достоверных различий в структуре артерий: СРПВ 10,58±0,1 м/с и 10,5±0,5 м/с (р=0,913), ТКИМ – 0,80±0,09 мм и 0,73±0,03 мм (р=0,12). А при сравнении групп с «короткими» теломерами выявлены достоверные различия в изменениях сосудистой стенки: СРПВ 15,08±1,3 м/с и 10,7±0,5 м/с (р=0,015). ТКИМ – 0,87±0,1 мм и 0,78±0,1 мм (р=0,03).

Заключение. Признаки сосудистого старения были более выражены у пациентов с СД2. Однако несмотря на наличие диабета, сосудистые изменения были минимальны у пациентов с «длинными» лимфоцитарными теломерами и сравнимы с состоянием сосудистой стенки здоровых людей. Длина лимфоцитарных теломер, возможно, обладает протективным действием на сосудистую стенку, предохраняя ее от повреждающих действий нарушений углеводного обмена.

Ключевые слова: длина теломер; сосудистое старение; сахарный диабет; инсулинорезистентность

Telomere length and vascular wall in patients with type 2 diabetes mellitus

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Aim. To study the relationship between changes in the artery structure and function and peripheral lymphocyte telomere length in patients with type 2 diabetes mellitus (DM2).

Materials and methods. A total of 50 patients with T2DM and without clinical manifestations of cardiovascular disease (CVD) were included in the study; the control group consisted of 49 people. The following tests were conducted for all study participants: carbohydrate metabolism evaluation, carotid artery duplex scan to measure intima—media complex thickness (IMT) and to determine the presence of atherosclerotic plaques, carotid—femoral pulse wave velocity (PWV) measurement and lymphocyte telomere length measurement.

Results. The vascular changes were more pronounced in patients with T2DM than in those without diabetes (9.53±0.1 vs 9.86±0.1, p=0.033). The participants were divided according to the telomere length. Among patients with T2DM, there were significant differences in the condition of the vascular wall [PWV: 10.58±0.1 m/s in patients with ‘long’ telomeres and 15.08±1.3 m/s in patients with ‘short’ telomeres; IMT: 0.80±0.09 mm in patients with ‘long’ telomeres and 0.87±0.05 mm in patients with ‘short’ telomeres (p=0.024)]. There were no significant differences in the arterial structure between the patient and control groups with ‘long’ telomeres [PWV: 10.58±0.1 m/s vs 10.7±0.5 m/s (p=0.913); IMT: 0.80±0.09 mm vs 0.73±0.03 mm (p=0.12). However, there were significant differences in the vascular wall condition between the patient and control groups with ‘short’ telomeres [PWV: 15.08±1.3 m/s vs, 10.7±0.5 m/s (p=0.015); IMT: 0.87±0.1 vs 0.78±0.1 (p=0.03)].
Diabetes mellitus (DM) is a chronic, noncommunicable disease that has reached epidemic proportions. Type 2 DM (T2DM) inevitably leads to the development of microvascular and macrovascular complications, which aggravate the course, worsen the prognosis of cardiovascular diseases (CVD) and lead to mortality.

A vast body of evidence indicates that age-related endothelial dysfunction and thickening and stiffening of the vascular wall create a metabolically and enzymatically active environment that contributes to the emergence or progression of vascular disease [1]. Several pathological processes contribute to the changes in the vascular wall: an increase in the collagen content accompanied by the formation of strong cross-links between fibres; fragmentation and a decrease in the elastin content; an increase in the concentrations of glycoxidation products and accumulation of advanced glycation end products (AGE) [2] and thickening of the intima–media complex (IMT) because of the accumulation of extracellular matrix proteins, collagen, glycosaminoglycans and smooth muscle cells and increased expression of adhesion molecules and monocyte adhesion to the endothelial surface [3].

Experimental data show that disturbances in carbohydrate metabolism contribute to accelerated changes in blood vessels. For example, Facchini et al. (2001) demonstrated that hyperinsulinemia may contribute to oxidative stress and thus accelerate age of blood vessels and the development of age-related diseases independently of hyperglycaemia [4]. In addition, insulin resistance (IR) is a predictor of atherosclerosis and CVD development independently of other risk factors, including high blood lipid content and high blood glucose in ageing endothelial cells, endothelial dysfunction and increased vascular wall stiffness [5]. Recently published studies demonstrate that increased vascular wall stiffness occurs in the initial stages of a carbohydrate metabolism disturbance, when IR is not yet accompanied by an increased blood glucose level [6, 7].

The different rates of ageing in patients with T2DM may be an inherently different level of ‘genetic protection’ of vessels from external factors. Some discoveries in vessel biology provide insight into the molecular mechanisms of ageing and allow us to prevent and slow down the arterial ageing process.

One of the most discussed genetic markers of ageing is the peripheral lymphocyte telomere length. Lymphocyte telomeres are the end portions of linear DNA molecules that have the repetitive nucleotide sequence TTAGGG. Telomeres protect linear chromosome ends from degrada-
the pathogenesis of changes in the cardiovascular system in patients with T2DM is highly relevant.

Aim

to study changes in arterial structure and function in relation to the peripheral lymphocyte telomere length and T2DM.

Materials and methods

Patients who previously underwent an outpatient examination at 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, who had no clinical manifestations of CVD and who contacted the NRCPM for preventive counselling.

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 with the goal to achieve the target HbA1c level; severe diabetic microangiopathy (proliferative and proliferative diabetic retinopathy and stage 3b, 4 or 5 chronic kidney disease); presence of CVD; 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 number 8, 29 November 2011, approved this study protocol.

All the patients received a standard clinical evaluation during screening: medical history; physical examination including height and weight measurements to calculate body mass index (BMI) and systolic (SBP) and diastolic blood pressure (DBP) measurement 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. In addition, rest and stress electrocardiograms (ECG) were recorded (treadmill test protocol BRUCE, Intertrack, Schiller, Miami, FL, USA). Patients with abnormalities on blood tests, heart rhythm and/or cardiac conduction on ECG and a positive stress test were excluded from the study.

Of the 158 patients screened, 99 met the inclusion criteria for the study. Additional tests were conducted on all study participants, including a carbohydrate metabolism evaluation; duplex scanning of carotid arteries to measure IMT and determine the presence of atherosclerotic plaques; measurement of carotid–femoral pulse wave velocity (PWV) and telomere length measurement.

Carbohydrate metabolism evaluation

The glucose concentration was measured for carbohydrate metabolism assessment using the glucose oxidase method on a SAPPHIRE-400 analyser using DiaSys diagnostic kits. The HbA1c level was measured by liquid chromatography on a Sapfire 400 (Niigata Mechatronics, Tokyo, Japan) analyser according to the manufacturer’s standard procedure.

Telomere length measurements

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

Arterial stiffness measurements

Applanation tonometry was used to measure carotid–femoral PWV for the vascular wall condition evaluation (SphygmoCor system, AtCor Medical, West Ryde, NSW, Australia). A high precision applanation tonometer 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 > 12 m/s was considered as an increase [1].

Evaluation of IMT and subclinical atherosclerosis

The Q-LAB special application program (Philips, Eindhoven, The Netherlands) was used for duplex scanning of extracranial brachiocephalic 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. sternoclidomastoideus. 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 visual maximum thickening for 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 ves-
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...levels in patients with T2DM were significantly (both p < 0.001) higher than those in the control group.

A significantly higher PWV (p < 0.001) and thickened IMT (p < 0.001) 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 lymphocyte telomere length. The median telomere length was 9.75. All the patients with a lymphocyte 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 carbohydrate metabolism parameters were compared with the telomere length in all the patients.

The severity of subclinical atherosclerosis and vascular stiffness was higher in patients with ‘short’ telomeres than in those with ‘long’ telomeres in both the 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 ath-

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### Table 1

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

Values are presented as mean ± SD.  
Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycated haemoglobin; FBG, fasting blood glucose.
erosclerosis 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). The results of this comparison are presented in Table 3.

Table 4 shows correlation analysis results for PWV and IMT with other parameters in participants with and without T2DM. A significant positive correlation was observed among PWV and SBP, IMT and HbA1c, while a significant negative correlation was detected between PWV and the relative lymphocyte telomere length in the T2DM group. IMT showed a significant positive correlation with SBP and a significant negative correlation with the relative lymphocyte telomere length.

A significant positive correlation was observed among PWV and age, SBP and IMT, while a significant negative correlation was detected between PWV and the relative lymphocyte telomere length in the control group. IMT showed a significant positive correlation with age and SBP.

Analysis of the relative lymphocyte telomere length in the T2DM group demonstrated an inverse correlation among the telomere length and HbA1c and PWV but no correlation with age, smoking status, BMI, FBG, IMT or the presence of atherosclerotic plaques (Table 5).

Multiple linear regression analysis with the relative lymphocyte 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 lymphocyte telomere length (Table 6).

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, 20, 21]. One of the possible explanations for increased vascular wall rigidity in patients with T2DM is AGE accumulation [20], which 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
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Table 6

<table>
<thead>
<tr>
<th>Parameter</th>
<th>β</th>
<th>Standard error</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>0.029</td>
<td>0.530</td>
<td>0.85</td>
</tr>
<tr>
<td>PWV, m/s</td>
<td>-0.15</td>
<td>2.721</td>
<td>0.037</td>
</tr>
<tr>
<td>FBG, mmol/l</td>
<td>-0.02</td>
<td>0.537</td>
<td>0.98</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>0.067</td>
<td>0.841</td>
<td>0.036</td>
</tr>
</tbody>
</table>

Therefore, in accelerated vascular wall ageing [19]. We demonstrated a correlation between arterial stiffness and HbA1c, which is the main indicator of carbohydrate 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 a study by Hovatta et al. [16]. However, Sampson et al. found no correlation between lymphocyte telomere shortening and carbohydrate metabolism indicators in a European study, possibly because of the small number of patients in that study [20]. Our results show not only significant differences in the HbA1c level between patients with T2DM and ‘long’ and ‘short’ telomeres but also a negative correlation between the lymphocyte 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 independent negative correlation between the telomere length and PWV and the independent positive correlation between the telomere length and HbA1c were the most important results of our study.

In other words, patients with T2DM and shorter telomeres are associated with stiffer vessels and poor diabetes control. The main reason for lymphocyte telomere shortening during the lifetime is oxidative stress and conditions associated with oxidative stress such as smoking, obesity, IR and chronic stress. Shortening of telomeres is accelerated in patients with T2DM because of the additional damaging effect of chronic hyperglycaemia and AGE accumulation. A strong correlation has been demonstrated between lymphocyte telomere shortening and the presence of T2DM [6]. Telomere shortening in patients with T2DM may be an important mechanism of blood vessel ageing and the development of diabetes-related CVD; however, this hypothesis requires further research and clarification.

Another important finding of our study is that the vascular wall status in patients with T2DM and ‘long’ lymphocyte telomeres was not significantly different from that in healthy individuals without T2DM. This finding indicates that a genetically greater telomere length may prevent accelerated vessel ageing in patients with a short diabetes duration (patients with established diabetes duration < 1 year were included). In contrast, patients with T2DM and ‘short’ lymphocyte telomeres demonstrated higher vascular stiffness and subclinical atherosclerosis severity despite a relatively short diabetes duration. Notably, participants with and without T2DM were comparable in age and SBP/DBP readings and ratio. Therefore, the influence of age and blood pressure on the lymphocyte telomere length was comparable. In other words, ‘short’ telomeres were associated with blood vessels that were more rigid 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 repairing damaged tissue and tissue differentiation and play an important role maintaining tissue homeostasis and ensuring the preservation of endothelial function. However, blood vessel stiffness is largely 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. It is also possible that the slow rate of telomere shortening affects the state of the matrix by some other mechanism and not by replicative activity.

Increasing evidence suggests that shortening of lymphocyte telomeres is a key component diminishing stem cell potential and age-related tissue degeneration, including increased vascular rigidity [20]. These findings have been established; however, the explanation is still lacking.

Conclusion

The correlation among T2DM, cellular ageing processes and the severity of subclinical morpho-functional changes in the vascular wall explains the higher incidence of CVD in patients with T2DM. Preventing these changes may help prevent CVD in patients with T2DM, particularly in patients with ‘short’ peripheral lymphocyte telomeres.

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