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An original biomarker for the risk of developing cardiovascular diseases and their complications: Telomere length

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ABSTRACT

Aim: The aim of this work was to study the effect of telomere length in the chromosomes of nuclear blood cells in individuals with coronary heart disease (CHD) on the development of cardiovascular complications (CVC).
Materials and methods: DNA was isolated from nuclear blood cells of 498 study participants. The telomere length was determined by real-time polymerase chain reaction. The investigation of each sample was repeated three times. Five years after the end of this study, a telephone survey of 119 patients with CHD was conducted in order to obtain data on the presence of CVC.
Results: According to the results obtained, a decrease in telomere length in patients with coronary heart disease increases the risk of subsequent development of cardiovascular complications.
Conclusion: Patients with coronary heart disease with shorter telomeres compared with conventionally healthy study participants had an increased risk of cardiovascular complications within 5 years after telomere analysis.

1. Introduction

The search for biomarkers which allow diagnosing various diseases and predicting the development of their complications is an important objective of modern medicine. This problem is especially relevant, both in general for cardiovascular diseases (CVD), and, in particular, for coronary heart disease (CHD). These pathologies are the most common among non-communicable diseases and are accompanied by significant disability of the population and high mortality resulting from complications. CHD is a multifactorial disease, the development of which is triggered by both genetic factors and lifestyle [1,2]. In addition, smoking, lack of physical activity, obesity and stress undoubtedly contribute to the development of cardiovascular diseases (CVD), obesity and stress all contribute to the development of CVD. One of the factors for the development of obesity and, as a consequence, development of diseases, connected with metabolic illnesses, is eating problems. It is shown that a correct diet, rich in fruit, vegetables, low in lipids and fast carbohydrates, has a cardioprotective effect [2,3]. At the same time, exposure to toxic substances leads to the occurrence and development of cardiovascular diseases and their risk factors. In particular, Abdulkareem A.O. et al. found cardiotoxic effects of oral exposure to untreated pharmaceutical effluent, which reduced Na+-K+-ATPase activity and decreased myocardial atrophy, therefore, drinking water contaminated with pharmaceutical effluent can promote the incidence of cardiovascular diseases [4]. Boyce G. R. et al. showed that the combined effects of welding fume inhalation and a high fat Western diet significantly altered the hepatic lipidome. Additionally, pulmonary exposure to welding fume alone increased lipid markers of inflammation [5]. Tsoukalas D. et al. found that urine organic acid levels related to the mechanisms of energy production and detoxification were associated with the presence of autoimmune diseases [6].

However, these factors do not fully explain the high prevalence of the

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disease. Telomere length can serve as one of the potential biomarkers of early diagnosis of coronary artery disease.

Telomeres are the ends of chromosomes (Fig. 1), composed of multiple repetitive six-nucleotide repeats [3]. Telomeres serve to maintain genome stability [7]. Somatic cell division is accompanied by natural shortening of telomeres due to terminal DNA underreplication [8]. When telomeres reach a critical length, the cell enters into replicative senescence, followed by apoptosis [9]. In addition, telomere shortening is associated with factors such as unfavorable environmental factors [10, 11], smoking [12], age-related diseases [13,14]; i.e. with states characterized by the intensification of oxidative stress [15,16]. Tsoukalas et al. found that regular consumption of omega-3 polyunsaturated fatty acids (PNFA), which can reduce inflammation and oxidative stress, associated with longer telomeres [17]. Oxidative stress can cause oxidative destruction of DNA molecules [18], to which tandem telomeric repeats are most susceptible [19,20]. In particular, mitochondrial dysfunction causes hyperproduction of reactive oxygen species (ROS) [21–25]. Such molecules damage DNA by oxidation of deoxyribose [26], this way contributing to shortening of telomeres [21], occurrence of mtDNA mutations and development of cardiovascular diseases [27–31], hypertension [32–36], neurodegenerative diseases [37], and also with such metabolic diseases as type 2 diabetes mellitus [35,38], chronic obstructive lung disease [35], arthritis [36] and different autoimmune diseases [40–42]. Besides, lifestyle of an individual can cause oxidative stress. For example, unhealthy eating [2]. In patients with these pathologies, shorter telomeres were found in comparison with conventionally healthy study participants [43–46]. However, it has not yet been established what is the root cause of this pathological process: shortening of telomere length is due to a disease, or shorter telomeres contribute to the development of age-related diseases. In case shorter telomeres of patients are one of the causes of disease, it would be useful to determine the size of telomeres associated with specific pathologies.

The aim of this work was to study telomere length in order to assess the possibility of using this parameter as a biomarker and/or predictor in the subsequent development of complications of diseases of the cardiovascular system.

2. Materials and methods

2.1. Study design

The study sample consisted of 498 study participants aged from 40 to 70 years (38 % of men), who were examined in National Medical Research Center of Cardiology. Material for this study was collected in accordance with the principles outlined in the Declaration of Helsinki and informed, written consent was obtained from each study participant.

Telomere length in nuclear blood cells was measured for all study participants. 5 years after the end of this study, a telephone survey of 119 patients with CVD was conducted in order to obtain data on the presence of cardiovascular complications: cardiovascular death, acute myocardial infarction (AMI), acute cerebrovascular accident (ACVI), which occurred in patients over the years.

To measure telomere length, whole blood was taken with subsequent isolation of DNA from nuclear cells. The kit for DNA isolation "DNA-Extran1" (CJSC "Syntol" Russia) was used.

The telomere length was determined by the method of quantitative real-time polymerase chain reaction (RT-PCR) using an amplifyer BIO-RADCFX 96 Real-Time System (Syngapore) [23–25]. The study of each sample was repeated three times. The calculation of the relative length of telomeres was carried out on the basis of the formula $2^{(-\Delta Ct)}$, $\Delta Ct = Ct_{telomeres}\text{-}Ct_{albumin}$, in which $Ct_{telomeres}$ – is a threshold cycle of a telomeric repeat, $Ct_{albumin}$ – is a threshold cycle of albumin gene. The albumin gene served as the internal control, relative to which the telomere length was determined. Results are presented as a percentage of the calibrator. DNA isolated from HeLa cells was used as a calibrator.

Since there were no significant age differences in all groups of study participants, normalisation of telomere length by age was not conducted.

2.2. Statistical processing of data

Data were statistically processed using the IBM SPSS Statistics 27.0 software. Results are presented as mean and error of the mean. To assess the difference in mean values in the compared groups, the Mann-Whitney test was used. Predictive significance was assessed by the area under the curve in ROC analysis. To predict the risk of development of cardiovascular complications, Cox regression, a model of proportional risks was used. To detect factors, which influence the length of telomeres, multiple-stage linear regression analysis was performed. To evaluate diagnostic significance of telomeric DNA length has a biomarker of cardiovascular complications in patients with CHD, a method of constructing ROC-curves was used for each parameter. Sensitivity and specificity of each of the constructed models was evaluated. Afterwards, power of the study was calculated. Statistically significant for each parameter were considered $p \leq 0{,}05$.

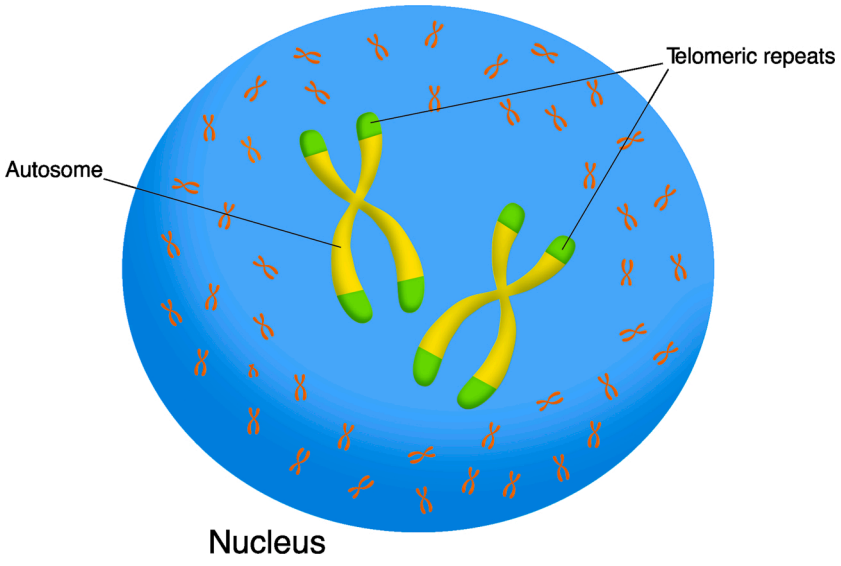

Fig. 1. Localization of telomeric repeats in the chromosomes of the cell nucleus.

3. Results

According to the results of clinical examination, all study participants were divided into three groups. The control group was formed of study participants with a low risk of developing coronary heart disease. It included 224 people (101 men and 113 women) aged from 40 to 70 years. The second group consisted of study participants without clinical manifestations of coronary heart disease, but with a high risk of its development and with an increased level of blood pressure (141 people). The third group included patients with coronary artery disease (133 people) and complications of this disease (myocardial infarction and acute cerebrovascular accident (ACVA). Demographical characteristics of study participants are presented in Table 1.

Statistically significant differences between study participants of the first and the second group and also between the first and the third groups were detected only for smoking status ($p \leq 0,05$) (Table 1).

The diagnosis of coronary artery disease was made based on the presence of a history of exertional angina and/or previous myocardial infarction or myocardial revascularization in anamnesis (Fig. 2). This Figure demonstrates that a decrease in telomere length with age occurs in all groups, regardless of the presence of the disease. Moreover, the length of telomeres in these groups turned out to be significantly smaller in patients with coronary artery disease, compared with conventionally healthy participants of the study.

Five years after the end of this study, a telephone survey of 119 patients with coronary artery disease was conducted in order to obtain data on the presence or absence of cardiovascular complications. The age of these study participants ranged 53–70 years. It was found that 21 patients had myocardial infarction, 7 people had ACVA, in 6 cases there was cardiovascular death (Table 2).

In patients with no CVC over the next five years, the initial mean telomere length was 60.8 ± 5.5 relative units (rel. units). In the study participants with rescued event of CVCs, the initial mean telomere length was 51.6 ± 6.5 relative units (Fig. 2) ($p \leq 0,048$). Therefore, the initial telomere length in CHD patients without CVC was significantly higher by 15 % than in patients with subsequent cardiovascular incidents.

A decrease in telomere length is observed at all CVC. The greatest shortening of telomeres was found in the group of individuals with a lethal outcome within 5 years after measuring telomere length. Comparison of the average telomere length between patients with the absence and presence of cardiovascular complications over the next five years showed that in individuals with MI, the average telomere length is shorter by 12 %, and with ACVI it was shorter by 15 %, and in the group with fatal outcomes it was shorter by 19 % (Fig. 3).

The authors of the article carried out a multifactor regression analysis to assess the influence of some parameters on the length of telomeres. As a dependent variable, relative length of telomeres was used, and as independent variables, the severity of a cardiovascular disease, age, IMT, burdened family anamnesis, smoking, the presence of type 2 diabetes mellitus, the level of systolic and diastolic arterial pressure, lipid profile (total cholesterol, triglycerides, lipoproteins of high and low density), the presence of cardiovascular complications. The initial linear regression model, containing all the above listed parameters, explained the variability of relative length of telomeres $r = 0.503$, $R^2 = 0.253$ ($P < 0,001$). In the process of gradual exclusion of less significant independent variables to obtain a prognostic linear regression model, the following qualities have left: the severity of a cardiovascular disease, burdened family anamnesis and smoking ($r = 0.446$, $R^2 = 0.199$ ($P < 0,001$). It should be noted that the exclusion of severity of a cardiovascular disease led to a reduction in explanatory possibilities of a model ($r = 0,341$, $R^2 = 0,196$ ($P < 0,001$)).

To assess the diagnostic significance of the length of telomeric DNA as a biomarker of CVC in patients with coronary artery disease, ROC curves were made for each investigated parameter (Fig. 4).

The analysis of the ROC curve revealed that the area under the curve for a biomarker such as telomere length was 0.66 ± 0.06 (95 % CI; 55.4–75.7; $p = 0.006$). Consequently, the model was significant. With the critical value of the relative telomere length equal to 58.4 relative units, the sensitivity of the method was 74.3 % (95 % CI; 56.7–87.5), specificity was 57.1 % (95 % CI 43.2–70, 3). Statistical power of the study was 0.75. The relation of probability for development of cardiovascular incidents was 3.8 ($P < 0.05$). Therefore, for study participants aged 53–70 years with a telomere length equal to or less than 58.4, the probability of CVC was increased by 3.8 times.

Table 1
Demographical characteristics of study participants: conditionally healthy individuals (group 1), people without clinical manifestations of coronary heart disease (CHD), but with high risk of its development (group 2) and people who have CHD (group 3).

Demographical characteristics	Group 1	Group 2	Group 3
Body mass index (kg/m^2)	28.4 ± 4.9	30 ± 4.2	30.0 ± 3.7
Systolic arterial pressure (mm Hg)	129.1 ± 17.8	139.8 ± 16.4	139.9 ± 17.0
Diastolic arterial pressure (mm Hg)	74.1 ± 8.1	85.2 ± 8.2	83.6 ± 9.1
Family anamnesis (%)	34.1	32.2	33.1
Total cholesterol (mg/dL)	5.7 ± 0.9	6.2 ± 1.0	6.4 ± 1.1
Triglycerides (mg/dL)	1.9 ± 0.7	2.0 ± 0.8	2.2 ± 0.7
Low density lipoproteins (mg/dL)	3.5 ± 0.8	3.8 ± 0.8	3.9 ± 0.9
High density lipoproteins (mg/dL)	1.4 ± 0.4	1.4 ± 0.3	1.4 ± 0.3
Smoking (%)	13.5	17.8	17.5
Diabetes (%)	10.1	11.6	12.7

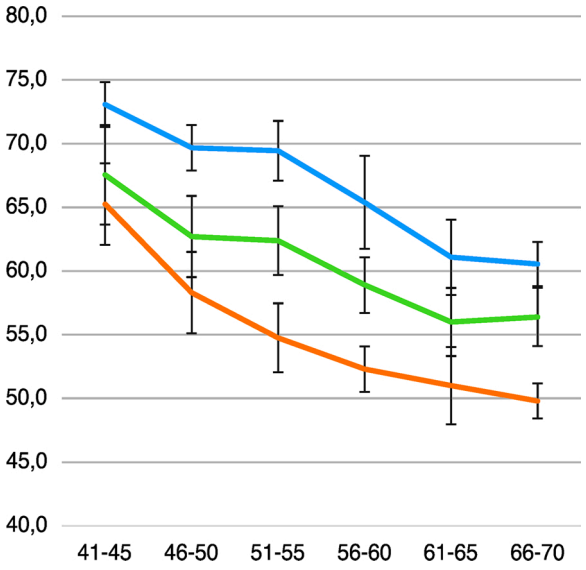

Fig. 2. The average telomere length depends on the age of conventionally healthy study participants (green curve), in patients without clinical manifestations of coronary heart disease, but with a high risk of its development (yellow curve), and in patients with coronary heart disease (red curve) * P < 0.05.

Table 2
Telomere length in blood leukocytes of conditionally healthy study participants (group 1), patients without clinical manifestations of coronary artery disease, but with a high risk of its development (group 2) and in patients with ischemic heart disease (group 3).

Group number	Number of persons in a group	Age	The relative length of the telomere
1	224	54 ± 1,5	67 ± 1
2	141	59 ± 1,38	62 ± 1,2
3	133	61 ± 1,3	57 ± 1,2

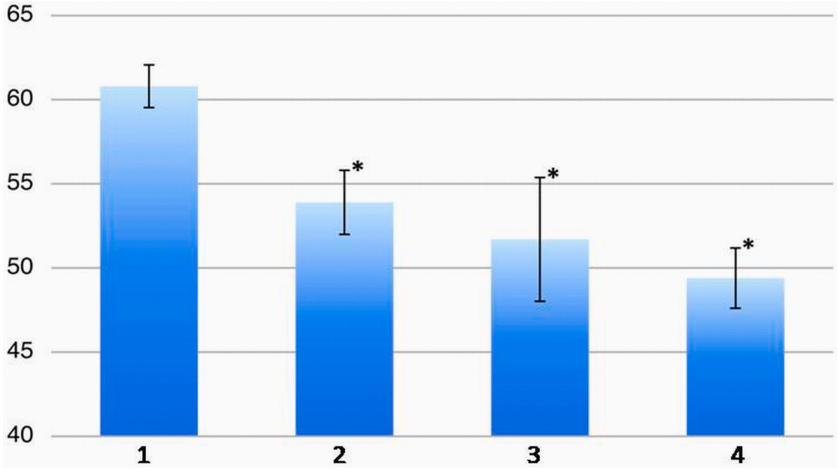

Fig. 3. Average values of the length of telomeric chromosome repeats in the group of respondents with various cardiovascular events which occurred within 5 years after the examination and without events: 1.No complications; 2. MI; 3. ACVI; 4. Cardiovascular death. Remark: * $P < 0.05$ compared with patients without CVC.

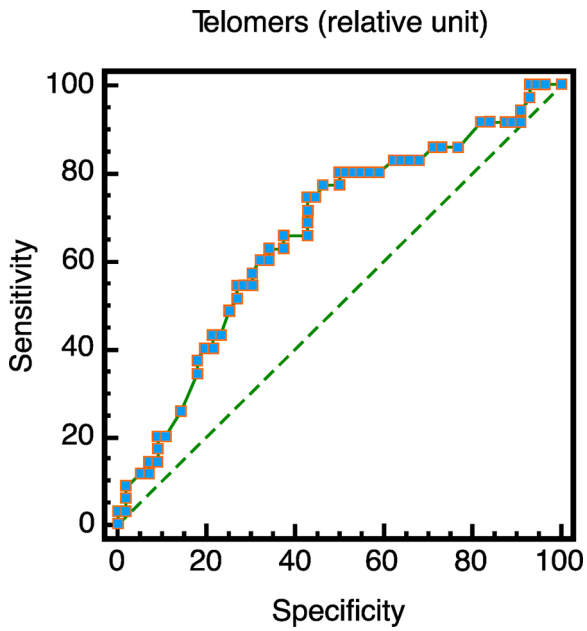

Fig. 4. ROC curve for determining the predictive value of telomere length in the development of cardiovascular complications. Area under the curve was 0.66; sensitivity was 73.3, specificity was 58.1 with telomere length less than 58.4 relative units.

To predict the risk of development of cardiovascular complications in patients with CHD and to assess the influence of independent variables on this risk, a model of proportional risks (Cox regression) was used. As an event, the occurrence of cardiovascular complications was considered. As independent variables, relative length of telomeres and classic risk factors were used: age, IMT, smoking, burdened family anamnesis, the presence of high blood pressure, the level of systolic and diastolic arterial pressure, lipid profile (total cholesterol, triglycerides, and lipoproteins of high and low density).For this analysis, a period was used from the examination of a patient to the occurrence of cardiovascular complications in years. Significant links were detected for two independent variables: the length of telomeres and burdened family anamnesis. This tandem of variables was associated with the occurrence of cardiovascular complications. Value Exp(B) for variable «relative length of telomeres» was detected to be equal to 0.9 ($P < 0{,}001$), i.e. the risk for development of complications when the length of telomeres was shorter than 57 relative units has an increase of 10 % each year. Value Exp(B) for variable «family anamnesis» turned out to be equal to 1.5 ($P < 0.001$), i.e. for patients with burdened family anamnesis the risk was 1.5 times higher than for people without such a family anamnesis.

4. Discussion

It is necessary to underline that the search of biomarkers for diagnostics and prediction of different diseases is a very relevant issue in the 21-st century. In particular, a promising biomarker may be the level of miRNA expression, as these molecules play an important role in many cellular processes, such as proliferation, apoptosis and differentiation [46–48]. It is shown that miRNA take part in the development of different pathologies: barrenness, diseases of cardiovascular system, neurological illnesses and oncological diseases [49,50]. However, the use of miRNA as diagnostic and therapeutic biomarkers in the clinical field is a very hard task.

Telomere length is considered a potential marker of biological aging of an organism, as it predictably shortens with age. At the same time, the change in telomere length, in addition to the terminal DNA under-replication, is influenced by many factors, for example, chronic inflammatory processes, extreme climatic conditions, and lifestyle [51]. Due to this, the length of telomeres can more objectively reflect the current state of the patient. In recent years, many studies have been carried out to identify the relationship between short telomere length and cardiovascular diseases [52,53]. Most authors have shown that short telomeres are associated with an increased risk of MI, CHD, atherosclerosis and other pathologies [39,41,52]. At the same time, many scientists showed an association of short telomeres with diseases, which are not connected with cardiovascular system, such as male and female sterility [44], obesity [54], stress [55], different oncological [56] and psychical diseases [57]. For the same nosologies, hyperproduction of reactive oxygen species was shown, i.e. intensification of free non-bound radicals processes. In this regard, it can be assumed that short length of telomeres is associated with the occurrence of oxidative stress. In its turn, oxidative stress can lead to the development of pathological processes in human organism.

It may be supposed that in the framework of one nosology, the length of telomeres may be used as a biomarker for prediction of development of a disease and its complications. After analyzing the obtained data, it was found that with such complications as ACVI, MI and cardiovascular death, the length of telomeres is significantly reduced.

Our research group decided to investigate the relationship between telomere length and the risk of complications in cardiovascular diseases. It was found that in study participants with ischemic heart disease, aged

53–70 years, had 12–15 % shorter telomeres, compared to patients with CHD, who had no documented cardiovascular complications (CVC). Such a size of telomeres increased the risk of development of cardiovascular complications 3.8 times. If the length of telomeres in such individuals decreases by 19 %, then the probability of cardiovascular death in the next 5 years increases significantly. It is necessary to note, that in the investigation by Zhang Y. et al., it was found that in a group of patients with atherosclerosis telomeres were 9 % shorter compared to a control group [58]. The group of scientists detected that in patients with coronary heart disease telomeres were 10 % shorter compared to control subjects [59]. These data confirm the results, obtained by our research group.

Therefore, the data obtained make it possible to use telomere length as a prognostic biomarker for predicting complications in diseases of the cardiovascular system.

5. Conclusion

According to the results of our study, patients with coronary heart disease with shorter telomeres compared with conventionally healthy study participants had an increased risk of cardiovascular complications during5 years after conducting telomere analysis. It possible to use telomere length as a prognostic biomarker for predicting complications in diseases of the cardiovascular system.

This work can be useful for doctors, medical geneticists and scientists engaged in research in the field of cardiovascular pathologies.

Conflict of Interest

The authors declare no conflict of interest.

CRediT authorship contribution statement

Natalya A. Doroschuk: Investigation, Validation, Formal analysis, Data curation, Writing - original draft. **Anton Yu Postnov:** Methodology, Formal analysis, Resources, Supervision. **Alexander D. Doroschuk:** Formal analysis, Software. **Anastasia I. Ryzhkova:** Investigation, Data curation. **Vasily V. Sinyov:** Investigation, Validation. **Marina D. Sazonova:** Investigation, Writing - original draft. **Victoria A. Khotina:** Visualization, Writing - original draft. **Alexander N. Orekhov:** Project administration, Supervision. **Igor A. Sobenin:** Supervision. **Margarita A. Sazonova:** Conceptualization, Methodology, Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

The authors report no declarations of interest.

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Glossary

CVD: Cardiovascular disease
CHD: Coronary heart disease
CVC: Cardiovascular complications
AMI: Acute myocardial infarction
ACVI: Acute cerebrovascular accident
MI: Myocardial infarction
RT-PCR: Real-time polymerase chain reaction
DNA: Deoxyribonucleic acid
CI: Confidential interval