ТЕОРЕТИЧНА І ЕКСПЕРИМЕНТАЛЬНА МЕДИЦИНА

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O.M. Bilovol, V.D. Nemtsova

Kharkiv National Medical University

EFFECT OF CARBOHYDRATE METABOLISM LEVELS ON THE RELATIVE LENGTH OF TELOMERES IN PATIENTS WITH HYPERTENSION AND IN COMBINATION WITH DIABETES MELLITUS TYPE 2

In 156 patients with stage II arterial hypertension, including 96 of them with diabetes mellitus type 2, the relationship between the relative telomeres length of blood leukocytes (RLTL) and buccal epithelium cells (RBTL) and different levels of carbohydrate metabolism have been determined and data in patients with arterial hypertension combined with diabetes mellitus type 2 and in patients with isolated hypertension have been compared. Carbohydrate metabolism parameters, blood pressure levels were evaluated. RLTL and RBTL were determined by a real time quantitative polymerase chain reaction. It is shown that a hypertension and diabetes mellitus type 2 combination was accompanied by greater significant RLTL ($p=0.009$) and RBTL ($p=0.001$) more than with isolated hypertension. In the patients with hypertension and diabetes mellitus type 2 the relative length of telomeres was assessed in the carbohydrate metabolism compensation and in its absence and no convincing data was obtained on the effect of glycemic control on the relative length of telomeres. In patients with isolated hypertension it was found that those with a lower glucose level than that within the risk factor (5.6–6.9 mmol/l) had a shorter mean relative telomeres length both of blood leukocytes ($p=0.05$) and the buccal epithelium cells ($p=0.03$) regarding indicators for those who had higher blood glucose levels. Patients with a comorbid course of hypertension and diabetes mellitus type 2 had more pronounced decrease in the RTL. Achieving glycemic control at hypertension and diabetes mellitus was accompanied by a significant increase in the RBTL. The relative length of blood leukocytes telomere was controversial. Additional determining the RBTL enhances the diagnostic and prognostic power when assessing the efficacy of correcting glycemic control in high cardiovascular risk patients.

Keywords: *hypertension, diabetes mellitus type 2, relative telomere length, carbohydrate metabolism.*

Introduction

Telomeres are gene sequences present at chromosomal ends and are responsible for maintaining genome integrity. Telomere length is considered as a biomarker of chronological aging. This age associated decrease in the telomere length is linked to various age-associated diseases like diabetes, hypertension, dyslipidemia, atherosclerosis etc., their complications and poor cardiovascular disease prognosis [1, 2]. Furthermore, a number of studies indicate that telomere attrition is positively correlated with diabetes and diabetic complications such as diabetic nephropathy [3]. There are studies which shows that the average telomere length of circulating leukocytes is shorter in hypertensive patients than in normotensive subjects [4].

Modern studies of telomere length dynamics also show that age-related diseases, in addition to synergistic effects, affect telomere length in-

dependently of one another. There are more and more reports that premature vascular aging, as measured by telomere length reduction, may be due to factors associated with the metabolic status changes.

Impact on modified risk factors, the most significant among them being considered blood pressure levels, the presence of dyslipidemia, overweight, carbohydrate metabolism disorders, is one of the strategic tasks of treating patients with the established diagnosis of cardiovascular disease, including arterial hypertension. It is believed that telomere length can be considered as a marker of cardiovascular aging [5], but its role as a marker of cardiovascular prognosis and quality control of the disease course remains to be clarified.

To study the length of telomeres, it is customary to use blood leukocytes, because can provide reliable data for determining the relative length of telomeres in other tissues [6] and shortening of telomeres in peripheral blood leukocytes can serve as a marker of the systemic pathological stress. In recent years, more and more scientific works have appeared, in which much attention is paid to the use of buccal epithelium as an alternative source of biological material for telomeric test due to the non-invasive procedure of material sampling [6, 7]. Now there is insufficient data on the length of telomeres, including that in the buccal epithelium, in the presence of the combined age-associated diseases, on the influence of various risk factors on the relative length of telomeres, including that of the Ukrainian population. Data on the effect of different levels of carbohydrate metabolism control on telomere length in the blood leukocytes and buccal epithelial cells in comorbid course of arterial hypertension and diabetes mellitus type 2 has not been found.

The purpose of this study was to determine the relationship between the relative telomeres length of blood leukocytes and buccal epithelium and different levels of carbohydrate metabolism in patients with hypertension in combination with diabetes mellitus type 2 in comparison to the patients with isolated hypertension.

Materials and methods

The study included 156 patients (62 men and 94 women), the average age is (62.66±4.21) years, with hypertension stage II, disease duration of (10.2±3.7) years. 96 of them had diabetes mellitus type 2, disease duration of (4.1±2.4) years. The control group consisted of 22 volunteers, identical to the examined patients by sex and age, and without cardiovascular and endocrinological pathology.

For the patients selection, the arterial hypertension diagnostic criteria, agreed with the ESC/ERS Guidelines for the diagnosis and treatment of arterial hypertension (2013) were applied [8]. The diagnosis of diabetes mellitus type 2 has been established in compliance with the international recommendations of the American Diabetes Association and the European Association for the Study of Diabetes [9].

Against the background of dietary recommendations, all patients received basic therapy in accordance with international and national guidelines for the management of patients with the relevant pathology [8, 9]. The patients with symptomatic arterial hypertension, diabetes mellitus type 1 and other endocrinological disorders, clinical signs of coronary heart disease or severe concomitant chronic diseases did not include in the study.

All patients were calculated anthropometric indices (height, body weight, body mass index calculation using the standard Quetelet formula). Arterial pressure level was recorded as the arithmetic mean, three measurements being carried out with the 2-minutes intervals in a sedentary position on the dominant hand.

Laboratory studies included the determination of glucose concentration (performed by the glucose oxidation method using the «Humolizer» analyzer, Germany). The concentration of blood serum insulin levels, the glycated hemoglobin (Hb_{A1c}) level were measured using the Hummer reagents kit (USA) by means of the immunoassay method. To determine insulin resistance (IR), the HOMA-IR index was used, which was calculated according to the formula

$$\text{HOMA-IR} = ((\text{fasting glucose, mmol/ml}) \times (\text{fasting insulin, } \mu\text{IU/ml})) / 22.5.$$

Telomere length was measured on a CFX96 touch real-time polymerase chain reaction detection system (Bio-Rad Laboratories) in DNA isolated from blood leukocytes and buccal epithelium using the DNA-sorb blood kit (Amplisens, RF). We used a modified monochrome multiplex quantitative polymerase chain reaction method [10], as described previously [11].

Statistical data processing was carried out using the computer software SPSS 21.0. To determine the nature of the data obtained

distribution, the Shapiro–Vilk criterion was used. When performing the statistical analysis, quantitative and qualitative variables were used. Qualitative data were presented as percentages; quantitative data were presented in the form of the mean and standard error (M±m).

Testing the significance of differences between the two groups was carried out using the Student's t-test. The frequency of traits in the groups was compared using the Pearson χ^2 test. The differences were statistically significant at $p<0.05$. When studying the influence of risk factors on the telomeres relative length, single-factor analysis of variance was used. Testing the hypothesis of dispersion homogeneity was performed using the Levene's test.

The work was performed in compliance with the basic provisions of the World Medical Association Declaration of Helsinki on ethical principles for medical research involving human subjects (1964–2000) and MOH of Ukraine Order № 690 dated September 23, 2009. The study was approved by the Bioethics Commission at the Kharkiv National Medical University in accordance with the principles set forth in the Helsinki Declaration.

Results

The chronologic age of the included patients did not have significant differences in the study design condition (*table 1*).

A comparative analysis of carbohydrate metabolism indices has expectedly revealed reliable differences in the studied groups of patients from the control group in almost all indices. Between groups 1 and 2, reliable differences were observed for fasting glucose levels ($p<0.001$), Hb_{A1c} ($p<0.001$) and HOMA-IR index ($p<0.001$). The levels of both systolic blood pressure (SBP) and diastolic blood pressure (DBP) in patients of groups 1 and 2 reliably differed from those in the control group. The presence of comorbid pathology was accompanied by higher rates of SBP and DBP than with isolated hypertension, however, reliable differences were only observed for SBP ($p=0.012$).

Patients with isolated hypertension showed a significant shortening of the telomere relative length, which is consistent with the results of other studies [4, 12]. The presence of combination of hypertension and diabetes mellitus type 2 was accompanied by an even greater shortening relative telomere length, reaching significant differences both in the buccal epithelium cells ($p=0.001$) and in blood plasma leukocytes ($p=0.009$).

To clarify the effect of different levels of carbohydrate control on the relative length of telomeres, we assessed the impact of the achieved target carbohydrate levels under the influence of

Table 1. Comparative characteristics of anthropometric and carbohydrate indices (M±m)

Index	Control (n=22)	Group 1 (n=60)	Group 2 (n=96)	Significance (p)
Chronologic age, years	58.30±1,96	60.59±1.37	62.66±4.21	*p>0.05 p_{1-2}>0.05
BMI, kg/m^2	22.12±2.51	29.01±0.90*	30.03±0.89*	*p<0.001
Fasting glucose, mmol/l	4.62±1.08	5.45±0.12	8.90±0.50	p_{1-2}<0.001
Hb_{A1c}, %	4.74±1.10	6.13±0.11*	7.61±0.19*	p_{1-2}<0.001 *p=0.001
Insulin fasting, μIU/ml	9.80±1.16	17.87±1.78	21.45±2.07*	*p<0.001
HOMA-IR	2.23±0.36	4.40±0.51*	8.07±0.68*	p_{1-2}<0.001 *p<0.001
SBP, mmHg	125.05±3.86	138.95±2.83*	146.65±2.74*	$p_{1-2=}$0.012 *p=0.001
DBP, mmHg	78.19±7.01	86.71±1.62	90.05±1.59*	*p<0.001
RLTL (T/S)	1.66±0.52	1.27±0.08	0.97±0.08*	p_{1-2}=0.009 *p=0.005
RBTL (T/S)	2.33±0.51	1.26±0.08*	0.90±0.08*	$p_{1-2=}$0.001 *p<0.001

Notes: 1. RLTL – relative blood leukocytes telomeres length (T/S); RBTL – relative buccal epithelium cells telomeres length (T/S); BMI – body mass index.

2.* Changes are reliable compared with the control group.

antidiabetic therapy on the relative telomeres length.

The relative telomeres length was assessed in the carbohydrate metabolism compensation and in its absence and no convincing data was obtained on the effect of glycemic control on the relative length of telomeres (*table 2*).

homogeneity of the statistical populations dispersions was tested using the Levene's test. Meanwhile, if the level P of Levene's test significance is less than 0.05, then the obtained difference in dispersions for the samples is unlikely to be the result of the research process randomness.

Table 2. Comparative characteristics of relative telomeres length in dependence of achieving target levels of carbohydrate metabolism in patients with hypertension and diabetes mellitus type 2 (M±m)

Index	RLTL (T/S)		RBTL (T/S)	
	with compensation	without compensation	with compensation	without compensation
Fasting glucose, mmol/l	0.83±0.06	0.96±0.03	0.95±0.04	0.78±0.06*
Hb_{A1c}, %	0.82±0.03	1.04±0.05*	1.03±0.03	0.60±0.04*

Notes: 1. RLTL – relative blood leukocytes telomeres length (T/S); RBTL – relative buccal epithelium cells telomeres length (T/S).

2. * p<0,05; changes are reliable compared with achieving target levels in appropriate subgroup and index.

In the case of carbohydrate compensation a longer relative telomere length was observed in the buccal epithelium cells, while in the blood leukocytes there was a shortening of the relative telomere length at the levels of fasting glucose close to normal and elongation at the Hb_{A1c} levels more than 7.5 %. The data of relative telomere length in the buccal epithelium cells in the absence of compensation of carbohydrate metabolism were significant as for fasting glucose, as for Hb_{A1c} ($p<0.05$).

When carrying out a similar analysis of carbohydrate metabolism among patients of group 1 it was found that a distribution of patients in group 1 according to the level of fasting glucose was accompanied by a paradoxical situation: those with a lower glucose level than that within the risk factor (5.6–6.9 mmol/l) had a shorter mean relative telomeres length both of blood leukocytes ($p=0.05$) and the buccal epithelium cells ($p=0.03$) in comparison with those who had higher blood glucose levels, which requires further study.

To determine the effect of carbohydrate metabolism levels changing in two directions: reaching the target levels and not reaching them, on the relative length of telomeres, the variance analysis which was carried out according to the traditional scheme, was used. The homogeneity of the dispersions between samples is the main prerequisite for the possibility of carrying out the dispersion analysis. The hypothesis about the

Performing the variance analysis in group 1 was not revealed a significant effect of different glucose levels on the distribution of the telomeres relative length both in the buccal epithelium and blood leukocytes.

The dispersion analysis in group 2 revealed the presence of a reliably significant effect of the levels of fasting glucose ($p=0.029$) and Hb_{A1c} ($p=0.034$) on the relative telomere length in the buccal epithelium cells. A reliably significant effect of glycemic control on the relative telomere length in the blood leukocytes was not found.

Discussion

Diabetes mellitus and essential hypertension are common conditions that are frequently present together. Both are considered risk factors for cardiovascular disease and microvascular complications and therefore treatment of both conditions is essential. In our work, we expectedly received shorter telomeres in patients with comorbid pathology compared with isolated hypertension, which is a confirmation of the assumption that hypertention together with diabetes mellitus greatly aggravate the processes of vascular aging, increasing the cardiovascular risk.

It is known that the acquiring the target values of blood pressure in patients with diabetes mellitus is no less important for predicting the further course of the disease and the development of its complications than the achievement of the target level of glycemic control [13, 14]. According

T. Mengden et al. (2017) study results a total of 13.9 % of patients with combined course of hypertension and diabetes mellitus type 2 in Germany were deemed to have controlled blood pressure (ambulatory blood pressure monitoring) [14]. We not seemed that we have a better situation in Ukraine. This may partly explain the contradictory results obtained in our work. It is possible that the achievement of blood pressure control, other factors that we did not take into account in our study, have a significantly more reliable impact on telomere length than the state of glycemic control. However, the presence of significant differences in the relative length of telomeres in buccal epithelium cells at glycemic levels close to normal allows us to speak about telomere length as a possible marker for given comorbidity course control.

Conclusions

1. In patients with combined course of hypertension and diabetes mellitus type 2 the telomeres relative length shortening was more pronounced than at isolated hypertension and normotensive individuals.

2. Achieving glycemic control at hypertension and diabetes mellitus was accompanied by a significant increase in the relative length of telomeres in the buccal epithelium cells. The relative length of blood leukocytes telomere was controversial.

3. Additional determining the telomeres relative length in buccal epithelial cells enhances the diagnostic and prognostic power when assessing the efficacy of correcting glycemic control in high cardiovascular risk patients.

Perspectives

To clarify the effect of carbohydrate metabolism on the factors of vascular aging, including telomere length, further research on a larger sample of patients is needed, taking into account various methods of diabetic and hypertensive control.

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А.Н. Беловол, В.Д. Немцова

ВЛИЯНИЕ СОСТОЯНИЯ УГЛЕВОДНОГО ОБМЕНА НА ОТНОСИТЕЛЬНУЮ ДЛИНУ ТЕЛОМЕР ПРИ ИЗОЛИРОВАННОЙ АРТЕРИАЛЬНОЙ ГИПЕРТЕНЗИИ И В СОЧЕТАНИИ С САХАРНЫМ ДИАБЕТОМ 2-ГО ТИПА

У 156 пациентов с артериальной гипертензией II стадии, в том числе у 96 из них с сахарным диабетом 2-го типа, определяли взаимосвязь между относительной длиной теломер лейкоцитов крови (ОДТЛ) и клеток буккального эпителия (ОДТБ) и различными уровнями углеводного обмена и сравнивали показатели у пациентов с сочетанной с сахарным диабетом 2-го типа и изолированной гипертензией. Измеряли показатели углеводного обмена, уровни артериального давления, ОДТЛ и ОДТБ определяли количественной полимеразной цепной реакцией в реальном времени. Показано, что комбинация артериальной гипертензии и сахарного диабета 2-го типа сопровождалась более значимым ОДТЛ (p=0,009) и ОДТБ (p=0,001), чем изолированная артериальная гипертензия. У пациентов с артериальной гипертензией и сахарным диабетом 2-го типа относительную длину теломер оценивали при компенсации углеводного обмена и без нее. Убедительных данных, касающихся влияния гликемического контроля на относительную длину теломер, не получено. У больных изолированной артериальной гипертензией с более низким уровнем глюкозы, чем в пределах фактора риска (5,6–6,9 ммоль/л), выявлено укорочение средней относительной длины теломер как лейкоцитов крови (p=0,05), так и клеток буккального эпителия (p=0,03) относительно показателей у тех, у кого уровень глюкозы в крови был выше. У пациентов с коморбидным течением артериальной гипертензии и сахарного диабета 2-го типа установлено более выраженное снижение относительной длины теломер, чем у больных изолированной артериальной гипертензией. Достижение гликемического контроля при артериальной гипертензии и сахарном диабете 2-го типа сопровождалось достоверным увеличением ОДТБ. Относительная длина теломер лейкоцитов крови носила противоречивый характер. Дополнительное определение ОДТБ повышает диагностическую и прогностическую силу при оценке эффективности коррекции гликемического контроля у пациентов с высоким сердечно-сосудистым риском.

Ключевые слова: *артериальная гипертензия, сахарный диабет 2-го типа, относительная длина теломер, углеводный обмен.*

О.М. Біловол, В.Д. Немцова

ВПЛИВ СТАНУ ВУГЛЕВОДНОГО ОБМІНУ НА ВІДНОСНУ ДОВЖИНУ ТЕЛОМЕР ПРИ ІЗОЛЬОВАНІЙ АРТЕРІАЛЬНІЙ ГІПЕРТЕНЗІЇ І В ПОЄДНАННІ З ЦУКРОВИМ ДІАБЕТОМ 2-ГО ТИПУ

У 156 пацієнтів з артеріальною гіпертензією II стадії, у тому числі у 96 з них із цукровом діабетом 2-го типу визначали взаємозв'язок між відносною довжиною теломер лейкоцитів крові (ВДТЛ) та клітин букального епітелію (ВДТБ) і різними рівнями вуглеводного обміну та порівнювали показники в пацієнтів із поєднаною з цукровим діабетом 2-го типу та ізольованою гіпертензією. Вимірювали показники вуглеводного обміну, рівні артеріального тиску, ВДТЛ та ВДТБ визначали кількісною полімеразною ланцюговою реакцією в реальному часі. Показано, що комбінація артеріальної гіпертензії і цукрового діабету 2-го типу супроводжувалась більш значущою ВДТЛ (p=0,009) і ВДТБ (p=0,001), ніж у хворих на ізольовану гіпертензію. У пацієнтів з артеріальною гіпертензією та цукровим діабетом 2-го типу відносну довжину теломер оцінювали при компенсації вуглеводного обміну й без неї. Переконливих даних щодо впливу глікемічного контролю на відносну довжину теломер не отримано. У пацієнтів з ізольованою гіпертензією з більш низьким рівнем глюкози, ніж у межах

фактора ризику (5,6–6,9 ммоль/л), виявлено укорочення середньої відносної довжини теломер як лейкоцитів крові (p=0,05), так і клітин букального епітелію (p=0,03) відносно показників у тих, у кого рівень глюкози в крові був вище. У пацієнтів з коморбідним перебігом артеріальної гіпертензії і цукрового діабету 2-го типу встановлено більш виражене зниження відносної довжини теломер, ніж у хворих на ізольовану артеріальну гіпертензію. Досягнення глікемічного контролю при артеріальній гіпертензії й цукровому діабеті 2-го типу супроводжувалось достовірним збільшенням ВДТБ. Відносна довжина теломер лейкоцитів крові носила суперечливий характер. Додаткове оцінювання ВДТБ підвищує діагностичну й прогностичну силу при оцінюванні ефективності корекції глікемічного контролю в пацієнтів з високим серцево-судинним ризиком.

Ключові слова: *артеріальна гіпертензія, цукровий діабет 2-го типу, відносна довжина теломер, вуглеводний обмін*.

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Відомості про авторів

Біловол Олександр Миколайович – доктор медичних наук, професор, професор кафедри клінічної фармакології та внутрішньої медицини Харківського національного медичного університету.

Адреса: 61022, м. Харків, пр. Науки, 4, ХНМУ.

Тел.: +38(050)406-81-23.

E-mail: valeriyan@ukr.net.

ORCID: https://orcid.org/0000-0002-7003-4551.

Немцова Валерія Даниілівна – кандидат медичних наук, доцент, доцент кафедри клінічної фармакології та внутрішньої медицини Харківського національного медичного університету.

Адреса: 61022, м. Харків, пр. Науки, 4, ХНМУ.

Тел.: +38(050)406-81-23.

E-mail: valeriyan@ukr.net.

ORCID: https://orcid.org/0000-0001-7916-3168.