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LEUKOCYTE TELOMERE LENGTH AND CLINICAL CHARACTERISTICS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS WITH AND WITHOUT CHRONIC KIDNEY DISEASE

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Background. *Telomeric mechanisms are considered important contributors to chronic kidney disease progression in patients with type 2 diabetes mellitus, although data on telomere length in diabetic kidney disease remain limited.* ***Objective.*** *To evaluate the relationship between telomere length and clinical characteristics in patients with type 2 diabetes mellitus with and without chronic kidney disease.* ***Methods.*** *The study included 100 patients with T2DM, divided into two groups: 50 with and 50 without CKD. Routine clinical and biochemical blood tests were performed for all subjects. Leukocyte telomere length was assessed by quantitative real-time polymerase chain reaction following the method described by Cawthon.* ***Results.*** *T2DM patients with CKD were significantly older, had a longer duration of diabetes, exhibited significantly lower estimated glomerular filtration rate (eGFR), higher urine albumin-to-creatinine ratio and frequency of cardiovascular complications compared with non-CKD patients. No significant correlations were found between telomere length and age, eGFR, albuminuria, or cardiovascular disease in either group. In patients with type 2 diabetes mellitus chronic kidney disease was associated with higher frequency of pathologically short telomeres (20.8%) versus non-CKD patients (2.1%), suggesting accelerated cellular aging in CKD independent of chronological age.* ***Conclusions.*** *Shortened telomeres in patients with type 2 diabetes mellitus and chronic kidney disease may reflect accelerated cellular aging and could serve as an additional marker for biological risk stratification beyond traditional renal indicators.*

K e y w o r d s: telomeres, type 2 diabetes mellitus, chronic kidney disease, cellular aging.

Chronic kidney disease (CKD) is one of the most significant global healthcare challenges, affecting more than 850 million people worldwide and associated with decreased quality of life, high treatment costs, and increased cardiovascular mortality [1]. According to WHO, CKD ranks among the top ten causes of death and, by 2040, is projected to become the fifth leading cause [2]. In Ukraine and other Eastern European countries, CKD prevalence is also rising, due to increasing rates of diabetes, hypertension, and population aging.

One of the main etiological causes of CKD is diabetic kidney disease (DKD), affecting up to 40% of patients with type 2 diabetes mellitus (T2DM) [3]. Despite the introduction of modern glucose-lowering and nephroprotective strategies, a substantial proportion of patients continue to experience progressive DKD.

Key mechanisms in CKD pathogenesis, especially in T2DM, include activation of the renin–angiotensin–aldosterone system, chronic inflammation, oxidative stress, epigenetic alterations, and impaired cellular regeneration [4,5]. Oxidative stress, in particular, triggers a cascade of renal cell injury - from activation of pro-inflammatory factors (NF-κB, TGF-β) to fibrosis and reduced nephron reserve [6].

In recent years, research attention has increasingly focused on molecular markers of biological aging – particularly telomeres. Telomeres are nucleoprotein structures at chromosome ends that ensure genomic stability and limit the number of cell divisions. Their progressive shortening reflects

cellular aging and is considered a hallmark of senescence [7]. Moreover, studies have confirmed elevated levels of senescence markers (γ-H2AX, p21, p16INK4a) in kidney tissue of CKD patients, underscoring the role of telomeric mechanisms in disease pathogenesis [10]. These changes may precede clinical alterations, such as reduced eGFR, and provide opportunities for early diagnosis.

Investigating telomere length as a biomarker associated with biological aging in patients with T2DM and CKD may help improve risk stratification, predict disease progression, and develop personalized therapeutic strategies. Importantly, telomeres are increasingly considered as therapeutic targets for emerging classes of drugs – geroprotectors and senolytics [11].

Patients with diabetes and CKD demonstrate accelerated telomere shortening, which correlates with lower eGFR, albuminuria, microvascular complications, and even mortality [8, 9]. However, available data on telomere length in T2DM with CKD remain fragmented.

Thus, studying the association between telomere length and CKD in patients with T2DM is highly relevant for understanding molecular mechanisms of the disease, improving early diagnostics, refining risk stratification, and identifying new therapeutic targets.

Despite growing interest in telomere biology in chronic kidney disease, data from Eastern European populations remain limited. This study provides evidence derived from a clinically well-characterized cohort of Ukrainian patients with type 2 diabetes mellitus, representing a population with a high burden of cardiometabolic risk and underrepresentation in international molecular aging research. By integrating clinical phenotyping with PCR-based telomere length assessment and age-adjusted analysis, the present work contributes region-specific data that may improve understanding of biological aging processes in CKD within real-world clinical settings. Our study aimed to assess the relationship between telomere length and clinical characteristics in patients with T2DM with and without CKD.

Materials and Methods

The study included 100 patients with T2DM, divided into two groups based on CKD status: 50 with CKD and 50 without. CKD was defined as eGFR <60 ml/min/1.73 m^2 (CKD-EPI) and/or urine albumin-to-creatinine ratio (ACR) ≥30 mg/g. In the non-CKD group, eGFR was ≥ 60 ml/min/1.73 m^2 and ACR < 30 mg/g.

All participants provided fasting venous blood samples following a minimum fasting period of eight hours. Routine clinical and biochemical blood tests were performed for all subjects. Plasma glucose levels were measured using an enzymatic glucose oxidase assay on a biochemical analyzer. Glycated hemoglobin (HbA1c) was determined by an immunochemical method employing an automated analytical system. Serum creatinine concentrations were obtained, and the estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI equation in accordance with the KDIGO 2024 recommendations. Urinary albumin excretion was evaluated by measuring the albumin-to-creatinine ratio (ACR) in a first-morning spot urine sample, analyzed with the URiSCAN Optima system. Chronic kidney disease was diagnosed in individuals presenting with an eGFR below 60 ml/min/1.73 m^2 and/or evidence of albuminuria.

Anthropometric assessment was conducted for all participants. Body weight and height were recorded using calibrated electronic scales and a portable stadiometer. Body mass index (BMI) was subsequently calculated as body weight in kilograms divided by the square of height in meters.

Leukocyte telomere length (LTL) was assessed by quantitative real-time polymerase chain reaction (qPCR) following the method described by Cawthon. DNA was isolated from PBMCs by standard phenol–chloroform extraction. The qPCR reaction mix was prepared with a commercial reagent kit (Solis BioDyne) and included betaine (Sigma, USA) at a final concentration of 1 M. Telomere primers (telg and telc) were used at a final concentration of 450 nM each, and albumin primers (albu and albd) at 250 nM each, in a master mix. List of primers used for MMqPCR. telg: 5′–ACA CTA AGG TTT GGG TTT GGG TTT GGG TTT GGG TTA GTG T–3′; telc: 5′–TGT TAG GTA TCC CTA TCC CTA TCC CTA TCC CTA TCC CTA ACA–3′; albd: 5′–GCC CGG CCC GCC GCG CCC GTC CCG CCG GAA AAG CAT GGT CGC CTG TT–3′; albu: 5′–CGG CGG CGG GCG GCG CGG GCT GGG CGG AAA TGC TGC ACA GAA TCC TTG–3′. Thermal cycling included: 12 min at 95°C; two cycles of 15 s at 94°C, 15 s at 49°C; 32 cycles of 15 s at 94°C, 10 s at 62°C, 15 s at 74°C with signal acquisition, 10 s at 84°C, and 15 s at 88°C with signal acquisition. Calibration curves were generated using four concentra-

tions of reference DNA, prepared by serial dilution, in duplicates covering a 27-fold range. DNA samples were analyzed in triplicates, and amplification curves were generated using an Opticon Monitor 3 Real-Time PCR System (Bio-Rad, Hercules,CA, USA). PCR was performed using a Bio-Rad Chromo4 thermal cycler (USA, Hercules). Amplification curves were generated using Opticon Monitor 3 software. LTL was expressed as the T/S ratio, the ratio of telomere repeats copy number (T) to single-copy gene (scg) copy number (S).

The study was conducted in accordance with the Declaration of Helsinki (WMA, 2013) and Ukrainian Ministry of Health Order No. 690 (23.09.2009). The Ethics Committee of Shupyk National Healthcare University of Ukraine approved the protocol (No. 11/7, 29.11.2024).

Informed consent. Written informed consent was obtained from all participants before enrollment in the study. All participants were informed about the purpose, procedures, potential benefits, and risks of the study and voluntarily agreed to participate. Confidentiality of personal and medical information was maintained throughout the study.

Statistical analysis was performed using Microsoft Excel.

Quantitative variables were presented as median (25^{th}; 75^{th} percentile), categorical variables as absolute numbers and percentages. Due to distribution asymmetry, quantitative variables were compared using the Mann-Whitney U test, and categorical variables by χ^2 or Fisher's exact test. Associations between telomere length and clinical parameters were evaluated with Pearson correlation; independent predictors were assessed using multiple linear regression. Statistical significance was set at $P < 0.05$.

Results

In accordance with the study objectives, patients with type 2 diabetes mellitus were stratified into two clinical groups based on the presence of chronic kidney disease. The first group included 50 patients with T2DM and CKD, while the second group comprised 50 patients with T2DM without evidence of CKD. This stratification enabled the evaluation of clinical, metabolic, and renal differences between groups, as well as their potential association with telomere length parameters.

As summarized in Table 1, patients with T2DM and CKD were significantly older than those without CKD and had a longer duration of diabetes. Body mass index and glycemic control, assessed by HbA1c levels, did not differ significantly between the groups. In contrast, renal function parameters showed marked differences: patients with CKD exhibited significantly lower eGFR values and higher urine albumin-to-creatinine ratios compared with non-CKD patients (Fig. 1). A more detailed characterization of cardiovascular comorbidities revealed a higher burden of macrovascular disease among patients with CKD. In this group, 34 individuals presented with cardiovascular pathology, including previous myocardial infarction (n = 8), prior coronary artery stenting due to obstructive atherosclerosis (n = 12), history of coronary artery bypass grafting (n = 3), cerebrovascular events (n = 4), and peripheral arterial disease (n = 3). In the non-CKD group, cardiovascular disease was documented in 17 patients and included prior myocardial infarction (n = 3), coronary artery stenting (n = 8), coronary artery bypass grafting (n = 1), cerebrovascular events (n = 3), and peripheral arterial disease (n = 2). All participants with cardiovascular disease received standard secondary prevention therapy, including antiplatelet treatment, statins (rosuvastatin in 80% and atorvastatin in 20% of cases), and antihypertensive medications according to current clinical practice.

Glucose-lowering therapy differed between groups in accordance with renal function status. In the non-CKD group, all patients received metformin at a therapeutic dose of 2000 mg/day. Additionally, half of the participants (n = 25) were treated with sodium–glucose cotransporter-2 inhibitors (SGLT2i), specifically dapagliflozin or empagliflozin. In the CKD group, SGLT2i therapy (dapagliflozin or empagliflozin) was administered to 25 patients. Metformin was prescribed to a smaller proportion of individuals (n = 20) at a reduced dose of 1000 mg/day, reflecting clinical recommendations to limit its use in the context of decreased estimated glomerular filtration rate.

Gender distribution was comparable between groups, with equal proportions of women and men.

Although mean telomere length was shorter in CKD patients (0.56 [0.38; 0.74]) compared with non-CKD (0.89 [0.60; 1.40]), the difference was not statistically significant (P = 0.21). No significant correlations were found between telomere length and age, eGFR, albuminuria, or CVD in either group (all $P > 0.05$).

Linear regression analysis of age vs telomere length showed no significant relationship in ei-

Table 1. Clinical and biochemical characteristics of patients with type 2 diabetes mellitus stratified by chronic kidney disease status

Parameters	CKD	non-CKD	*P* value
Age, years	71 [65–78]	52 [47–56]	<0.001
Female sex, *n* (%)	20 (40%)	20 (40%)	1.00
Male sex, *n* (%)	30 (60%)	30 (60%)	1.00
Diabetes duration, years	6 [2–15]	3 [2–5]	0.02
BMI, kg/m^2	32.8 [28.7–36.2]	32.5 [26.9–36.7]	0.21
HbA1c, %	6.7 [6.1–7.7]	6.1 [5.6–7.5]	0.32
eGFR, ml/min/1.73 m^2	46 [36–53]	80 [73–89]	<0.00001
ACR, mg/g	28 [7.2–56]	5.4 [2.7–11.1]	<0.00001
Cardiovascular disease, *n* (%)	30 (60%)	17 (34%)	0.009
Telomere length (T/S)	0.56 [0.38–0.74]	0.89 [0.60–1.40]	0.21

Note. Quantitative variables were presented as median (25th; 75th percentile), categorical variables as absolute numbers and percentages; *n* = 50

ther group (*P* = 0.598 and *P* = 0.645, respectively; R^2 = 0.006 and 0.005).

Residual values (ΔT/S) were calculated to normalize telomere length for age. CKD patients had significantly lower ΔT/S compared with non-CKD (z = –2.11; *P* = 0.035) (Fig. 2). Pathologically short telomeres (Z < –1) were found in 20.8% of CKD patients versus 2.1% of non-CKD patients (*P* = 0.0076), suggesting accelerated cellular aging in CKD independent of chronological age (Fig. 3).

To further evaluate independent predictors of telomere length, a multiple linear regression model was constructed including age, eGFR, albuminuria, BMI, diabetes duration, HbA1c, cardiovascular disease status, metformin use, and SGLT2 inhibitor therapy. The model explained 51% of the variability in telomere length, indicating that the included clinical variables collectively contributed significantly to telomere length variation (R^2 = 0.51; *P* < 0.001).

Within this multivariable framework, reduced eGFR remained independently associated with shorter telomere length (*P* < 0.05), whereas diabetes duration did not demonstrate a significant association with telomere length (*P* > 0.05).

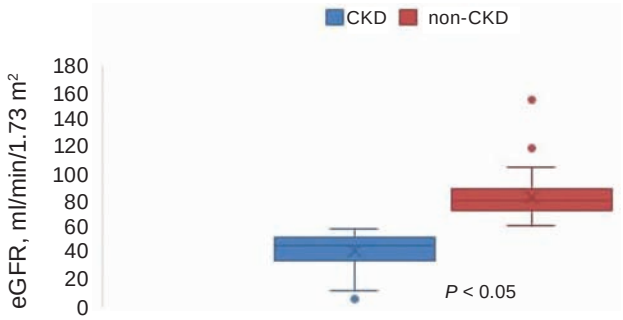

Fig. 1. Estimated glomerular filtration rate (eGFR) in patients with and without chronic kidney disease

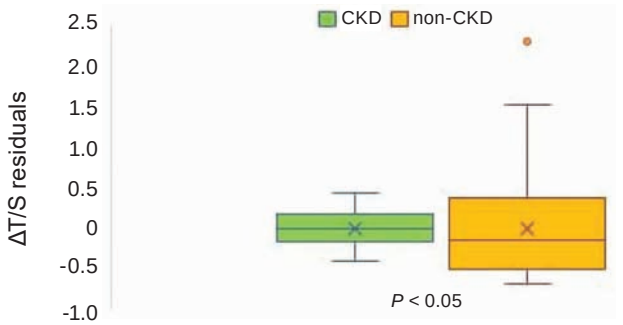

Fig. 2. Age-adjusted telomere length (ΔT/S) in CKD and non-CKD patients

Discussion

Although absolute telomere length did not differ significantly between groups, age-adjusted analysis revealed significantly shorter residual telomere length (ΔT/S) in patients with T2DM and CKD. This suggests alterations in telomere dynamics beyond chronological aging effects [12].

No significant associations were observed between telomere length and metabolic parameters (HbA1c, BMI), suggesting that telomere variation in this cohort may be more closely related to CKD status than to glycemic or anthropometric factors. Nevertheless, causal interpretation is limited by

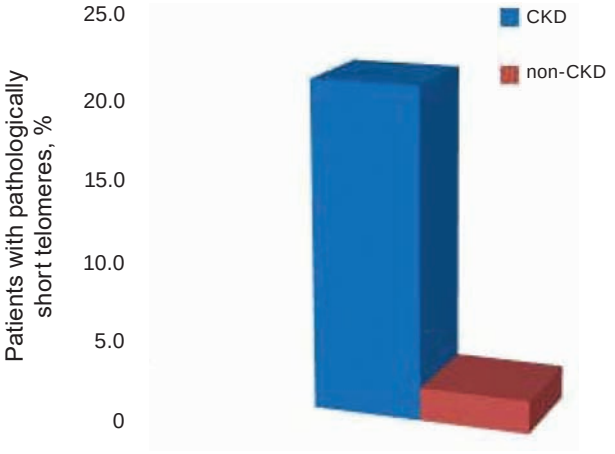

Fig. 3. Prevalence of pathologically short telomeres in CKD and non-CKD patients

the cross-sectional design and potential residual confounding.

Our approach of analyzing residual telomere length (ΔT/S) corrected for age effects offers a more refined assessment of "pathological" telomere attrition [13]. Patients with CKD had a longer duration of type 2 diabetes, which could potentially influence telomere dynamics. To address this, diabetes duration was included as a covariate in the multiple linear regression model evaluating independent predictors of telomere length. In this multivariable framework, SGLT2 inhibitor use remained the only significant independent predictor, while diabetes duration did not demonstrate a significant association with telomere length. Future longitudinal studies should further explore the interaction between diabetes duration, CKD progression, and telomere attrition.

These findings suggest that the observed telomere differences between CKD and non-CKD groups are unlikely to be explained solely by longer diabetes duration, although residual confounding cannot be fully excluded due to the cross-sectional study design.

While the present study was not designed to evaluate health-economic outcomes, the use of age-adjusted telomere residuals (ΔT/S) may have potential clinical relevance. Identification of patients exhibiting disproportionately shortened telomeres relative to age could support improved biological risk stratification beyond traditional renal markers such as eGFR and albuminuria. This may facilitate earlier recognition of individuals with increased vulnerability to systemic complications and guide closer monitoring or inclusion in preventive or interventional programs.

From a translational perspective, incorporating molecular aging markers into patient characterization may contribute to future personalized management frameworks rather than immediate cost reduction. Further longitudinal and cost-effectiveness studies are required to determine whether telomere-based stratification can influence clinical decision-making or healthcare resource utilization in CKD populations.

An additional key observation was the potential link between telomere dynamics and cardiovascular complications. Telomere shortening has been associated with ischemic heart disease, heart failure, and atrial fibrillation, supporting the concept of a "cardio-renal aging continuum" [14].

Emerging evidence also suggests that certain drug classes (e.g., SGLT2 inhibitors, metformin) may exert anti-senescence effects and preserve telomere length [15]. Although our study was not interventional, the pharmacological modulation of telomere stability warrants further investigation.

Strengths of this study include detailed patient characterization, age-adjusted analysis, and PCR-based telomere measurement.

Limitations include the cross-sectional design, lack of longitudinal data, and relatively small sample size.

Overall, our results underscore the need to integrate telomere analysis into long-term observational studies in T2DM and CKD, particularly in early disease stages.

Conclusions.

1. After adjustment for age, patients with T2DM and CKD demonstrated significantly shorter residual telomere length (ΔT/S) compared to patients with T2DM without CKD, indicating altered telomere dynamics beyond chronological aging effects.

2. The prevalence of pathologically short telomeres ($Z < -1$) was significantly higher in CKD patients, suggesting involvement of telomere-related mechanisms in disease pathophysiology.

3. CKD was associated with an increased burden of cardiovascular events, supporting the concept of a cardio-renal continuum and emphasizing the systemic context of telomere alterations.

4. Telomere length analysis, particularly using age-adjusted residuals (ΔT/S), may represent a promising tool for biological risk stratification and

personalized prognostic assessment in T2DM patients with CKD.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

Author contributions. Y.E. Rebrova: patient recruitment, data collection, data interpretation, manuscript drafting, and preparation of the final version of the manuscript. Y.A. Saienko: critical revision of the manuscript for important intellectual content and editing of the manuscript. K.K. Midlovets: statistical analysis, data interpretation. B.M. Mankovsky: study conception and design, supervision of the research project.

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ДОВЖИНА ТЕЛОМЕР ЛЕЙКОЦИТІВ ТА КЛІНІЧНІ ХАРАКТЕРИСТИКИ У ПАЦІЄНТІВ ІЗ ЦУКРОВИМ ДІАБЕТОМ 2 ТИПУ З ХРОНІЧНОЮ ХВОРОБОЮ НИРОК ТА БЕЗ НЕЇ

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Вступ. Теломерні механізми розглядаються як важливі чинники прогресування хронічної хвороби нирок у пацієнтів із цукровим діабетом 2 типу, однак дані щодо довжини теломер за діабетичного ураження нирок залишаються обмеженими. **Мета.** Оцінити взаємозв'язок між довжиною теломер та клінічними характеристиками у пацієнтів із цукровим діабетом 2 типу за наявності та відсутності хронічної хвороби нирок. **Методи.** У дослідження включено 100 пацієнтів із цукровим діабетом 2 типу, яких було розподілено на дві групи: 50 пацієнтів із хронічною хворобою нирок та 50 пацієнтів без неї. Усі пацієнти пройшли стандартне клінічне та біохімічне обстеження. Довжину теломер лейкоцитів визначали методом кількісної полімеразної ланцюгової реакції в реальному часі відповідно до методики Cawthon. **Результати.** Пацієнти з цукровим діабетом 2 типу та хронічною хворобою нирок були достовірно старшими, мали більшу тривалість діабету, нижчу розрахункову швидкість клубочкової фільтрації, вищий рівень співвідношення альбумін/креатинін сечі та більшу частоту серцево-судинних ускладнень порівняно з пацієнтами без хронічної хвороби нирок. У жодній із груп не виявлено достовірної кореляції між довжиною теломер та віком, швидкістю клубочкової фільтрації, альбумінурією або наявністю серцево-судинних захворювань. Водночас у пацієнтів із цукровим діабетом 2 типу та хронічною хворобою нирок значно частіше виявляли патологічно короткі теломери (20,8 проти 2,1% у групі без хронічної хвороби нирок), що може свідчити про прискорене клітинне старіння незалежно від хронологічного віку. **Висновки.** Скорочення довжини теломер у пацієнтів із цукровим діабетом 2 типу та хронічною хворобою нирок може відображати процеси прискореного клітинного старіння та потенційно розглядатися як додатковий маркер біологічного ризику поряд із традиційними показниками функції нирок.

Ключові слова: теломери, цукровий діабет 2 типу, хронічна хвороба нирок, клітинне старіння.

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