UDC 616.379-008.64 : 616.61 : 575.1 doi: https://doi.org/10.15407/ubj97.02.041

MITOCHONDRIAL DNA COPY NUMBER IN LEUKOCYTES OF PATIENTS WITH TYPE 2 DIABETES MELLITUS AND CHRONIC KIDNEY DISEASE

Y. A. SAIENKO[1,2]✉, D. S. KRASNENKOV[1], K. K. MIDLOVETS[1], V. V. KORCHEVA[1], Y. E. REBROVA[1,3], D. D. YEPISHYNA[1], B. M. MANKOVSKY[1]

[1]SI "D.F. Chebotarev Institute of Gerontology, National Academy of Medical Sciences of Ukraine", Kyiv;
[2]SI "Center for Cardiology and Cardiac Surgery of the Ministry of Health of Ukraine", Kyiv
[3]P.L. Shupyk National University of Health Care of Ukraine, Kyiv;
✉e-mail: ysaenko1981@gmail.com

Received: 04 April 2025; **Revised:** 23 April 2024; **Accepted:** 25 April 2025

Chronic kidney disease (CKD) as one of the most common complications of type 2 diabetes mellitus (T2DM) significantly increases the risk of cardiovascular disease and mortality. Mitochondrial dysfunction, in particular a reduction in mitochondrial DNA copy number (mtDNA-CN), plays an important role in the development of diabetic complications, including nephropathy. The aim of this study was to determine the mtDNA-CN in peripheral blood leukocytes of patients with T2DM depending on the presence of CKD. A total of 109 individuals were examined, including 20 healthy controls and 89 patients with T2DM divided into groups based on the presence or absence of CKD. The mtDNA-CN in leukocytes was determined using quantitative real-time PCR. Biochemical markers of T2DM and CKD were evaluated, non-parametric tests and correlation analysis were performed. No statistically significant differences in mtDNA-CN level were observed between patients with T2DM and CKD, patients with T2DM without CKD, and the control group ($P > 0.05$). No associations between mtDNA-CN and kidney function parameters were identified. The absence of mtDNA-CN alterations is assumed to contribute to the relatively satisfactory glycemic control in diabetic groups.

K e y w o r d s: type 2 diabetes mellitus, chronic kidney disease, mitochondrial DNA, mtDNA copy number, leukocytes.

Chronic kidney disease (CKD) is a common complication of type 2 diabetes mellitus (T2DM) and plays a key role in increasing cardiovascular risk and mortality among diabetic patients [1]. According to epidemiological studies, CKD develops in 30–40% of individuals with diabetes, highlighting the importance of investigating its pathogenesis and progression mechanisms [2]. Despite modern advances in the treatment and prevention of CKD, it remains the leading cause of end-stage renal disease and the need for renal replacement therapy worldwide [3].

Mitochondrial dysfunction is recognized as a critical factor in the pathogenesis of diabetes and its complications, particularly kidney involvement. One of the key biomarkers of mitochondrial function is mitochondrial DNA copy number (mtDNA-CN) in leukocytes. A decreased mtDNA-CN may indicate impaired energy metabolism, increased oxidative stress, and chronic inflammation [4-8].

Previous studies have demonstrated reduced mtDNA-CN levels in leukocytes in diabetes and its complications, including retinopathy, cardiomyopathy, and nephropathy [9]. The kidneys are highly energy-dependent organs due to the reabsorption processes that require substantial ATP. Although kidneys represent less than 1% of total body mass, they consume nearly 10% of the body's oxygen required for cellular respiration. Accordingly, the kidneys contain a high number of mitochondrial DNA copies. Mutations or reductions in mtDNA-CN can lead to bioenergetic deficiencies associated with CKD development [9].

Mitochondrial DNA is present in all nucleated cells (as well as platelets) and is particularly abundant in cells with high-energy needs, such as kidney

cells. Measurement of mtDNA-CN in blood leukocytes can indirectly reflect mtDNA-CN in various tissues, including the kidneys [10]. Studies investigating mtDNA-CN in patients with CKD suggest its potential prognostic value for adverse clinical outcomes. Some findings indicate that reduced mtDNA-CN is correlated with a decline in glomerular filtration rate (GFR) and elevated serum creatinine in patients with CKD; however, results remain inconsistent [11]. These discrepancies may be due to differences in patient populations, methods for mtDNA-CN quantification, and other mitochondrial function-modifying factors.

A reduced mtDNA-CN has also been associated with an increased risk of mortality and infections in large CKD cohorts [12]. Determining mtDNA-CN levels in the blood leukocytes of patients with T2DM and CKD may help elucidate the underlying pathophysiological mechanisms and could serve as a potential biomarker for disease progression. Moreover, mtDNA-CN might be considered a predictive factor for CKD development, enabling a more personalized treatment approach and early identification of individuals at high risk of renal dysfunction [8, 11, 13].

However, current evidence regarding the role of mtDNA-CN in CKD development remains inconclusive and warrants further investigation.

The aim of the study was to determine the mtDNA copy number in blood leukocytes of patients with T2DM depending on the presence of CKD.

Materials and Methods

The study was conducted in accordance with ethical standards and the principles of the Declaration of Helsinki. All participants received detailed written information about the study prior to inclusion. The study protocol, patient information sheet, and informed consent form were reviewed and approved by the Institutional Ethics Committee (protocol No 1 of 10.01.2022). All individuals included in the study voluntarily signed informed consent forms before participation.

A total of 109 individuals were enrolled in the study, including 89 patients with type 2 diabetes mellitus (T2DM) and 20 healthy individuals in the control group.

All subjects underwent fasting venous blood sampling after at least eight hours of fasting. General clinical and biochemical blood analyses were performed. Plasma glucose concentration was determined using the glucose oxidase method on a biochemical analyzer. Glycated hemoglobin (HbA1c) was measured using an immunochemical method on an automated analyzer. Serum creatinine levels were assessed, and the estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI formula in accordance with KDIGO 2024 guidelines. The urine albumin-to-creatinine ratio (ACR) was measured in a morning spot urine sample using the URiSCAN Optima device. A diagnosis of CKD was established in patients with an eGFR <60 ml/min/1.73 m² and/or the presence of albuminuria [15].

Anthropometric measurements were performed on all participants. Body weight and height were measured using calibrated electronic scales and a portable stadiometer. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared [16]. Systolic (SBP) and diastolic (DBP) blood pressure were measured in a seated position at rest [17].

MtDNA-CN in peripheral blood leukocytes was determined using quantitative polymerase chain reaction (qPCR). The leukocyte fraction was isolated from venous blood by centrifugation, followed by DNA isolation by the phenol-chloroform method [18]. Quantification was performed by real-time qPCR according to the method developed by Hsieh [19]. Fragments of the mitochondrial displacement loop (D-loop) and albumin (ALB) were used as mtDNA and nuclear DNA sequences. PCR was performed using the "HOT FIREPol EvaGreen qPCR Mix Plus" kit (Solis BioDyne, Estonia). PCR was carried out using a Bio-RAD Chromo 4 thermocycler. Amplification curves were generated with the OpticonMonitor 3 software.

For all variables under investigation, the median (Me) and interquartile range (QI-QIII) were calculated. For the clinical and demographic characteristics of the patients, the arithmetic mean and standard deviation were used. Normality was assessed using the Kolmogorov-Smirnov and Lilliefors tests for experimental and control groups. For subgroups based on sex, normality was tested using skewness and kurtosis coefficients (As and Ex) for samples up to 50 data points, and the Shapiro–Wilk test for samples with fewer than 30 data points. Sample homogeneity was evaluated using the Kruskal-Wallis H-test and the Mann-Whitney U-test (for diabetes duration). Group comparisons were made using the Mann–Whitney U-test (for unpaired samples) [20]. For experimental and control groups, the Jonckheere–Terpstra test was also applied [21, 22].

Differences were considered statistically significant at a *P*-value < 0.05.

Statistical analysis and data visualization were performed using OriginPro 2016 32-bit (OriginLab Corporation, USA).

Results

In accordance with the study objective, all patients with T2DM were divided into two clinical groups based on the presence of CKD. The first group included 65 patients with T2DM and CKD. The second clinical group consisted of 24 individuals with T2DM without signs of CKD. This grouping enabled us to assess potential differences in mtDNA-CN levels and their association with renal function in patients with T2DM. The control group comprised individuals without T2DM or CKD. As shown in Table 1, patients with T2DM and CKD were significantly older compared to those without CKD and the control group. The duration of diabetes was also significantly longer in the CKD group than in patients without CKD. There were no significant differences in body weight, BMI, systolic or diastolic blood pressure between the groups. Patients with type 2 diabetes had a significantly lower height compared to individuals in the control group. Blood glucose and HbA1c levels were significantly higher in both T2DM groups compared to the control group; however, no significant differences were observed between patients with and without CKD. Renal function parameters differed significantly between groups: patients with T2DM and CKD had higher serum creatinine levels, lower eGFR, and elevated urine albumin-to-creatinine ratio. Notably, the mean eGFR in the CKD group was 44.15 ± 11.66 ml/min/1.73 m², corresponding to CKD stages G3a-b. Patients with CKD stages 4 - 5 were not included in the study. In the group of patients with T2DM and CKD, 6 individuals (9%) received insulin therapy, while 59 (91%) were treated with oral glucose-lowering medications. Among patients with type 2 diabetes without chronic kidney disease, 2 (8%) individuals were administered insulin, and 22 (92%) were treated with oral glucose-lowering agents.

Figure presents mtDNA-CN levels in leukocytes of the three study groups: control, T2DM without CKD, and T2DM with CKD. The box-plot chart compares mtDNA-CN values in peripheral blood leukocytes with individual data points indicated. The red boxes represent patients with T2DM (with and without CKD), while the green box indicates the control group.

The mtDNA-CN values in the three groups were: 1.29 ± 0.60 in the control group, 1.28 ± 0.36

Table 1. Clinical and demographic characteristics of patients with T2DM depending on the presence of CKD compared to the control group (M ± SD)

Parameters	Control (*n* = 20; man – 16, woman – 4)	T2DM without CKD (*n* = 24; man – 17, woman – 7)	T2DM with CKD (*n* = 65; man – 40, woman – 25)
Age, years	50.30 ± 3.81	52.95 ± 6.88	71.08 ± 8.08*#
Diabetes duration, years	–	4.91 ± 5.09	9.74 ± 7.31#
Body weight, kg	94.10 ± 24.29	97.04 ± 22.07	93.09 ± 16.78
Height, cm	174.60 ± 9.24	170.75 ± 8.11	167.70 ± 8.72*
BMI, kg/m²	30.65 ± 7.73	32.50 ± 4.92	33.30 ± 6.46
SBP, mm Hg	131.55 ± 11.66	136.63 ± 17.45	133.28 ± 18.84
DBP, mm Hg	82.35 ± 7.75	83.63 ± 9.45	80.69 ± 10.34
Plasma glucose, mmol/l	5.72 ± 0.40	9.19 ± 3.98*	10.14 ± 3.95*
HbA1c, %	5.46 ± 0.32	7.66 ± 2.26*	7.75 ± 1.72*
Creatinine, μmol/l	100.26 ± 22.09	88.74 ± 14.55*	145.03 ± 87.80*#
eGFR, ml/min/1.73 m²	83.04 ± 22.84	81.96 ± 12.20	44.15 ± 11.66*#
Urine albumin-to-creatinine ratio (ACR)	6.85 ± 4.81	10.29 ± 4.64	36.89 ± 74.34*#

Note: *$P < 0.05$ compared to the control group; #$P < 0.05$ compared to the group of patients with T2DM without CKD

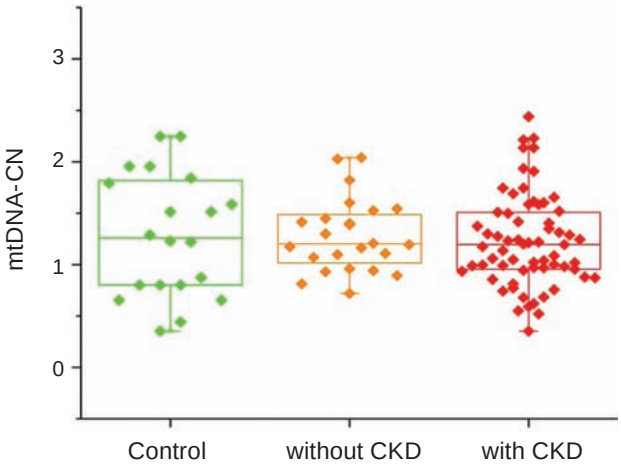

Fig. mtDNA-CN values in patients with T2DM and CKD, T2DM without CKD, and the control group

in T2DM without CKD, and 1.23 ± 0.45 in T2DM with CKD. According to the Kruskal–Wallis H-test, no statistically significant differences were observed between the groups ($P > 0.05$), indicating no association between mtDNA-CN levels and CKD status in patients with T2DM.

Discussion

In this study, we analyzed the potential association between mitochondrial DNA copy number (mtDNA-CN) in peripheral blood leukocytes and the presence of chronic kidney disease (CKD) in patients with T2DM. While some published studies have reported a link between reduced mtDNA-CN and declining kidney function, we did not observe such an association in our cohort.

Several authors have described a decrease in mtDNA-CN in patients with metabolic disorders, including T2DM and CKD [12]. Some studies have reported a significant reduction in mtDNA-CN in women with T2DM and CKD compared to healthy controls, suggesting that mitochondrial biogenesis might be suppressed or mitochondrial degradation increased due to chronic metabolic stress and systemic inflammation. These findings support the hypothesis that mitochondrial dysfunction contributes to the pathogenesis of the cardiorenal syndrome [23].

The precise mechanisms of mitochondrial dysfunction in CKD remain unclear. Although mtDNA mutations may occur as a result of chronic cellular damage, they are likely secondary events rather than primary causes of renal pathology [25].

Research on mtDNA-CN in CKD has been conducted using various methods and different biological samples, with most studies showing a decrease in mtDNA-CN in both renal tissues and peripheral blood (plasma, PBMCs) as CKD progresses [14, 24, 28]. However, some investigations involving patients with non-diabetic CKD did not find a consistent association between mtDNA-CN and the rate of renal function decline [27].

Among the biochemical mechanisms underlying decreased mtDNA-CN, one hypothesis involves the suppression of HIF-1α in the context of hyperglycemia. Zheng et al. (2022) demonstrated that persistent hyperglycemia induces excessive production of mitochondrial reactive oxygen species (mtROS), leading to mitochondrial damage and suppression of mitochondrial biogenesis. This hypothesis has been supported by a series of experimental studies [26].

In our study, no significant differences in mtDNA-CN were found between patients with T2DM and CKD, patients with T2DM without CKD, and healthy controls. We hypothesize that the relatively satisfactory glycemic control observed in both diabetic groups may have contributed to the absence of mtDNA-CN alterations. The average HbA1c levels in T2DM patients with and without CKD were 7.75% and 7.66%, respectively, which may have helped reduce the intensity of chronic oxidative stress – one of the key mechanisms of mitochondrial damage and mtDNA-CN depletion. As a result, the impact of persistent hyperglycemia-related damaging factors was likely attenuated. Moreover, patients with advanced CKD (stages 4–5) were not included in our study, which may have limited the ability to detect more pronounced mitochondrial alterations.

Conclusions. 1. No significant differences in mitochondrial DNA copy number (mtDNA-CN) in peripheral blood leukocytes were found between patients with type 2 diabetes mellitus (T2DM) and comorbid chronic kidney disease (CKD), patients with T2DM without CKD, and healthy individuals. 2. The absence of association may be attributed to relatively satisfactory metabolic control in both diabetic groups, as evidenced by similar HbA1c levels. 3. Another potential reason for the lack of differences in mtDNA-CN levels is the exclusion of patients with end-stage CKD (stages G4–G5), in whom mitochondrial alterations might be more pronounced. 4. Further studies involving a broader range of patients, including those with advanced CKD stages, are warranted to better understand the role of mtDNA-CN as a potential biomarker of renal dysfunction.

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.

Funding. The article was carried out within the framework of the research project "Study of phenotypic and genetic factors, clinical course features of age-associated cardiometabolic diseases, and their impact on the development of complications", state registration number: 0124U004408.

КІЛЬКІСТЬ КОПІЙ МІТОХОНДРІАЛЬНОЇ ДНК У ЛЕЙКОЦИТАХ ПАЦІЄНТІВ ІЗ ЦУКРОВИМ ДІАБЕТОМ 2 ТИПУ ТА ХРОНІЧНОЮ ХВОРОБОЮ НИРОК

Я. А. Саєнко[1,2]✉, *Д. С. Красненков*[1], *К. К. Мідловець*[1], *В. В. Корчева*[1], *Ю. Є. Реброва*[1,3], *Д. Д. Єпішина*[1], *Б. М. Маньковський*[1]

[1]ДУ «Інститут геронтології імені Д. Ф. Чеботарьова НАМН України», Київ;
[2]ДУ «Центр кардіології та кардіохірургії МОЗ України», Київ;
[3]Національний університет охорони здоров'я України імені П. Л. Шупика, Київ;
✉e-mail: ysaenko1981@gmail.com

Хронічна хвороба нирок (ХХН) є одним із найпоширеніших ускладнень цукрового діабету 2 типу (ЦД2Т), що суттєво підвищує ризик серцево-судинної патології та смертності. Мітохондріальна дисфункція, зокрема зниження кількості копій мітохондріальної ДНК (mtDNA-CN), відіграє важливу роль у розвитку діабетичних ускладнень, включаючи нефропатію. Метою нашого дослідження було визначення mtDNA-CN у лейкоцитах периферичної крові пацієнтів із ЦД2Т залежно від наявності ХХН. У дослідження було включено 109 осіб, серед яких – 20 здорових осіб (група контролю) та 89 пацієнтів із ЦД2Т, розподілених на групи залежно від наявності або відсутності ХХН. Кількість копій mtDNA у лейкоцитах визначали методом кількісної полімеразної ланцюгової реакції в реальному часі. Також оцінювали біохімічні маркери ЦД2Т та ХХН, проводили непараметричні тести й кореляційний аналіз. Статистично значущих відмінностей рівня mtDNA-CN між пацієнтами з ЦД2Т і ХХН, пацієнтами з ЦД2Т без ХХН та контрольною групою не виявлено ($P > 0,05$). Зв'язку між mtDNA-CN та показниками функції нирок встановлено не було. Відсутність змін mtDNA-CN може бути пов'язана з відносно задовільним глікемічним контролем у групах пацієнтів із ЦД2Т.

Ключові слова: цукровий діабет 2 типу, хронічна хвороба нирок, кількість копій мітохондріальної ДНК, лейкоцити.

References

1. Hong YS, Longchamps RJ, Zhao D, Castellan CA, Loehr LR, Chang PP, Matsushita K, Grove ML, Boerwinkle E, Arking DE, Guallar E. Mitochondrial DNA Copy Number and Incident Heart Failure: The Atherosclerosis Risk in Communities (ARIC) Study. *Circulation*. 2020; 141(22): 1823-1825.
2. Johansen KL, Gilbertson DT, Li S, Li S, Liu J, Roetker NS, Ku E, Schulman IH, Greer RC, Chan K, Abbott KC, Butler CR, O'Hare AM, Powe NR, Reddy YNV, Snyder J, St Peter W, Taylor JS, Weinhandl ED, Wetmore JB. US Renal Data System 2023 Annual Data Report: Epidemiology of Kidney Disease in the United States. *Am J Kidney Dis*. 2024; 83(4 Suppl 1): A8-A13.
3. Mitrofanova A, Fontanella AM, Burke GW, Merscher S, Fornoni A. Mitochondrial Contribution to Inflammation in Diabetic Kidney Disease. *Cells*. 2022; 11(22): 3635.
4. Lowes H, Pyle A, Santibanez-Koref M, Hudson G. Circulating cell-free mitochondrial DNA levels in Parkinson's disease are influenced by treatment. *Mol Neurodegener*. 2020; 15(1): 10.
5. Al-Kafaji G, Aljadaan A, Kamal A, Bakhiet M. Peripheral blood mitochondrial DNA copy number as a novel potential biomarker for diabetic nephropathy in type 2 diabetes patients. *Exp Ther Med*. 2018; 16(2): 1483-1492.
6. Yao L, Liang X, Qiao Y, Chen B, Wang P, Liu Z. Mitochondrial dysfunction in diabetic tubulopathy. *Metabolism*. 2022; 131: 155195.
7. Sifuentes-Franco S, Padilla-Tejeda DE, Carrillo-Ibarra S, Miranda-Díaz AG. Oxidative Stress, Apoptosis, and Mitochondrial Function in Diabetic Nephropathy. *Int J Endocrinol*. 2018; 2018: 1875870.

8. Castellani CA, Longchamps RJ, Sun J, Guallar E, Arking DE. Thinking outside the nucleus: Mitochondrial DNA copy number in health and disease. *Mitochondrion*. 2020; 53: 214-223.
9. Forbes JM, Thorburn DR. Mitochondrial dysfunction in diabetic kidney disease. *Nat Rev Nephrol*. 2018; 14(5): 291-312.
10. Basu M, Wang K, Ruppin E, Hannenhalli S. Predicting tissue-specific gene expression from whole blood transcriptome. *Sci Adv*. 2021; 7(14): eabd6991.
11. He WJ, Li C, Huang Z, Geng S, Rao VS, Kelly TN, Hamm LL, Grams ME, Arking DE, Appel LJ, Rebholz CM; CRIC Study Investigators. Association of Mitochondrial DNA Copy Number with Risk of Progression of Kidney Disease. *Clin J Am Soc Nephrol*. 2022; 17(7): 966-975.
12. Fazzini F, Lamina C, Fendt L, Schultheiss UT, Kotsis F, Hicks AA, Meiselbach H, Weissensteiner H, Forer L, Krane V, Eckardt KU, Köttgen A, Kronenberg F; GCKD Investigators. Mitochondrial DNA copy number is associated with mortality and infections in a large cohort of patients with chronic kidney disease. *Kidney Int*. 2019; 96(2): 480-488.
13. Sharma P, Sampath H. Mitochondrial DNA Integrity: Role in Health and Disease. *Cells*. 2019; 8(2): 00.
14. Malik AN. Mitochondrial DNA - novel mechanisms of kidney damage and potential biomarker. *Curr Opin Nephrol Hypertens*. 2023; 32(6): 528-536.
15. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2024 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. *Kidney Int*. 2024; 105(4S): S117-S314.
16. Flint AJ, Rexrode KM, Hu FB, Glynn RJ, Caspard H, Manson JE, Willett WC, Rimm EB. Body mass index, waist circumference, and risk of coronary heart disease: a prospective study among men and women. *Obes Res Clin Pract*. 2010; 4(3): e171-e181.
17. Campbell NRC, Paccot Burnens M, Whelton PK, Angell SY, Jaffe MG, Cohn J, Espinosa Brito A, Irazola V, Brettler JW, Roccella EJ, Maldonado Figueredo JI, Rosende A, Ordunez P. 2021 World Health Organization guideline on pharmacological treatment of hypertension: Policy implications for the region of the Americas. *Lancet Reg Health Am*. 2022; 9: 100219.
18. Midlovets KK, Korcheva VV, Shevchuk OI, Barsukov OO. Theory and practice of biological research. In: Krasnenkov DS, editor. Kyiv: D.F. Chebotaryov Institute of Gerontology NAMS of Ukraine; 2025. p. 54. (Ukrainian). Available from: https://www.geront.kiev.ua/methodological-recommendations/methodological-recommendations-for-students-on-working-in-molecular-biology-laboratory/
19. Hsieh AYY, Budd M, Deng D, Gadawska I, Côté HCF. A Monochrome Multiplex Real-Time Quantitative PCR Assay for the Measurement of Mitochondrial DNA Content. *J Mol Diagn*. 2018; 20(5): 612-620.
20. Atramentova LO, Utevskaya OM. Statistical methods in biology. (In Ukrainian). Kharkiv: V. N. Karazin Kharkiv National University; 2007. 288 p.
21. Hollander M, Wolfe DA, Chicken E. Nonparametric Statistical Methods. 3rd ed. Hoboken (NJ): Wiley; 2014.
22. van Belle G, Fisher LD, Heagerty PJ, Lumley T. Biostatistics: A Methodology for the Health Sciences. 2nd ed. Hoboken (NJ): Wiley-Interscience; 2004.23. Ahmad AA, Draves SO, Rosca M. Mitochondria in Diabetic Kidney Disease. *Cells*. 2021; 10(11): 2945.
24. Liu Y, Pan Y, Tian Z, Wang J, Chen F, Geng Z, Li Q, Liu Z, Zhou X, Zhou K. Association of mitochondrial DNA copy number with chronic kidney disease in older adults. *BMC Geriatr*. 2023; 23(1): 514.
25. Kanazashi Y, Maejima K, Johnson TA, Sasagawa S, Jikuya R, Hasumi H, Matsumoto N, Maekawa S, Obara W, Nakagawa H. Mitochondrial DNA Variants at Low-Level Heteroplasmy and Decreased Copy Numbers in Chronic Kidney Disease (CKD) Tissues with Kidney Cancer. *Int J Mol Sci*. 2023; 24(24): 17212.
26. Zheng X, Narayanan S, Xu C, Eliasson Angelstig S, Grünler J, Zhao A, Di Toro A, Bernardi L, Mazzone M, Carmeliet P, Del Sole M, Solaini G, Forsberg EA, Zhang A, Brismar K, Schiffer TA, Rajamand Ekberg N, Botusan IR, Palm F, Catrina SB. Repression

of hypoxia-inducible factor-1 contributes to increased mitochondrial reactive oxygen species production in diabetes. *Elife*. 2022; 11: e70714.
27. Irazabal MV, Chade AR, Eirin A. Renal mitochondrial injury in the pathogenesis of CKD: mtDNA and mitomiRs. *Clin Sci (Lond).* 2022; 136(5): 345-360.
28. Feng J, Chen Z, Liang W, Wei Z, Ding G. Roles of Mitochondrial DNA Damage in Kidney Diseases: A New Biomarker. *Int J Mol Sci*. 2022; 23(23): 15166.