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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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URINARY EXCRETION OF ALPHA-ACTININ-4 AND TIGHT JUNCTION PROTEIN 1 IN PATIENTS WITH TYPE 2 DIABETES AND DIFFERENT PATTERNS OF CHRONIC KIDNEY DISEASE

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Abstract.

Background and Aims: Abnormalities of the cytoskeleton and the slit diaphragm of podocytes have been attributed to diabetic nephropathy. In this study, we assessed urinary excretion of alpha-actinin-4 (ACTN-4), a cytoskeleton protein and a component of the slit diaphragm, and tight junction protein 1 (TJP-1, or ZO-1), a peripheral membrane protein that forms molecular complexes with actin filaments, in patients with type 2 diabetes (T2D) and albuminuric or non-albuminuric chronic kidney disease (CKD). **Material and Methods:** The study included 140 patients with long-term T2D (≥10 years) and 20 healthy subjects as control. Patterns of CKD were identified based on the estimated glomerular filtration rate (eGFR) and urinary albumin-to-creatinine ratio (UACR). Urinary ACTN-4 and TJP-1 were assessed by ELISA. **Results.** Patients with T2D had increased urinary excretion of ACTN-4 (p=0.03) and TJP-1 (p=0.006). In logistic regression models, both ACTN-4 and TJP-1 demonstrated associations with albuminuric CKD (UACR ≥3.0 mg/mmol and eGFR <60 mL/min×1.73 m^2) after adjusting to age, sex, diabetes duration, HbA1c, and smoking. In ROC-analysis, TJP-1 excretion ≥70 pg/mmol was associated with albuminuric CKD (OR 5.45, 95% CI 1.96–15.18, p=0.001). **Conclusions:** The results demonstrate that elevated urinary ACTN-4 and TJP-1 are associated specifically with albuminuric CKD, but not with non-albuminuric CKD, in T2D patients.

Key words. Type 2 diabetes, chronic kidney disease, albuminuria, glomerular filtration rate, alpha-actinin-4, tight junction protein 1.

Introduction.

About 20–40% of patients with type 2 diabetes (T2D) are estimated to have chronic kidney disease (CKD) [1,2]. Recent data indicate increasing proportion of non-albuminuric CKD phenotype in these subjects [3-5]. The prevalence of non-albuminuric CKD varies from 8 up to 28%. Among patients with reduced renal function, the prevalence of this pattern is even higher [5,6]. Non-albuminuric and albuminuric CKD differ in their risk factors and pathogenic mechanisms [5-8]. While tubulointerstitial and vascular lesions are supposed to be more advanced in non-albuminuric CKD, an increase in albuminuria is associated primarily with glomerular injury [9,10] and, especially, with the damage and loss of podocytes [11,12].

Podocytopathy in diabetic kidney disease is characterized by the effacement of the foot processes and disruption of the slit diaphragm, resulting in podocyteuria and elevated permeability for albumin [13,14]. Patients with diabetes demonstrate elevated urinary excretion of nephrin and podocin, two principal components of the slit diaphragm [15]. In this study, we assessed urinary excretion of alpha-actinin-4 (ACTN-4) and tight junction protein 1 (TJP-1), the molecules essential for podocyte physiology, in patients with T2D and different patterns of CKD.

ACTN-4 is a 100 kDa rod shaped protein. This is a minor component of the slit diaphragm and a part of the nephrin multiprotein complexes [16,17]. It was demonstrated that ACNT-4 plays an important role in the integrity of the podocyte foot processes by the filamentous actin crosslinking and providing structural support for podocytes [17-19]. Mutations in ACTN-4 gene and disrupted phosphorylation of ACTN-4 are associated with proteinuria [20,21].

Tight junction proteins were recognized to be a structural component of the slit diaphragm [22]. These proteins are essential for the interdigitation of the foot processes [23]. It was postulated that abnormalities of the tight junction protein synthesis contribute to the development of diabetic podocytopathy [24]. TJP-1, also known as ZO-1, is a 225 kD protein localized exclusively on the cytoplasmic surface of the tight junctions [22,25]. Apart from being responsible for organizing components of the tight junctions by linking them to the cortical actin cytoskeleton, ACTN-4 regulates structural and functional organization of the slit diaphragm [23]. Similar to ACTN-4, genetic disruption or decreased glomerular expression of TJP-1 can lead to the development of podocytopathy and proteinuria [23,26,27].

According to results of bioinformatics studies, both ACTN-4 and TJP-1 may be involved in the progression of diabetic kidney disease [28-30]. We hypothesized that urinary excretion of these molecules may change differentially in people with T2D depending on the CKD patterns.

The aim of our study was to assess the urinary excretion of ACTN-4 and TJP-1 in patients with long-term T2D depending on the presence of albuminuric or non-albuminuric CKD.

Materials and Methods.

Design: We performed observational single-center cross-sectional study. Adult patients with T2D duration more than 10 years since the diagnosis were selected from the institutional database. Verified non-diabetic CKD, renal replacement therapy or kidney transplantation in anamnesis, congestive heart failure (class IV by NYHA), acute kidney injury, severe disease or trauma required hospitalization within the last 3 months, cancer, chronic autoimmune or inflammatory diseases in medical history were used as exclusion criteria. We also did not include individuals with body mass index (BMI) ≥40 kg/m^2

or <18.5 kg/m^2, and those with major amputations or bariatric surgery in anamnesis.

Based on the matching of estimated glomerular filtration rate (eGFR) and urinary albumin/creatinine ratio (UACR), four groups of patients were formed. Each group included 35 subjects. Those with eGFR ≥60 mL/min×1.73 m^2 and UACR <3.0 mg/mmol were referred as a normal renal function / normal albuminuria (NF/NA) group. Participants with eGFR <60 mL/min×1.73 m^2 and UACR <3.0 mg/mmol formed the declined renal function / normal albuminuria (DF/NA) group. Patients with eGFR ≥60 mL/min×1.73 m^2 and UACR ≥3.0 mg/mmol were assigned into the normal renal function / elevated albuminuria (NF/EA) group. Finally, individuals with eGFR <60 mL/min×1.73 m^2 and UACR ≥3.0 mg/mmol were included in the declined renal function / elevated albuminuria (DF/NA) group.

Subjects without diabetes, obesity, and CKD, matched by sex and age with diabetic group, were considered as control.

Laboratory measurements: Hemoglobin A1c (HbA1c), serum creatinine and UACR were assessed with AU680 Chemistry Analyzer (Beckman Coulter, USA). eGFR was calculated according to CKD-EPI formula (2012). The urine samples were stored at –20°C without melt-freeze cycles for following research. The concentrations of ACTN-4 and TJP-1 were assessed with the use of commercially available kits (SEC223Hu and SEC262Hu, respectively, Cloud Clone Corp., China) according to the manufacturer`s manuals. Raw data were adjusted to the urinary creatinine concentrations.

Statistical analysis: The continuous variables were tested for normal distribution with Shapiro–Wilk test (SPSS Statistics, IBM, USA). As most of the studied parameters were not distributed normally, the data are presented as medians and interquartile ranges (IQRs), unless stated otherwise. The statistical significance of differences between groups was tested with Mann–Whitney U-tests for comparison of two groups. We used Kruskal–Wallis H-test for multiple group comparisons. Multiple comparisons of mean ranks for groups were applied for post-hoc analysis with a Bonferroni adjustment. The χ^2 test was applied for categorical data (Statistica, Dell, USA). The differences were noted as significant with two-sided p-value below 0.05.

The associations between continuous parameters were assessed with Spearman correlation analysis (Statistica, Dell, USA). The associations of urinary excretion of ACNT-4 and TJP-1 with declined renal function and elevated albuminuria were tested in ROC-analysis (SPSS Statistics, IBM, USA) and in multiple logistic regression models (Statistica, Dell, USA).

Ethical issues: The study was approved by the Ethical Committee of RICEL – Branch of IC&G SB RAS (Protocol 88, 22 November 2012; Protocol 166, 24 June 2021). Informed consent was obtained from all subjects involved in the study.

Results.

Clinical characteristics of the study participants: One hundred and forty subjects with T2D, 70 men and 70 women, aged from 44 to 83 years (median 65 years), were enrolled. Diabetes duration since the diagnosis varied from 10 to 48 years (median 15 years). Median HbA1c was 8.28%, or 67 mmol/mol (from 5.19%, or 33.2 mmol/mol to 15.8%, or 149 mmol/mol). Clinical characteristics of patient groups are presented

Table 1. *Clinical characteristics of T2D groups.*

Parameter	NF/NA (N=35)	DF/NA (N=35)	NF/EA (N=35)	DF/EA (N=35)
Age, years	62 (56 – 66)	71 (65 – 75)^^^	63 (58 – 68)##	68 (61 – 71)
Sex (F/M), n	18/17	17/18	18/17	70/18
BMI, kg/m^2	31.6 (29.0 – 35.7)*	31.4 (27.9 – 35.7)*	31.9 (28.7 – 35.7)	32.4 (27.5 – 35.7)
Smoking, n	7 (20.0)	10 (28.6)	7 (20.0)	3 (8.6)
Duration of T2D, years	13 (10 – 15)	15 (11 – 20)	16 (12 – 21)	18 (12 – 23)^
HbA1c, %	8.0 (7.3 – 9.3)	7.8 (6.8 – 8.8)	9.2 (8.0 – 10.9)^##	8.6 (8.0 – 9.9)
Insulin, n (%)	21 (60)	22 (62.9)	24 (68.6)	29 (82.9)
Metformin, n (%)	34 (97.1)	24 (68.6)	31 (88.6)	16 (45.7)^*
Sulfonylurea, n (%)	16 (45.7)	15 (42.9)	15 (42.9)	9 (25.7)
DPP-4 inhibitors, n (%)	8 (22.9)	4 (11.4)	3 (8.6)	3 (8.6)
GLP-1 analogues, n (%)	1 (2.86)	1 (2.86)	0 (0)	0 (0)
SGLT-2 inhibitors, n (%)	7 (20)	11 (31.4)	13 (37.1)	4 (11.4)
Arterial hypertension, n (%)	35 (100)	35 (100)	35 (100)	35 (100)
ACE inhibitor / ARB, n (%)	12/16 (34.3/45.7)	11/19 (31.4/54.3)	9/17 (25.7/48.6)	14/17 (40/48.6)
Beta-blockers, n (%)	15 (42.9)	24 (68.6)	25 (71.4)	24 (68.6)
Calcium channel blockers, n (%)	8 (22.9)	16 (45.7)	14 (40)	20 (57.1)^
Diuretics, n (%)	16 (45.7)	19 (54.3)	14 (40)	24 (68.6)
Statins, n (%)	23 (65.7)	22 (62.9)	21 (60.0)	24 (68.6)
Creatinine, μmol/L	81 (70 – 89)	113 (98 – 122)^^^	84 (70 – 97)	114 (101 – 149)^^^
eGFR, mL/min×1.73 m2	78 (72 – 90)	52 (46–58)^^^	75 (66 – 88)	50 (40 – 56)^^^
CKD G1/G2/G3a/G3b/G4, n (%)	7/28/0/0/0 (20/80/0/0/0)	0/0/27/8/0 (0/0/80/20/0)	7/28/0/0/0 (20/80/0/0/0)	0/0/22/11/2 (0/0/62.9/31.4/5.71)
UACR, mg/mmol	0.3 (0.2 – 0.4)	0.4 (0.3 – 0.6)	21.1 (7.3 – 57.7)^^^###	43.6 (9.9 – 99.7)^^^###
CKD A3, n (%)	0 (0)	0 (0)	15 (42.8)	18 (51.4)

*Data are presented as medians and IQRs. ^ p<0.05, ^^^ p<0.001 vs. NF/NA, ## p<0.01, ### p<0.001 vs. DF/NA, * p<0.05 vs. NF/EA.*

in Table 1. Mean age was the highest in DF/NA group, while DF/EA patients had the longest diabetes duration, and NF/EA individuals had the highest HbA1c levels.

All patients received antihyperglycemic agents, including metformin (n=105), sulfonylurea (n=55), DPP-4 inhibitors (n=18), GPL-1 analogues (n=2), SGLT2 inhibitors (n=35) and insulin (n=96). Most patients (n=115) were treated by the renin-angiotensin system blockers. Compared to other groups, lower proportion of DF/EA patients received metformin, while calcium channel blockers were used more frequently in this group. There were no significant differences in other treatment modalities between diabetic groups.

The control group comprised of 20 subjects, 10 men and 10 women. The median age of these subjects was 62.5 years (from 45 years to 78 years, IQR 59.5–67.4), and median BMI was 25.7 kg/m^2 (from 23.1 kg/m^2 to 29.7 kg/m^2, IQR 24.7–28.6).

Urinary excretion of ACTN-4 and TJP-1: Patients with T2D had elevated urinary excretion of ACTN-4 and TJP-1 as compared to control (Figure 1). Specifically, ACTN-4 excretion was increased by 1.64-fold (p=0.03) and excretion of TJP-1 was elevated by 1.81-fold (p=0.006). We found no significant differences in the urinary ACNT-4 excretion between the diabetic groups (all p>0.05). Excretion of TJP-1 was significantly elevated in DF/EA patients as compared to control (p=0.008).

NF/NA group: Estimated glomerular filtration rate (eGFR) ≥60 mL/min×1.73 m^2 and urinary albumin-to-creatinine ratio (UACR) <3.0 mg/mmol; DF/NA group: eGFR <60 mL/min×1.73 m^2 and UACR <3.0 mg/mmol; NF/EA group: eGFR ≥60 mL/min×1.73 m^2 and UACR ≥3.0 mg/mmol; DF/EA group: eGFR <60 mL/min×1.73 m^2 and UACR ≥3.0 mg/mmol.

Data are presented as medians and IQRs. * p<0.05, ** p<0.01 vs. control.

Both molecules demonstrated week correlations with diabetes duration (ACTN-4: r=0.3, p<0.001; TJP-1: r=0.29, p<0.001), eGFR (ACTN-4: r=–0.21, p=0.02; TJP-1: r=–0.17, p=0.04) and UACR (ACTN-4: r=0.24, p=0.005; TJP-1: r=0.3, p<0.001).

In ROC-analysis, urinary TJP-1 concentration ≥70.0 pg/mmol was associated with UACR ≥3.0 mg/mmol, eGFR <60 mL/min×1.73 m^2, and combination of these parameters (Table 2). We found no significant cut-off point for ACNT-4 as a factor associated with albuminuria or eGFR <60 mL/min×1.73 m^2.

In univariate logistic regression models, ACTN-4 and TJP-1 demonstrated associations with albuminuric CKD patterns with normal or declined renal function (Table 3). After adjusting to age, sex, diabetes duration, HbA1c, and smoking, we found an association between TJP-1 and UACR ≥3.0 mg/mmol. In multivariate models, both ACTN-4 and TJP-1 were associated with DF/EA phenotype.

Parameters of the models: [1]Intercept (constant, β0) –3.15; β regression coefficients for age –0.036 (p=0.166), male sex 0.32 (p=0.12), for BMI 0.006 (p=0.89), for diabetes duration 0.055 (p=0.06), for HbA1c 0.36 (p=0.002), for smoking 0.45 (p=0.09), for TJP-1 excretion 0.013 (p=0.006); KS p-level <0.001, area under ROC (AUC) 0.75, sensitivity (Se) 0.69, specificity (S_p) 0.69 for cut-off point of logistic function (L_p) 0.49;

[2]β0 = –7.42; β regression coefficients for age 0.035 (p=0.38), for female sex 0.69 (p=0.046), for BMI –0.088 (p=0.20), for diabetes duration 0.12 (p=0.02), for HbA1c 0.46 (p=0.008), for smoking 0.59 (p=0.23), for ACTN-4 excretion 2.877 (p=0.022); KS p-level < 0.001, AUC=0.81, Se=0.71, S_p=0.71 for L_p=0.47.

[3]β0 = –7.03; β regression coefficients for age 0.026 (p=0.52), for female sex 0.76 (p=0.04), for BMI –0.09 (p=0.20), for diabetes duration 0.12 (p=0.02), for HbA1c 0.46 (p=0.009), for smoking 0.55 (p=0.25), for TJP-1 excretion 0.023 (p=0.009); KS p-level < 0.001, AUC=0.83, Se=0.71, S_p=0.71 for L_p=0.50.

Discussion.

In this study, we assessed the urinary excretion of ACTN-4 and TJP-1, two molecules that are necessary for the interaction of the cytoskeleton and the slit diaphragm, in patients with long-term T2D depending on CKD phenotypes. We found an association between elevated excretion of both molecules and albuminuric CKD. Urinary TJP-1 was associated with albuminuria more closely. At the same time, both molecules demonstrated no associations with non-albuminuric CKD pattern.

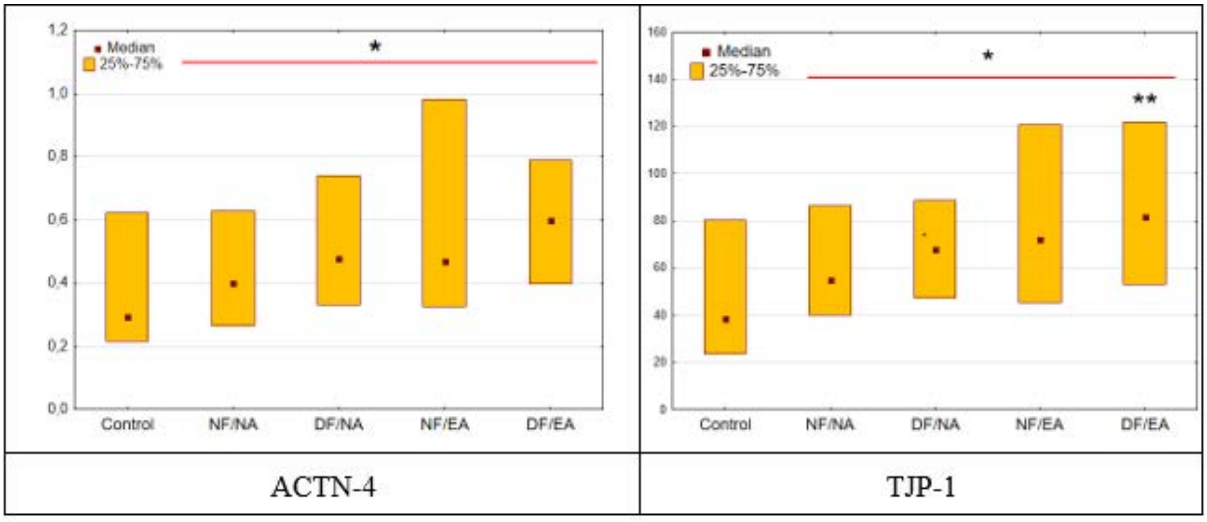

Figure 1. *Urinary excretion of alpha-actinin-4 (ACTN-4, ng/mmol) and tight junction protein 1 (TJP-1, pg/mmol) in patients with type 2 diabetes (T2D).*

Table 2. *Associations of urinary ACTN-4 and TJP-1 with CKD signs and patterns in ROC-analysis.*

Parameter	Cut-off point	AUC ± SE, 95% CI, p-value	OR, 95% CI, p-value	Se	Sp
eGFR <60 mL/min×1.73 m^2					
ACNT-4, ng/mmol	≥ 0.48	0.58±0.05 (0.48 – 0.67), p=0.12	1.41 (0.73 – 2.74), p=0.31	0.54	0.54
TJP-1, pg/mmol	≥ 70.0	0.58±0.05 (0.49 – 0.68), p=0.10	2.00 (1.02 – 3.92), p=0.04	0.59	0.59
UACR ≥3.0 mg/mmol					
ACNT-4, ng/mmol	≥ 0.48	0.59±0.05 (0.50 – 0.69), p=0.06	1.41 (0.73 – 2.74), p=0.31	0.54	0.54
TJP-1, pg/mmol	≥ 70.0	0.61±0.05 (0.52 – 0.71), p=0.02	2.54 (1.28 – 5.01), p=0.007	0.61	0.61
DF/NA pattern					
ACNT-4, ng/mmol	≥ 0.41	0.61±0.07 (0.47 – 0.74), p=0.13	1.41 (0.55 – 3.62), p=0.47	0.57	0.54
TJP-1, pg/mmol	≥ 56.9	0.61±0.07 (0.47 – 0.74), p=0.13	2.88 (1.09 – 7.60), p=0.03	0.66	0.60
NF/EA pattern					
ACNT-4, ng/mmol	≥ 0.41	0.62±0.07 (0.49 – 0.75), p=0.08	1.41 (0.55 – 3.62), p=0.47	0.54	0.51
TJP-1, pg/mmol	≥ 58.0	0.62±0.07 (0.49 – 0.75), p=0.08	2.54 (0.97 – 6.65), p=0.06	0.63	0.63
DF/EA pattern					
ACNT-4, ng/mmol	≥ 0.488	0.67±0.06 (0.54 – 0.79), p=0.02	2.25 (0.86 – 5.85); p=0.10	0.60	0.60
TJP-1, pg/mmol	≥ 70.0	0.69±0.06 (0.56 – 0.81), p=0.006	5.45 (1.96 – 15.18), p=0.001	0.71	0.69

Table 3. *Associations of urinary ACTN-4 and TJP-1 with CKD signs and patterns in logistic regression models.*

Parameter	Crude OR, 95% CI, p-value	Adjusted OR, 95% CI, p-value
eGFR <60 mL/min×1.73 m^2		
ACNT-4, each 0.1 ng/mmol	1.06 (0.97 – 1.15), p=0.21	1.03 (0.96 – 1.10), p=0.41
TJP-1, each 10 pg/mmol	1.04 (0.97 – 1.11), p=0.26	1.00 (0.93 – 1.08), p=0.99
UACR ≥3.0 mg/mmol		
ACNT-4, each 0.1 ng/mmol	1.07 (0.98 – 1.17), p=0.16	1.06 (0.97 – 1.17), p=0.22
TJP-1, each 10 pg/mmol	1.11 (1.03 – 1.20), p=0.008	1.13 (1.04 – 1.24), p=0.006[1]
DF/NA pattern		
ACNT-4, each 0.1 ng/mmol	1.15 (0.98 – 1.35), p=0.09	1.10 (0.89 – 1.37), p=0.39
TJP-1, each 10 pg/mmol	1.10 (0.96 – 1.26), p=0.17	0.99 (0.82 – 1.19), p=0.90
NF/EA pattern		
ACNT-4, each 0.1 ng/mmol	1.18 (1.004 – 1.39), p=0.04	1.14 (0.95 – 1.36), p=0.16
TJP-1, each 10 pg/mmol	1.13 (1.005 – 1.27), p=0.04	1.11 (0.97 – 1.26), p=0.13
DF/EA pattern		
ACTN-4, each 0.1 ng/mmol	1.24 (1.02 – 1.51), p=0.03	1.33 (1.04 – 1.71), p=0.02[2]
TJP-1, each 10 pg/mmol	1.19 (1.04 – 1.35), p=0.008	1.26 (1.06 – 1.50), p=0.009[3]

Some literature data indicate changes in the renal expression of ACTN-4 in hyperglycemic conditions. A decreased ACTN-4 mRNA expression was found in podocyte cultures in the presence of high glucose and/or advanced glycation end products [31,32]. In patients with diabetic nephropathy, a decreased renal ACTN-4 mRNA and protein expression correlated inversely with proteinuria [33]. An elevated urinary excretion of ACTN-4 mRNA was observed in patients with T2D; the excretion correlated positively with serum creatinine. In this study, we found an association between urinary ACTN-4 and albuminuric CKD pattern (UACR <3.0 mg/mmol and eGFR <60 mL/min×1.73 m^2) in individuals with T2D. The association was significant after adjustment to age, sex, diabetes duration, HbA1c, and smoking. These data are in agreement with the results of the aforementioned studies, indicating the changes in the expression and excretion of ACTN-4 in diabetic kidney disease.

In our study, urinary excretion of TJP-1 turned out to be associated with increased albuminuria (UACR ≥3.0 mg/mmol) and albuminuric CKD (UACR ≥3.0 mg/mmol and eGFR <60 mL/min×1.73 m^2). These associations were significant after adjustment to traditional risk factors for diabetic nephropathy. Previously it was demonstrated that decreased TJP-1 expression elevates permeability of monolayered cultured rat glomerular epithelial cells [34]. High glucose and advanced glycation end products decrease phosphorylation and internalization of TJP-1 [35]. The loss of TJP-1 in podocytes contributes to epithelial-to-mesenchymal transition of these cells [36,37]. Activation of the signalling pathways of transforming growth factor-β and connective tissue growth factor decreases expression of TJP-1 in tubular cells contributing to the epithelial-to-mesenchymal transition [38]. Loss of TJP-1 in renal tubular cells can be a consequence of albumin overload [39]. Therefore, the association we found between the excretion of TJP-1 and albuminuric CKD may have a pathogenetic basis.

In our study, urinary ACNT-4 and TJP-1 were not associated independently with DF/NA pattern of CKD in subjects with T2D. Previously, we did not reveal the elevation of nephrin and podocin, the components of the slit diaphragm, in patients with T2D and non-albuminuric CKD [15]. Based on these data, we

can hypothesize that podocyte dysfunction is more pronounced in the albuminuric than in the non-albuminuric diabetic CKD. However, abnormalities of the cytoskeleton and tight junction proteins may be important not only for podocyte dysfunction, but also for the dysfunction of other types of kidney cells, including tubular epitheliocytes [39,40]. In this study, we did not find an association between the urinary excretion of the studied markers and the non-albuminuric CKD. Apparently, this pattern of kidney damage has its own pathogenetic features, among which vascular and tubulointerstitial lesions are discussed [9].

The limitations of our study include cross-sectional design and single-site recruitment of the patients. Taken into account the variability in UACR and eGFR, some patients could be misclassified between the groups. We did not verify morphological changes in the kidneys in our patients.

At the same time, as far as we know, this is the first study evaluated the changes in ACTN-4 and TJP-1 excretion in T2D subjects with different CKD phenotypes. The results provide further support to notion of differences in the pathogenetic mechanisms of albuminuric and non-albuminuric CKD in diabetes.

Conclusion.

In people with long-term T2D, urinary excretion of ACTN-4 and TJP-1 is associated with albuminuric CKD, but not with non-albuminuric CKD pattern. The results support the role of the proteins mediating the link between cytoskeleton and the slit diaphragm in the pathogenesis of diabetic kidney disease. Future prospective studies are needed to elucidate the value of urinary ACTN-4 and TJP-1 as possible predictors of albuminuric CKD development and progression in people with diabetes.

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