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ABSTRACT

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SIGNIFICANCE OF URINARY AMINOPEPTIDASE N AND DIPEPTIDYL PEPTIDASE IV IN EARLY DIAGNOSIS OF KIDNEY DAMAGE IN CHILDREN WITH TYPE 1 DIABETES MELLITUS IN NORTH-EASTERN REGION OF UKRAINE

Introduction. Compared to adults, diabetes in children and adolescents follows a more aggressive clinical course. This is characterized by a reduced response to current treatments, a faster decline in β-cell function, the rapid progression of insulin resistance, and an accelerated development of both microvascular and macrovascular complications. Diabetic nephropathy stands out as one of the most critical and common complications of diabetes and is the leading cause of end-stage renal disease. This makes type 1 diabetes mellitus particularly significant for pediatric nephrologists. While clinical signs of diabetic nephropathy, such as albuminuria and a decline in glomerular filtration rate, typically manifest over a longer period (10–25 years), specific structural changes in the kidneys, such as glomerular basement membrane thickening and mesangial expansion can occur much earlier, within 1.5 to 5 years of diabetes onset. Notably, diabetic nephropathy affects not only the glomeruli but also involves tubular damage. Tubulointerstitial lesions often precede glomerular injury, suggesting that tubular biomarkers might be more sensitive for early detection. Markers with peptidase activity have proven effective in identifying early tubular injury. Aminopeptidase N, an ectopeptidase widely expressed in the kidneys, is a recognized urinary marker for proximal tubule damage. Similarly, Dipeptidyl peptidase IV is expressed in glomerular visceral epithelial cells, endothelial cells, and the brush border of proximal tubules. Elevated urinary Dipeptidyl peptidase IV levels have been detected in diabetic patients with normoalbuminuria, indicating its potential as an early biomarker for the onset of diabetic nephropathy.

Objective. To determine urinary Aminopeptidase N and Dipeptidyl peptidase IV levels in children from north-eastern region of Ukraine depending on the diabetes duration.

Materials and methods. A total of 55 participants were included in the study, comprising 47 children with type 1 diabetes mellitus and 8 children with no history of diabetes and kidney disease. The patients with type 1 diabetes mellitus were divided into three groups based on disease duration: less than 1 year (11 participants), 1–5 years (24 participants), and more than 5 years (12 participants). The chemiluminescence signals of Aminopeptidase N and Dipeptidyl peptidase IV in urine were analyzed using the Proteome Profiler Human Kidney Biomarker Antibody Array (R&D Systems, Minneapolis, USA) and the Bio-Rad ChemiDoc Touch imaging system. Statistical analysis was conducted using descriptive statistics and nonparametric methods, including contingency tables and Spearman's rank correlation coefficient (r). Results with $p < 0.05$ were considered statistically significant.

Results. Urinary Aminopeptidase N and Dipeptidyl peptidase IV levels statistically increased in children with the duration of type 1 diabetes mellitus less than one year. Aminopeptidase N showed moderate correlation with glomerular filtration rate (r=0.589, p=0.044). While Dipeptidyl peptidase IV was strongly positive correlated with glomerular filtration rate (r=0.869, p=0.0001) and weaker correlation with Aminopeptidase N (r=0.467, p=0.126).

Conclusions. Serum creatinine levels rise significantly only 1–5 years after the onset of type 1 diabetes mellitus, making it unsuitable as an early predictor of kidney damage in children with type 1 diabetes mellitus. Both Aminopeptidase N and Dipeptidyl peptidase IV are reliable markers for the early detection of renal injury in children with type 1 diabetes mellitus in north-eastern region of Ukraine. Among these, Dipeptidyl peptidase IV is a preferable non-invasive marker for early kidney damage due to its specific localization in the proximal tubules and glomerular epithelium, as well as its strong positive correlation with glomerular filtration rate.

Keywords. Diabetes mellitus, children, diabetic nephropathy, biomarkers, Aminopeptidase N, DPP IV.

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РЕЗЮМЕ

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ЗНАЧЕННЯ АМІНОПЕПТИДАЗИ N ТА ДИПЕПТИДИЛ-ПЕПТИДАЗИ IV СЕЧІ ДЛЯ РАННЬОЇ ДІАГНОСТИКИ УРАЖЕННЯ НИРОК У ДІТЕЙ ІЗ ЦУКРОВИМ ДІАБЕТОМ 1 ТИПУ У ПІВНІЧНО-СХІДНОМУ РЕГІОНІ УКРАЇНИ

Вступ. У дітей та підлітків діабет має більш агресивний клінічний перебіг порівняно з дорослими. Це характеризується зниженою відповіддю на сучасні методи лікування, швидшим зниженням функції β-клітин, швидким прогресуванням інсулінорезистентності та прискореним розвитком як мікроваскулярних, так і макроваскулярних ускладнень. Діабетична нефропатія є одним з найважливіших та найпоширеніших ускладнень діабету і є основною причиною термінальної стадії ниркової недостатності. Що робить цукровий діабет 1 типу

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особливо актуальною проблемою для дитячих нефрологів. Клінічні прояви діабетичної нефропатії, такі як альбумінурія та зниження швидкості клубочкової фільтрації, зазвичай розвиваються протягом тривалого періоду (10–25 років). Водночас специфічні структурні зміни в нирках, такі як потовщення базальної мембрани клубочків і мезангіальне розширення, можуть виникати значно раніше — через 1,5–5 років після початку діабету. Важливо зазначити, що при діабетичній нефропатії вражаються не лише клубочки, а також канальці. Тубулоінтерстиціальні ураження виникають раніше, ніж ушкодження клубочків, що вказує на те, що тубулярні біомаркери можуть бути більш чутливими для раннього виявлення. Маркери із пептидазною активністю було визнано ефективними для діагностики ранніх пошкоджень канальців. Амінопептидаза N, яка є ектопептидазою, широко експресується в нирках і є визнаним уринарним маркером пошкодження проксимальних канальців. Аналогічно, Дипептидилпептидаза IV експресується у вісцеральних епітеліальних клітинах клубочків, ендотеліальних клітинах та щітковій облямівці проксимальних канальців. Підвищені рівні Дипептидилпептидази IV у сечі відмічалися у діабетичних пацієнтів із нормоальбумінурією, що свідчить про її потенціал як раннього біомаркера початку діабетичної нефропатії.

Мета. Визначити рівень Амінопептидази N та Дипептидилпептидази IV у сечі дітей з північно-східного регіону України залежно від тривалості діабету.

Матеріали та методи. Обстежено 55 осіб, з них 47 дітей з цукровим діабетом 1-го типу та 8 практично здорових дітей без діабету та патології нирок. Пацієнтів було поділено на 3 групи залежно від тривалості захворювання: з вперше виявленим діабетом до одного року – 11 осіб, з перебігом захворювання від 1 до 5 років – 24 дитини, із тривалістю хвороби понад 5 років – 12 осіб. Для визначення інтенсивності хемілюмінесценції Амінопептидази N та Дипептидилпептидази IV використовували Proteome Profiler Human Kidney Biomarker Antibody Array (R&D Systems, Minneapolis, USA) та систему візуалізації сигналу Bio-Rad ChemiDoc Touch. Для статистичного аналізу отриманих даних використовували дескриптивну статистику та непараметричні методи, зокрема таблиці спряженості та коефіцієнт рангової кореляції Спірмена (r). Результати з $p < 0.05$ вважалися статистично значущими.

Результати. Рівні Амінопептидази N та Дипептидилпептидази IV у сечі були статистично підвищені у дітей з тривалістю цукрового діабету 1 типу менше одного року. Амінопептидаза N показала помірну кореляцію зі швидкістю клубочкової фільтрації (r=0.589, p=0.044). Натомість Дипептидилпептидаза IV мала сильну позитивну кореляцію зі швидкістю клубочкової фільтрації (r=0.869, p=0.0001) та помірну кореляцію з Амінопептидазою N (r=0.467, p=0.126).

Висновки. Рівень сироваткового креатиніну значно підвищується лише через 1–5 років після початку цукрового діабету 1 типу, що робить його не придатним для раннього виявлення пошкодження нирок у дітей. На відміну від нього, Амінопептидаза N та Дипептидилпептидаза IV є надійними предикторами ураження нирок у дітей з цукровим діабетом 1 типу у північно-східному регіоні України. Серед них Дипептидилпептидаза IV виділяється як кращий неінвазивний

маркер завдяки специфічній локалізації в проксимальних канальцях та епітелії клубочків, а також вираженій позитивній кореляції зі швидкістю клубочкової фільтрації.

Ключові слова. Цукровий діабет, діти, діабетична нефропатія, біомаркери, Амінопептидаза N, DPP IV.

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ABBREVIATIONS: type 1 diabetes mellitus – T1DM, advanced glycation end products – AGEs, diabetic nephropathy – DN, end-stage renal disease – ESRD, albumin excretion rate – AER, glomerular filtration rate – GFR, Aminopeptidase N – ANPEP, renin angiotensin system – RAS, angiotensin III – Ang III, angiotensin IV – Ang IV, Dipeptidyl peptidase IV – DPP IV, body mass index – BMI, enzyme-linked immunosorbent assay – ELISA, type 2 diabetes mellitus – T2DM, insulin-like growth factor-1 – IGF-1, nitric oxide – NO, vascular endothelial growth factor – VEGF, sodium-glucose transport protein 2 – SGLT2, nicotinamide adenine dinucleotide phosphate – NADPH, transforming growth factor-β1 – TGF-β1, reactive oxygen species – ROS, reactive nitrogen species – RNS, protein kinase C – PKC, mitogen-activated protein – MAP, nuclear factor-kB – Nf-kB, extracellular matrix proteins – ECM, mannose-6-phosphate/insulin-like growth factor 2 receptor – M6P/IGF2-R, glucagon-like peptide – GLP-1, microRNA-29 –miR29, vascular endothelial growth factor receptor type 1 – VEGFR1, endothelial-mesenchymal transition – EndMT

INTRODUCTION

Diabetes mellitus remains a significant global health concern. According International Diabetes Federation in 2021, approximately 537 million people worldwide are living with diabetes, a number expected to increase to 643 million by 2030 and 783 million by 2045. The prevalence of diabetes among children and adolescents (under 19 years old) is also steadily rising, with more than 1.2 million affected by type 1 diabetes mellitus (T1DM). The European Region has the highest number of children and adolescents with T1DM, totaling 295,000 [1].

In children and adolescents, diabetes tends to progress more aggressively than in adults. It is characterized by a weaker response to current treatments, rapid decline in β-cell function, accelerated insulin resistance, and early development of microvascular and macrovascular complications. Elevated levels of advanced glycation end products (AGEs) contribute to the activation of several pathological processes, such as inflammation, oxidative stress, endothelial dysfunction, and fibrosis. These mechanisms lead to structural changes in the glomeruli and renal tubules, negatively impacting kidney function [2, 3].

Diabetic nephropathy (DN) is one of the most common and severe complications of diabetes, as well as a leading cause of end-stage renal disease (ESRD). This makes the management of T1DM especially critical for pediatric nephrologists. Structural kidney changes associated with DN, such as thickening of the glomerular basement membrane and mesangial expansion, may appear within the first few years after the onset of diabetes. However, clinical symptoms of DN often remain absent for 10–15 years. The gold standard for DN diagnosis includes measuring urinary albumin excretion rate (AER) and glomerular filtration rate (GFR). Microalbuminuria (AER 30–300 mg/24 hr) occurs in 26% of children after 10 years of diabetes and in 51% of adolescents after 19 years of diabetes, while macroalbuminuria (AER >300 mg/24 hr) is observed in 14% of children with T1DM after a median duration of 10 years. Hyperfiltration (GFR 120–150 mL/min/1.73 m^2) is detected in 25–40% of young individuals with diabetes and is considered a strong predictor of GFR decline and DN progression [4]. Clinical manifestations, albuminuria and decrease GFR, usually emerge over a prolonged period of 10 to 25 years. While specific structural changes in the kidney in DM patients, namely thickening of the glomerular basement membrane and mesangial expansion are often detected earlier, typically within 1.5 to 5 years of diabetes onset [3].

So, traditional laboratory panels based on markers that describe glomerular structure damage (measurement of albuminuria and GFR) cannot be considered a standard for the early detection of DN. DN is not only a disease of the glomeruli but also involves damage to the tubules, and tubulointerstitial lesions occur earlier than glomerular injury, so tubular biomarkers may be more sensitive. Early tubular injury can be detected using the following markers with peptidase activity.

Aminopeptidase N (ANPEP) is an ectopeptidase, widely expressed in the kidneys, and urinary ANPEP is a determined marker of the proximal tubule damage. ANPEP is involved in the regulation of tissue and

systemic renin angiotensin system (RAS) through hydrolysis of the N-terminal arginine angiotensin III (Ang III) to form angiotensin IV (Ang IV). ANPEP takes part in the process of regulating sodium excretion and, therefore, salt sensitivity and protection against hypertension [5–7].

Dipeptidyl peptidase IV (DPP IV) is a glycoprotein with serine exopeptidase activity. Its involved in immune system modulation, activation of intracellular signal transduction pathways, natriuresis, cell–cell interactions, and cellular interactions with the extracellular matrix. In the kidney, DPP IV is expressed by glomerular visceral epithelial cells, endothelial cells and the brush border of proximal tubule. Increased urinary DPP IV was observed in diabetic patients with normoalbuminuria, suggesting that urinary DPP IV levels may serve as a biomarker for early onset DN [5,8–10]. There aren't publications on PubMed Central® which are associated with combination of the such three phrases: peptidase, diabetic nephropathy, children. Only DPP IV inhibitors were mentioned in diabetic guidelines for adults [11,12].

The aim of this study is to explore the characteristics of ANPEP and DPP IV levels in the urine of children from north-eastern region of Ukraine, in relation to the duration of T1DM.

MATERIALS AND METHODS

A total of 55 participants (22 girls and 33 boys), aged 7 to 17 years, with a mean age of 13.7±0.4 years. Among them, 47 children had type 1 diabetes mellitus (T1DM), and they were divided into three groups based on the duration of the disease: less than 1 year (11 participants), 1-5 years (24 participants), and more than 5 years (12 participants). The comparison group consisted of eight children without T1DM and kidney disease. The study was conducted in compliance with the ethical principles outlined in the Declaration of Helsinki, adopted at the 18th WMA General Assembly in Helsinki, Finland, in June 1964, and amended at the 52nd WMA General Assembly in Edinburgh, Scotland, in October 2000. The research received approval from the Ethics Committee of Sumy State University[13].

Parents and children were informed about the study's purpose and provided written consent to participate. At the time of the study the diagnosis of T1DM was determined according to the Ministry of Health of Ukraine's Order No. 254 (dated April 27, 2006) on medical care provision in the field of "Pediatric Endocrinology" [14]. The "Bedside Schwartz" formula based on creatinine was used to estimate GFR, which, according to the National Kidney Foundation (USA), is considered the best method of assessing GFR in children (https://www.kidney.org/professionals/kdoqi/gfr_calculatorPed). Body mass index (BMI) was determined to detect obesity in children (https://kidshealth.org/en/parents/bmi-charts.html).

The urine sample from involved children was carried out in the Communal non-profit enterprise of Sumy Regional Council "Regional Children's Clinical Hospital". The samples were centrifuged, frizzed and stored at −20°C until providing an analysis. We analyzed intensity of the chemiluminescence of ANPEP and DPP IV in the urine of children depending on the T1DM duration using a Proteome Profiler Human Kidney Biomarker Antibody Array (R&D Systems, Minneapolis, USA). This assay is similar to an enzyme-linked immunosorbent assay (ELISA), but uses a membrane instead of a plate. The analysis procedure involved diluting urine samples, mixing with biotinylated detection antibodies, and incubating with the membranes overnight. Streptavidin-Horseradish Peroxidase and chemiluminescent detection reagents were then applied, generating a signal at each capture sport. The capture antibodies were immobilized on the membrane in duplicate spots for each biomarker. Chemiluminescence signals from each spot of the membranes were detected with BioRad ChemiDoc Touch system (https://www.bio-rad.com/) and analyzed semi-quantitatively using BioRad Image Lab Software. We pooled urine samples from each group based on T1DM duration (< 1 year, 1-5 years, > 5 years) and comparison (control) group. The resulting data were processed using GraphPad Prism 7.04 (https://www.graphpad.com/) and Microsoft Excel 2016 software package.

Statistical analysis included descriptive statistics (mean (M), the mean error (m), confidence interval (CI)) and non-parametric methods (contingency tables for difference between the comparison group and patients with T1DM). The statistical relationship between the rankings of two variables was evaluated using Spearman's rank correlation coefficient (r). Statistically significant differences were indicated by p values <0.05.

RESULTS

Table 1 summarizes the clinical and demographic characteristics of the studied population [15]. The findings revealed a predominance of males with T1DM across all groups, with an overall male-to-female ratio of 1.76:1. The mean age of the patients with T1DM was 13.73±0.41 years.

Serum creatinine is the most widely used marker of the GFR for assessing renal function, but his levels can be influenced by age, sex, muscle mass, diet and chronic illness [16]. Serum creatinine levels were significantly elevated in two groups of children with T1DM (1–5 years and more 5 years of diabetes duration).

Table 1 – Clinical and demographic characteristics of studied groups (M ± m, CI)

Variables	Comparison group	Duration of T1DM		
		< 1 year	1-5 years	> 5 years
Number of subjects, n (male/female)	8 (3/5)	11 (6/5)	24 (17/7)	12 (7/5)
Age (years)	12.50±0.76 10.71-14.29	12.27±1.02 10.00-14.54	13.63±0.65 12.28-14.97	16.08±0.38 15.25-16.92
Duration of DM (years)	-	0.8±0.06 0.7-0.9	3.6±0.25 3.08-4.12	11.9±0.97 9.76-14.04
Creatinine (μmol/l)	80.24±4.47 69.68-90.79	86.22±3.92 77.35-95.09	97.52±6.26 84.57-110.47 p=0.032	96.36±6.18 75.90-116.82 p=0.049
GFR (ml/min/1.73m^2)	108.49±15.75 71.25-145.74	88.38±7.58 71.49-105.26	97.53±6.84 83.38-111.68	96.46±10.42 73.51-119.40
BMI	19.07±1.04 17.03-21.11	17.45±1.3 14.85-20.01	19.08±0.6 18.62-20.26	19.83±0.86 18.14-21.52

Notes: p – statistical significance relative to the comparison group

GFR, calculated using the Schwartz creatinine-based formula showed no significant changes among patients with varying T1DM duration relative to the comparison group. Consequently, serum creatinine and creatinine-based GFR were not effective to predict the occurrence of hyperfiltration in children with T1DM.

The analysis of pixel density (Table 2) showed that urinary levels of ANPEP and DPP IV were statistically increased in the very first year of diabetes onset in children (Figure 1).

Table 2 – Pixel density of the markers depending on the duration of T1DM in children (M, SD)

	ANPEP		DPP IV	
	mean (M)	standard deviation (SD)	mean (M)	standard deviation (SD)
Control	169173	14013.44	73854	9584.125
Less 1 year	441142*	29144.11	257674.5*	8864.998
1–5 year	544086*	11811.51	274522.5*	6866.714
More 5 year	468958.5*	26531.35	342810*	20924.7

*Notes: *– statistical significance relative to the comparison group, p <0.05*

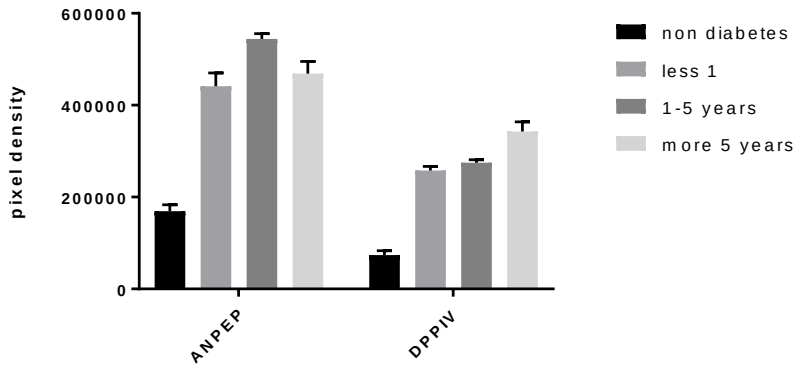

Figure 1 – The intensity of the chemiluminescence signal of the markers

The comparison of the two markers ANPEP and DPP IV (Table 3) reveals that DPP IV increase relatively early – already during 1st year of onset of T1DM – but also to a greater extent throughout the entire observation period.

Table 3 – Increase in the intensity of signal/pixel density depending on the duration of T1DM in children

	Duration of T1DM		
	< 1 year	1–5 years	> 5 years
ANPEP	2.6 times*	3.2 times*	2.7 times*
DPP IV	3.5 times*	3.7 times*	4.6 times*

*Notes: * – statistical significance relative to the comparison group, p <0.05*

DPP IV shows a strong positive correlation with GFR (r=0.869, p=0.0001), in contrast to ANPEP, where the correlation is moderate (Table 4). Thus, both indicators increase with rising GFR and may use as markers of hyperfiltration as an early sign of kidney damage. The stronger correlation of GFR with DPP IV may be due to the fact that this exopeptidase is located not only in the brush border of the proximal tubule but also in glomerular visceral epithelial cells. As a result, DPP IV may have a weaker correlation with ANPEP, which is predominantly localized in the proximal tubule. So, DPP IV is a preferable non-invasive marker of early kidney damage in children with T1DM.

Table 4 – Spearman's rank correlation coefficient (r) between GFR and urinary markers in children with T1DM

	r
ANPEP: DPP IV	0.467 p=0.126
GFR: ANPEP	0.589* p=0.044
GFR: DPP IV	0.869* p=0.0001

*Notes: * – statistical significance between parameters*

DISCUSSION

DN is increasingly encountered in children and requires earlier identification of the disease process to allow more intensive intervention and improved treatment [1]. Microalbuminuria and decreased GFR are considered the first markers of renal failure in diabetic patients. But, it is necessary to take into account the growing prevalence of the nonalbuminiric phenotype among people with T1DM and type 2 diabetes mellitus (T2DM). Several biomarkers of kidney injury are present early and precede the onset of albuminuria in diabetic patients [17–19].

Hyperglycemia activates metabolic and hemodynamic pathological processes that contribute to the proliferation and hypertrophy of renal cells. Diabetic condition triggers release of insulin-like growth factor-1 (IGF-1), glucagon, nitric oxide (NO), prostaglandin, and vascular endothelial growth factor (VEGF), which cause vasodilatory effect in the afferent arterioles of the glomeruli. Increases of the endothelin-1 secretion leading to glomerular basement membrane thickening, glomerular endothelial cell dysfunction, podocyte foot process effacement, loss of glycocalyx and mesangial expansion. In tubular epithelial cells hyperglycemia induces upregulation of sodium-glucose transport protein 2 (SGLT2), leading to increased reabsorption glucose, hypoxic injury and causes AGE production. Activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase causing increased intracellular oxidative stress, mitochondrial dysfunction, endothelial injury and RAS activation. RAS hyperactivity promotes renal tissue fibrosis through mediators such as transforming growth factor-β1 (TGF-β1), angiotensin II, and aldosterone. Overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) activates protein kinase C (PKC), mitogen-activated protein (MAP) kinase, and nuclear factor-kB (Nf-kB) which eventually results in overproduction of extracellular matrix proteins (ECM) [2, 20–22].

The renal tubule and interstitium are crucial in the pathogenesis of DN and are closely linked to the progressive decline in renal function [23]. Accompanied by inflammation, oxidative stress, and disturbance in blood flow, renal tubular epithelial cells demonstrate proliferation with subsequent hypertrophy and finally cell death [24, 25].

Tubular damage appears in the early stages of DN and some markers of proximal tubular cell injury can be detected in the urine of early diabetic patients. ANPEP is a renal tubular brush border enzyme, its participate in the regulation of RAS. Renal ANPEP generates Ang IV, which reduces basolateral tubular Na^+/K^+-ATPase activity, hence increasing sodium excretion. Urinary excretion of ANPEP is a potential biomarker for the early diagnosis of proximal tubule brush border injury [7, 23]. Mitic B. et al. have found that urinary ANPEP activity was significantly ($p< 0.01$) higher in both type 1 and type 2 diabetic patients compared to healthy controls [26]. In the kidney, ANPEP contributes to the extracellular catabolism of glutathione [27], so may influence on oxidative stress activity and progression of DN. ANPEP is an indicator of the development of diabetic vascular

complications, i.e. retinopathy, among patients without proteinuria [28].

We found a moderate correlation between GFR, measured by creatinine clearance, and increased urinary excretion of ANPEP was observed. The data could suggest that increased urinary ANPEP activity early represent renal injury despite normal or increased GFR. In the kidney, ANPEP represents approximately 8% of the brush border membrane protein [27], that's why we should mention that no less than this percentage is affected in case of enzyme increasing. Since ANPEP is a zinc-dependent metalloproteinase, studying zinc levels and related trace elements is essential for understanding the pathogenesis of DN.

DPP IV is involved in immune system modulation, cellular interactions with the extracellular matrix, natriuresis and activation of intracellular signal transduction pathways. The urinary DPP IV activity was significantly higher in patients with T2DM and albuminuria compared to patients with non-albuminuric diabetes or healthy people [8]. It is expressed predominantly in the glomeruli and S1 to S3 segments of the proximal tubules where regulates the absorption of cleaved dipeptides. In the proximal tubules DPP IV facilitates the function of sodium/hydrogen exchanger-3 [29]. So, strong positive correlation between urinary DPP IV and GFR indicates possible affection of abovementioned kidney areas in case of elevation DPP IV in the early stage of T1DM despite normal or increased GFR. Unlike ANPEP, which is localized only in the proximal tubules, increased urinary excretion of DPP IV, which is found on the brush border of the proximal tubule and in glomerular visceral epithelial cells, may indicate not only damage to the proximal tubule epithelium but also injury of glomerular cells, thereby increasing the informational value of its measurement in urine. Enzyme binds to the mannose-6-phosphate/insulin-like growth factor 2 receptor (M6P/IGF2-R) to initiate signal transduction. DPP IV cleaves glucagon-like peptide (GLP-1) and glucose-dependent insulinotropic peptide, which are responsible for the enhancing of insulin secretion. This data describes direct influence of DPP IV on insulin metabolism. This is the basis for the usage of DPP IV inhibitors in case of diabetes [9].

DN is associated with increased expression of surface DPP IV on endothelial and tubular epithelial cells, through decreased levels of microRNA-29 (miR29) in hyperglycemic environment. Activated DPP IV interacts with integrin β1 and induces its phosphorylation. The resulted complex stimulates the activation vascular endothelial growth factor receptor type 1 (VEGFR1) and dimerization of the TGFβ receptor, which eventually stimulates endothelial-mesenchymal transition (EndMT) and the development of diabetic vascular complications [20, 30, 31].

According to the Diabetes Control and Complications Trial in T1DM patients, 12.9% of patients did not have diabetic retinopathy progression, but had DN development; 10.7% showed progression of retinopathy, without developing DN, and 7.3% of patients experienced both diabetic retinopathy progression and DN development [32]. So, urinary DPP IV monitoring on the early stage of T1DM in children can avoid such complication as diabetic retinopathy and DN.

Our study has showed elevated urinary DPP IV levels, which may indicate kidney damage (proximal tubule and glomerular epithelium) in children with T1DM.

CONCLUSIONS

Serum creatinine cannot serve as an early predictor of DN because its level showed significant elevation only between 1 to 5 years compared to the initial manifestation of T1DM in the children from north-eastern region of Ukraine. Both investigated peptidases are suitable for early detection of kidney damage in the children with T1DM. DPP IV is a preferable non-invasive marker of early kidney damage in children with T1DM due to its localization (proximal tubule and glomerular epithelium) and strong positive correlation with GFR.

PROSPECTS FOR FUTURE RESEARCH

We would like to evaluate the levels of kidney damage biomarkers in the urine of each patient with T1DM on an individual basis.

AUTHOR CONTRIBUTIONS

Concept and design of research – AL, writing the first version – IV, IZ, final approval of the version for publication – AL, agree to be responsible for all aspects of the work – IV.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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