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Value of urinary adiponectin, VCAM-1 and RBP 4 in early diagnosis of kidney damage in children with type 1 diabetes mellitus

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Key words: diabetes mellitus, diabetic nephropathy, children, biomarkers, adiponectin, VCAM-1, RBP 4.

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Aim. The aim of the current study was to investigate urinary adiponectin, VCAM-1, and RBP 4 levels in children depending on the diabetes duration.

Materials and methods. The study involved 55 subjects, including 47 children with type 1 diabetes mellitus and eight children without diabetes and kidney disease history. Participants with diabetes were stratified into three groups, depending on the diabetes duration: <1 year (11 people), 1–5 years (24 people) and >5 years (12 people). According to the Order of the Ministry of Health of Ukraine, dated April 27, 2006, No. 254 on providing medical care to children in the specialty "Pediatric Endocrinology", we examined the children and diagnosed type 1 diabetes mellitus. Chemiluminescence signals of adiponectin, VCAM-1, and RBP4 in urine were analyzed with Bio-Rad ChemiDoc Touch using a Proteome Profiler Human Kidney Biomarker Antibody Array (R&D Systems, Minneapolis, USA). We used descriptive statistics and nonparametric methods (contingency tables and Spearman's rank correlation coefficient (r)) for the statistical analysis of study materials. Statistically significant differences were indicated by P values <0.05.

Results. Urinary adiponectin, VCAM-1, and RBP 4 levels statistically increased within the first year after diagnosing type 1 diabetes in children. Adiponectin was strongly correlated with VCAM-1 (r = 0.636, P = 0.026), and RBP 4 (r = 0.650, P = 0.022). Urinary adiponectin levels showed a statistically significant correlation with GFR (r = 0.007).

Conclusions. Serum creatinine and GFR are ineffective as diagnostic indicators of kidney damage in children with diabetes mellitus at the incipient stages. Adiponectin in children's urine can be used as a non-invasive kidney damage marker in the early years of type 1 diabetes. Adiponectin, VCAM-1, and RBP 4 measurements would allow an early prediction and evaluation of both tubular and glomerular kidney damage in children with diabetes.

Ключові слова: цукровий діабет, діабетична нефропатія, діти, біомаркери, адипонектин, VCAM-1, RBP 4.

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Значення адипонектину, VCAM-1 і RBP 4 сечі для ранньої діагностики ураження нирок у дітей із цукровим діабетом 1 типу

І. О. Віхрова, А. М. Лобода

Мета роботи – дослідити рівень адипонектину, VCAM-1 та RBP 4 у сечі дітей залежно від тривалості діабету.

Матеріали та методи. Здійснили комплексне обстеження 55 осіб: 47 дітей із цукровим діабетом 1 типу та 8 практично здорових дітей без діабету, репрезентативних за віком та статтю. Залежно від тривалості захворювання пацієнтів поділили так: з уперше виявленим цукровим діабетом 1 типу – 11 осіб, із тривалістю хвороби від 1 до 5 років – 24 дитини, з перебігом захворювання понад 5 років – 12 осіб. Дітей обстежили згідно з наказом МОЗ України № 254 від 27.04.2006 р. щодо надання медичної допомоги дітям за спеціальністю «Дитяча ендокринологія». Проаналізували інтенсивність хемілюмінесценції адипонектину, VCAM-1 і RBP 4 за допомогою Bio-Rad ChemiDoc Touch, використовуючи Proteome Profiler Human Kidney Biomarker Array (R&D Systems, Minneapolis, USA). Матеріал для дослідження біомаркерів – сеча здорових дітей і хворих на цукровий діабет 1 типу. Для статистичного аналізу результатів використовували дескриптивну статистику та непараметричні методи (таблиці спряженості, коефіцієнт рангової кореляції Спірмена).

Результати. Рівень адипонектину, VCAM-1 і RBP 4 у сечі дітей статистично збільшувався вже в перший рік захворювання на цукровий діабет 1 типу. Уринарний адипонектин показав істотну динаміку протягом усього періоду дослідження. Рівень адипонектину в сечі значно корелював із VCAM-1 (r = 0,636, p = 0,026) і RBP 4 (r = 0,650, p = 0,022). Коефіцієнт кореляції Спірмена між швидкістю клубочкової фільтрації та уринарними маркерами показав статистично значущу кореляцію тільки з адипонектином (p = 0,007).

Висновки. Сироватковий креатинін і швидкість клубочкової фільтрації не є ефективними діагностичними показниками пошкодження нирок на ранніх етапах захворювання на цукровий діабет 1 типу в дітей. Визначення рівня адипонектину в сечі дітей можна використовувати як неінвазивний індикатор ураження нирок у перші роки захворювання на цукровий діабет 1 типу. Визначення вмісту адипонектину, VCAM-1 і RBP 4 у сечі дасть змогу на ранніх стадіях прогнозувати й оцінювати стан клубочкових і тубулярних ниркових структур у дітей із цукровим діабетом 1 типу.

Значение адипонектина, VCAM-1 и RBP 4 мочи в ранней диагностике повреждения почек у детей с сахарным диабетом 1 типа

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Цель работы – изучить уровень уринарного адипонектина, VCAM-1 и RBP 4 у детей в зависимости от продолжительности диабета.

Материалы и методы. Проведено комплексное обследование 55 человек: 47 детей с сахарным диабетом 1 типа и 8 детей без диабета, репрезентативных по возрасту и полу. В зависимости от продолжительности заболевания пациентов поделили так: с впервые диагностированным сахарным диабетом 1 типа – 11 человек, с продолжительностью заболевания от 1 до 5 лет – 24 ребенка, течением болезни больше 5 лет – 12 детей. Детей обследовали согласно приказу МОЗ Украины № 254 от 27.04.2006 г. об оказании медицинской помощи детям по специальности «Детская эндокринология». Проанализировали интенсивность хемилюминесценции адипонектина, VCAM-1 и RBP 4 с помощью Bio-Rad ChemiDoc Touch, используя Proteome Profiler Human Kidney Biomarker Array (R&D Systems, Minneapolis, USA). Материал для исследования биомаркеров – моча здоровых детей и больных сахарным диабетом 1 типа. Для статистического анализа использовали дескриптивную статистику и непараметрические методы (таблицы сопряженности, коэффициент ранговой корреляции Спирмена).

Результаты. Уровень адипонектина, VCAM-1 и RBP 4 в моче детей статистически увеличивался уже в первый год заболевания сахарным диабетом 1 типа. Адипонектин мочи показал значительную динамику на протяжении всего периода исследования. Уровень адипонектина значительно коррелировал с VCAM-1 ($r = 0,636$, $p = 0,026$) и RBP 4 ($r = 0,650$, $p = 0,022$). Коэффициент корреляции Спирмена между скоростью клубочковой фильтрации и маркерами в моче показал статистически значимую корреляцию только с адипонектином ($p = 0,007$).

Выводы. Сывороточный креатинин и скорость клубочковой фильтрации неэффективны в качестве диагностических маркеров повреждения почек на ранних этапах заболевания сахарным диабетом 1 типа у детей. Определение уровня адипонектина в моче детей может быть использовано как неинвазивный индикатор повреждения почек в первые годы заболевания сахарным диабетом 1 типа. Определение содержания адипонектина, VCAM-1 и RBP 4 в моче позволяет на ранних стадиях прогнозировать и оценивать состояние клубочковых и тубулярных почечных структур у детей с сахарным диабетом 1 типа.

Ключевые слова: сахарный диабет, диабетическая нефропатия, дети, биомаркеры, адипонектин, VCAM-1 и RBP 4.

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Diabetes mellitus is a global health problem resulting in social and economic effects. In 2019, according to the International Diabetes Federation, 463 million adults lived with diabetes, and this number is supposed to reach 578 million by 2030. More than 1.1 million children suffer from type 1 diabetes mellitus (T1DM). The overall annual increase is estimated to be around 3 %. The age-sex standardized incidence rate of T1DM in Ukrainian children and adolescents aged 0–14 years lies between 5–10 per 100,000 population per annum, and it is not so high as in the other EU countries such as Finland (62.3) or Sweden (43.2) [1]. However, the possibility of early formation (in five years of the disease duration) of micro- and macrovascular complications makes the problem of T1DM extremely actual.

Diabetic nephropathy (DN) is one of the most common complications of diabetes and the leading cause of the end-stage renal disease (ESRD). The prevalence of DN among US children with T1DM is 5.8 % [2]. DN is caused by alterations in the glomerular and tubular structure and function under the influence of hyperglycemia, high blood pressure, and generates advanced glycation end products (AGEs) [3]. Uncertainty remains regarding the diagnosis of early pathological changes in the kidneys of children with diabetes. Non-invasive methods of routine screening for DN in children and adolescents with T1DM must be preferable. The gold standard for DN identification consists in the measurement of urine albumin levels. Microalbuminuria (30–300 mg/24 hr) or macroalbuminuria (>300 mg/24 hr) are conventional biomarkers of DN and its progression to ESRD, but kidney structural damage might precede the albumin excretion [2,4]. Nowadays, significant numbers of novel biomarkers were detected in urine and can be used for early identification of DN, thus improving the in-time interpretation of the disease stage and adjusting therapy, unlike with traditional diagnostics.

Adiponectin is an anti-inflammatory cytokine produced by adipose tissue, and urinary adiponectin is an independent predictor of end-stage renal disease in diabetic patients Adiponectin reduces angiotensin-induced inflammation, has an anti-fibrotic function, and decreases oxidative stress in renal-cells [5,6]. Vascular cell adhesion molecule-1 (VCAM-1) is a glycoprotein expressed by endothelial cells and is a major regulator of leukocyte adhesion through interaction with α4β1 integrin [7]. VCAM-1 expression is activated by pro-inflammatory cytokines, high glucose concentration, and stress [8]. Its expression increases in the kidneys of DN patients and correlates with albuminuria in diabetic patients. VCAM-1 urine levels indicate an early inception of DN in T1DM patients [9].

Retinol-binding protein 4 (RBP 4) represents the family of lipocalins produced in the liver and mature adipocytes. Its filtration processes occur in the glomerulus, and then the RBP 4 is almost completely reabsorbed in the renal proximal tubules. Some sources claim, that an increase in RBP 4 in the urine was observed in diabetes patients with macro- and microvascular complications, which confirmed the predictive role of RBP 4. Increased urinary RBP 4 levels were also present in patients with diabetes and normal albumin in urine [10,11].

Aim

The aim of this study was to investigate the features of urinary adiponectin, VCAM-1, and RBP 4 levels in children depending on the diabetes duration.

Materials and methods

The study involved 55 participants (22 girls and 33 boys) aged from 7 to 17 years, the mean age was 13.7 ± 0.4 years, including 47 patients with T1DM and eight children without diabetes.

Children with T1DM were divided into three groups depending on the diabetes duration: <1 year (11 people), 1–5 years (24 people) and >5 years (12 people). The comparison group included individuals without T1DM and with urine clinical analysis within normal physiological ranges.

Complying with the Declaration of Helsinki (adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964 held in Helsinki, Finland in June 1964, and amended by the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000 and revised in October 2000, Edinburg, Scotland), the study was performed following the approval of the Ethics Committee of Sumy State University. The children and their parents were informed of the study purpose and gave a written informed consent to participate in the study.

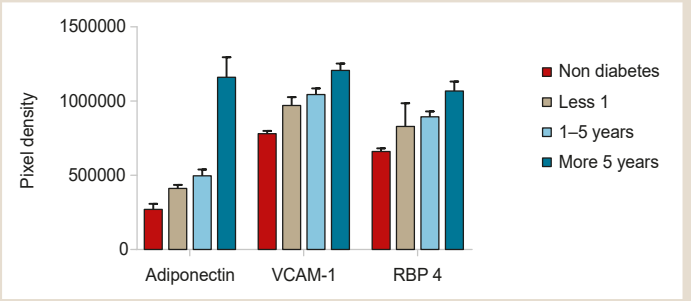

Fig. 1. The intensity of the chemiluminescence signal of the markers.

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

Variables, units	Comparison group	Duration of T1DM		
		less than a year	1–5 years	over 5 years
Number of subjects, n (male/female)	8 (3/5)	11 (6/5)	24 (17/7)	12 (7/5)
Age, years old	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	–	0.80 ± 0.06 0.7–0.9	3.60 ± 0.25 3.08–4.12	11.90 ± 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/1.73 m^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

P: statistical significance relative to the comparison group.

Table 2. 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
Adiponectin	1.5 times*	1.7 times*	3.9 times*
VCAM-1	1.3 times*	1.4 times*	1.6 times*
RBP 4	1.3 times*	1.3 times*	1.5 times*

*: statistical significance relative to the comparison group.

Table 3. Spearman's rank correlation coefficient (r) between GFR and urinary markers in children with T1DM

	r
GFR: Adiponectin	0.734*; P = 0.007
GFR: VCAM-1	0.345; P = 0.271
GFR: RBP 4	0.329; P = 0.189

*: statistical significance between parameters.

We diagnosed T1DM according to the Order of the Ministry of Health of Ukraine dated April 27, 2006, No. 254 on providing medical care to children in the specialty "Pediatric Endocrinology" [12]. Creatinine-based "bedside Schwartz" formula was used to estimate glomerular filtration rate (GFR). According to the National Kidney Foundation (USA), this formula is currently considered to be the best method for assessing GFR in children (https://www.kidney.org/professionals/kdoqi/gfr_calculatorPed).

The first morning urine sample from the enrolled children was collected in the Regional Children's Clinical Hospital in Sumy, centrifuged, and stored at -20 °C until providing an analysis. We analyzed the chemiluminescence intensity for urine adiponectin, VCAM-1 and RBP 4 in the children depending on the T1DM duration using a Proteome Profiler Human Kidney Biomarker Antibody Array (R&D Systems, Minneapolis, USA) (https://www.rndsystems.com/products/proteome-profiler-human-kidney-biomarker-array-kit_ary019). This assay is analogous to enzyme-linked immunosorbent assay (ELISA), but uses a membrane as a substrate rather than a plate. Urine samples were diluted, mixed with biotinylated detection antibodies, and incubated overnight with the membranes. Streptavidin-horseradish peroxidase and chemiluminescent detection reagents were applied, and a signal was amplified at each capture spot. Capture antibodies were spotted onto the membrane, with each pair of spots representing a different analyte. The membranes contained duplicate spots that represented each biomarker. The chemiluminescence signals (pixel density) from each spot of the membranes were detected with Bio-Rad ChemiDoc Touch (https://www.bio-rad.com/) and analyzed semi-quantitatively using Bio-Rad Image Lab Software. We pooled urine samples from each group (less than 1 year, 1–5 years, over 5 years, and the comparison). The data obtained from the study were analyzed using the GraphPad Prism 7.04 (https://www.graphpad.com/) and Microsoft Excel 2016 software package.

For statistical analysis of the study materials, we used descriptive statistics (mean (M), the mean error (m), confidence interval (CI)), and nonparametric methods (contingency tables for the difference between the comparison group and patients with T1DM). We calculated the statistical dependence between the rankings of two variables using Spearman's rank correlation coefficient (r). Statistically significant differences were indicated by P values <0.05.

Results

Table 1 represents clinical and demographic characteristics of the examined population. The study indicated the prevalence of males with T1DM in all groups, the general male-to-female ratio was 1.76 : 1.00. Such gender difference with a male excess is typical for populations of European origin [13]. The mean age of examined patients with T1DM was 13.73 ± 0.41 years. This result confirms that the highest incidence of T1DM is observed in 10–14-year-old patients [13].

Serum creatinine can not be an early predictor of DN because its level significantly elevated only between 1 to 5 years compared to the initial manifestation of T1DM in children.

One of the characteristic early renal functional changes in of DN includes hyperfiltration [14]. GFR, calculated according to the Schwartz creatinine-based formula, did not demonstrate any significant changes in patients with different duration of T1DM relative to the comparison group. That is why this marker and creatinine-based GFR were not effective to predict the occurrence of hyperfiltration in children with T1DM.

The analysis of contingency tables showed that urinary levels of adiponectin, VCAM-1, and RBP 4 were statistically increased in the very first year of diabetes onset in children (*Fig. 1*).

However, the urine adiponectin levels demonstrated greater dynamics throughout the study (*Table 2*).

Thus, we recommend this marker as a first line non-invasive indicator of kidney damage in children with T1DM. Urinary adiponectin was strongly correlated with

VCAM-1 (r = 0.636, P = 0.026) and RBP 4 (r = 0.650, P = 0.022). Therefore, their high levels may be foreseen in case of increased concentration of adiponectin in the urine. However, a statistically significant correlation with GFR was relevant only for urinary adiponectin. Neither VCAM-1 nor RBP 4 indicated changes of GFR (*Table 3*).

Thus, urinary adiponectin is more indicative of glomerular dysfunction in patients with T1DM, while VCAM-1 and RBP 4 illustrate the tubular apparatus impairment. The subsequent development of a biomarker panel that contains these three biomarkers would reliably and early predict and evaluate both tubular and glomerular kidney damage in children with T1DM.

Discussion

The prevalence of diabetes is increasing and has already pandemic proportions. Diagnosis of DN at incipient stages can improve treatment and the quality of life in patients with T1DM [1–3]. Albuminuria is a standard biomarker for the diagnosis of DN, but it has different limitations. Several biomarkers of renal dysfunction can precede microalbuminuria, assuming its presence after overt kidney failure has already occurred [15]. Hyperglycemia leads to the formation of AGEs that contribute to the proliferation and hypertrophy of renal cells. There occurs an activation of NADPH oxidase and increased release of reactive oxygen species (ROS), which activates protein kinase C (PKC), a mitogen-activated protein (MAP), and NF-kB resulting in the overproduction of extracellular matrix proteins. Extracellular matrix accumulation in the tubular cells may be a significant factor for renal failure progression in diabetic patients [3,16]. Nevertheless, both renal tubules and glomeruli are heavily involved in the pathogenesis of DN [10]. We have identified several glomerular and tubular biomarkers predicting DN onset or progression in line with this. They are essential in early diagnostics, especially in normoalbuminuric diabetic patients with normal GFR [10,17].

Decreased concentration of plasma adiponectin is associated with insulin resistance in diabetes and metabolic syndrome [6]. Adiponectin prevents kidney podocytes from cell death by apoptosis, therefore, it is involved in recovery of renal function in diabetic conditions [18]. It also protects both kidney glomeruli and tubules. Glomerular cells express adiponectin receptor 1 (AdipoR1). AdipoR1 can activate adenosine monophosphate-activated protein kinase (AMPK) and plays a vital role in controlling oxidative stress and cell survival within the glomerulus [6,19]. The serum level of adiponectin was significantly elevated in children with poor T1DM control; it was directly associated with HbA1c and negatively correlated with the disease/year [20]. Elevated adiponectin levels in urine were associated with rapid GFR decline, CKD incidence, and persistent DN over six years in adults with T1DM [5]. Yamakado S. et al. have found that urinary adiponectin could be a new diagnostic index for assessing CKD related to diabetic nephropathy in adults [21]. Our study has established an essential role of urinary adiponectin as an early marker of glomerular damage in pediatric patients with T1DM.

Hyperglycemia impairs vascular endothelial cell function, probably in part owing to oxidation of low-density lipoprotein (LDL) and increased formation of free radicals. Free radicals stimulate the release of proinflammatory cytokines, which induce the expression of adhesion molecules, including VCAM-1 [22]. VCAM-1 expression is directly activated by high glucose concentration [8]. Urine proteome analysis indicates that VCAM-1 is the most relevant protein for earlier stages DN diagnosis. The renal filtration function declines, and urinary albumin excretion levels increase progressively with an elevation in serum VCAM-1 levels [9,23,24]. VCAM-1 level was significantly higher in microalbuminuric patients, so it may be used as a predictive marker for risk stratification for nephropathy development and progression [24]. However, there are no studies that describe the role of urinary VCAM-1 in DN in children with T1DM. Therefore, our finding of VCAM-1 as a marker of kidney tubular apparatus lesion is novel and important.

Similar to adiponectin, RBP 4 is involved in mechanisms of insulin resistance. It induces an enzyme expression in hepatocytes (mainly phosphoenolpyruvate carboxykinase) in the gluconeogenesis and impairs insulin signaling pathways in skeletal muscle [11,25]. Serum RBP 4 positively correlates with diabetes duration, glucose level, HbA1c, and urine albumin-to-creatinine ratio (ACR). Negative correlations between serum RBP 4 and GFR illustrate that a decrease in GFR could lead to accumulation of RBP 4 in the systemic circulation [11]. This marker is filtered at the glomerulus and entirely reabsorbed in the proximal tubule. Thus, RBP 4 is one of the most sensitive functional biomarkers of proximal tubule damage [10,11]. Urinary RBP 4 excretion is elevated in diabetic subjects and correlates with urinary albumin excretion, serum and urine creatinine, GFR, indicating its potential clinical application as a marker of early DN [10,26]. Increased urinary excretion of RBP 4 could suggest that proximal tubular dysfunction may occur independently of glomerular alteration [10]. Our study has also shown that RBP 4 in the urine may indicate a tubular injury and serve as a tool for clinical monitoring of DN development and progression in children with T1DM.

Adiponectin, VCAM-1, and RBP 4 can serve as indicators of treatment effectiveness. Reducing angiotensin II levels by ACE inhibition may have a multifactorial effect on decreasing albuminuria, including reducing adhesion molecules and diminishing glomerular filtration pressure, as well as preventing the promotion of profibrotic pathways [22].

Conclusions

1. Serum creatinine could not be an early predictor of DN because its level was significantly elevated only between 1 to 5 years from the initial manifestation of T1DM in children.

2. Creatinine-based glomerular filtration rate calculation did not effectively predict hyperfiltration occurrence in children with T1DM.

3. Urinary adiponectin can be recommended as a first-line non-invasive kidney damage indicator in children with T1DM.

4. Urinary adiponectin is more indicative of glomerular dysfunction in patients with T1DM, while VCAM-1 and RBP 4 illustrate the tubular apparatus impairment.

Prospects for further research. It seems promising to assess the level of kidney damage biomarkers in urine individually for each patient with T1DM.

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