Serum Cystatin C as a Biomarker for Early Diabetic Nephropathy and Dyslipidemia in Young Type 1 Diabetes Patients

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Abstract

Background. Early capture of initial stages of complications is the destination of long term follow up of type 1 diabetes (T1D) patients. The aim of this study was to assess the clinical significance of serum cystatin C in the early diagnosis of renal injury and its association with dyslipidemia in young T1D patients.

Methods. 779 subjects were evaluated for kidney function by estimating glomerular filtration rate (eGFR) based on serum creatinine (eGFRcreat) and cystatin C (eGFRcys).

Results. Median age of study subjects was 16.2 years (2.1;26.4), diabetes duration – 5.3 years (0.51;24.0). The median of HbA1c was 8% (5.2;19.9) (64 mmol/mol (33.3;194)); 24.2% of participants had HbA1c <7% (53 mmol/mol). Elevated albumin excretion rate was found in 13.5% of subjects. The median of cystatin C was 0.8 mg/L (0.13;1.71), the median of creatinine – 63 µmol/L (6;126). Median of eGFRcys was lower than eGFRcreat (92 ml/min/1.73m² vs. 101 ml/min/1.73m², p<0.001). 30.2% of all patients were classified as having worse kidney function when using cystatin C vs. creatinine for eGFR calculation. Linear correlations were found between cystatin C and HbA1c, r=0.088, p<0.05, as well as cystatin C and HDL, r=-0.097, p<0.01.

Conclusion. This study showed that cystatin C might be used as an additional biomarker of early kidney injury for young patients with T1D.

1. Background

Diabetic nephropathy is a chronic kidney disease defined as renal dysfunction or structural abnormalities lasting for 3 or more months and highly affecting patient's quality of life and mortality risk [1]. Estimated glomerular filtration rate (eGFR) below 60 ml/min/1.73 m² determines chronic kidney disease. Recent data suggest the benefits of GFR calculated with serum cystatin C for evaluation of early risk for end-stage renal disease [2].

Cystatin C is a low-molecular-weight protein (Mw 13 343 Da), an inhibitor of endogenous cysteine protease [3]. It is produced in all tissues and found in all biological fluids. Through regulating cysteine protease activity, cystatin C has been reported to be involved in many disease processes, such as inflammation and tumor metastasis [4]. Recent studies showed that cystatin C is an ideal endogenous glomerular filtration rate marker [5, 6] is more sensitive parameter for renal function assessment compared to serum creatinine [3].

Increased cystatin C and disorders of lipid metabolism are associated with higher risk of cardiovascular diseases (CVD) which are the leading cause of mortality in patients with type 1 diabetes (T1D) [7, 8, 9]. Early observations of lipid profile in patients with T1D, revealing pro-atherogenic features such as hypercholesterolemia and hypertriglyceridemia are associated with poor glycemic control and nephropathy [7, 8, 10].

Recent studies showed that cystatin C is a valuable marker for acute kidney injury in pediatric population and even helps to stratify CVD risk in children with chronic kidney disease [11, 12]. However, in the SEARCH for Diabetes in Youth study higher levels of cystatin C were found in healthy subjects compared to type 1 and type 2 diabetes patients [13].

Since there are controversies regarding the role of cystatin C in the assessment of kidney function, we aimed to assess the clinical significance of serum cystatin C in the early diagnosis of renal injury and its association with dyslipidemia in young T1D patients.

2. Methods

2.1. Study design and subjects

A cross sectional population-based study was carried out in Lithuanian University of Health Sciences, Department of Endocrinology, the whole project is described previously [14]. In this study we included 531 children and 248 adults younger than 26 years of age with T1D diagnosis in Lithuania. All patients had been confirmed with T1D diagnosis with at least one positive islet autoimmune marker: glutamic acid decarboxylase 65-kilodalton isoform, tyrosine phosphatase-related islet antigen 2 and/or insulin autoantibodies; and were treated with insulin. We included patients with disease duration more than 6 months because of known metabolic instability at the onset of diabetes [15].

Detailed clinical assessment and examination of this cohort is described previously [16], briefly at the study entry, data on onset, duration, and treatment of diabetes were collected, clinical examination included height (cm), weight (kg), waist circumference (cm) and body mass index (BMI) (kg/m²). BMI values were converted to Z-scores and stratified to 4 weight status groups: underweight, normal weight, overweight and obesity, using World Health Organization approach [17]. Waist circumference (cm) divided by height (cm) values were used to evaluate Waist-to-Height ratio (WtHR): optimal WtHR was considered < 0.5 [18].

Diabetes control was assessed by glycated hemoglobin (HbA1c). Fasting blood samples were taken for analysis of lipid profile, creatinine and cystatin C. All patients were screened for diabetic retinopathy and neuropathy, and the level of albuminuria in 24-hour urine was assessed.

2.2. Laboratory analyses

HbA1c, lipids, serum creatinine were measured using UniCel DxC 800 Synchron system (Beckman Coulter, USA). Normal cut-off values for HbA1c were 4–6% (20 mmol/mol – 42 mmol/mol). Optimal metabolic control was defined as HbA1c below 7% (53 mmol/mol) without severe hypoglycemas as recommended by International Society for Pediatric and Adolescent Diabetes (ISPAD) [19]. Normal values for low density-lipoprotein cholesterol (LDL), high density-lipoprotein cholesterol (HDL) and triglycerides (Tg) were defined as < 2.6 mmol/L, > 1.1 mmol/L and < 1.7 mmol/L, respectively. Normal values for total cholesterol (TCh) were < 5.2 mmol/L for patients ≥ 16 years and < 5.5 mmol/L for children under 16 years. At least one abnormal lipid level indicated
dyslipidemia. Normal cut-off values for creatinine were 39–91 µmol/L. Cystatin C was measured by an automated enzyme immunoassay system AIA 2000 (TOSOH Corporation, Japan) with a normal range of 0.52–0.97 mg/L.

2.3. Evaluation of kidney function

The estimated glomerular filtration rate (eGFR) values were calculated using serum creatinine (eGFRcreat) and serum cystatin C (eGFRcys) for all participants. Creatinine-based Schwartz formula [20] for eGFRcreat was used to calculate eGFRcreat in children. For eGFRcreat and eGFRcys in adults and eGFRcys in children equations from the Chronic Kidney Disease Epidemiology Collaboration were used [21, 22].

Kidney function was staged according American Diabetes Association (ADA) guidelines [23]: Level 1 if GFR ≥ 90 ml/min./1.73 m²; Level 2 - GFR 60–89 ml/min/1.73 m²; Level 3 - GFR 30–59 ml/min/1.73 m²; Level 4 - GFR 15–29 ml/min/1,73 m² and Level 5 - GFR < 15 ml/min/1,73 m².

Further, we classified patients according to eGFRcreat and eGFRcys levels: Group 1 - if eGFRcreat level was equal to eGFRcys level; Group 2 - if eGFRcreat level < eGFRcys level; Group 3 - if eGFRcreat level > eGFRcys level.

2.4. Evaluation of microvascular complications

Digital fundus photos were taken and evaluated by a single ophthalmologist specialized in diabetes retinopathy.

All patients were screened for the level of albuminuria in 24-hour urine as reported earlier [24]. Normal values were determined if albumin excretion rate (AER) was below 30 mg/24 h; microalbuminuria - between 30 and 300 mg/24 h; macroalbuminuria - exceeding 300 mg/24 h.

All patients were screened for the presence of symptoms or signs of symmetrical peripheral neuropathy. We applied Michigan Neuropathy Screening Questionnaire and evaluated sensations of vibration, pressure and temperature. Diagnosis of peripheral neuropathy was confirmed if two or more of these tests were abnormal [25, 26].

2.5. Statistical analyses

IBM SPSS Statistics software was used for statistical analyses. Student’s 2-tailed t test, χ² statistics, parametric one-way ANOVA (for normally distributed data) and Mann-Whitney U-test (for non-normal data distribution) or Kruskal-Wallis one-way ANOVA (in the case of ordinal data) were used. Pearson correlation coefficient (for normally distributed data) and Spearman's coefficient (for non-normal data) were used while testing associations between continuous variables. P values < 0.05 were considered statistically significant. All P values were 2-tailed.

3. Results

3.1. General characteristics of the cohort

Overall 779 patients with T1D were included into the study, 51.2% (n = 399) were females. The median of age at the inclusion was 16.2 years (2.1;26.4), age at the onset of T1D - 9.4 years (0.8;24.9), the median of diabetes duration - 5.3 years (0.5;24.0). There were 51.9% (n = 404) of patients with diabetes duration ≥ 5 years.

Normal BMI was found in 75.3% (n = 581) of participants, 20.1% (n = 155) were overweight, 3.6% (n = 28) - obese and 1% (n = 8) - underweight. Optimal WHR was found in 85.3% (n = 622) of participants.

Optimal glycemic control was found in 24.2% (n = 188) of patients. Higher than normal serum cystatin C concentration was present in 10.8% (n = 84) of patients.

General characteristics and comparison of children vs. adults’ groups and males vs. females are presented in Table 1.
| Category          | Gender/Total | Median (range), total cohort | Age groups | Median (range) in children (<18 years) | Median (range) in adults (≥18 years) | p value (comparing children vs. adults) |
|-------------------|--------------|------------------------------|------------|----------------------------------------|--------------------------------------|----------------------------------------|
| **BMI Z-score**   | Males        | 0.23 (-3.66;4.34)            |            | 0.32 (-3.66;4.34)                     | 0.07 (-1.91;3.16)                    | NS                                     |
|                   | Females      | 0.36 (-3.96;2.97)            |            | 0.36 (-3.96;2.8)                      | 0.37 (-1.87;2.97)                    | NS                                     |
|                   | Total        | 0.29 (-3.96;4.34)            |            | 0.34 (-3.96;4.34)                     | 0.29 (-1.91;3.16)                    | NS                                     |
| **WtHR**          | Males        | 0.44 (0.36;0.8)              |            | 0.44 (0.38;0.8)                       | 0.44 (0.36;0.61)                     | NS                                     |
|                   | Females      | 0.45 (0.25;0.63)             |            | 0.44 (0.25;0.59)                      | 0.45 (0.37;0.63)                     | NS                                     |
|                   | Total        | 0.44 (0.25;0.8)              |            | 0.44 (0.25;0.8)                       | 0.45 (0.36;0.63)                     | NS                                     |
| **HbA1c, %**      | Males        | 7.8 (5.2;14.8)               |            | 7.8 (5.3;14.8)                        | 7.9 (5.2;14.1)                       | 0.047                                  |
|                   | Females      | 8.3 (5.4;19.9)               |            | 8.3 (5.5;19.9)                        | 8.3 (5.4;14.1)                       | NS                                     |
|                   | Total        | 8.0 (5.2;19.9)               |            | 8.0 (5.3;19.9)                        | 8.0 (5.2;14.1)                       | NS                                     |
| **Serum creatinine, µmol/L** | Males   | 70 (7;126)                  |            | 62 (7;107)                            | 80 (35;113)                          | < 0.001                                |
|                   | Females      | 59 (6;111)                   |            | 58 (6;94)                             | 64 (36;111)                          | < 0.001                                |
|                   | Total        | 63 (6;126)                   |            | 63 (6;107)                            | 70 (35;113)                          | < 0.001                                |
| **Serum cystatin C, mg/L** | Males | 0.84 (0.08;1.71)            |            | 0.85 (0.54;1.27)                      | 0.82 (0.08;1.71)                     | NS                                     |
|                   | Females      | 0.78 (0.33;1.19)             |            | 0.80 (0.33;1.11)                      | 0.76 (0.52;1.19)                     | 0.004                                  |
|                   | Total        | 0.81 (0.08;1.71)             |            | 0.83 (0.33;1.27)                      | 0.79 (0.08;1.71)                     | 0.008                                  |
| **Total cholesterol, mmol/L** | Males | 4.6 (1.9;8.9)               |            | 4.7 (1.9;8.9)                         | 4.6 (2.9;7.9)                        | NS                                     |
|                   | Females      | 4.9 (2.5;9.3)                |            | 4.9 (3.1;8.5)                         | 5.0 (2.5;9.3)                        | NS                                     |
|                   | Total        | 4.8 (1.9;9.3)                |            | 4.8 (1.9;8.9)                         | 4.7 (2.5;9.3)                        | NS                                     |
| **LDL-cholesterol, mmol/L** | Males | 2.6 (0.5;5.8)               |            | 2.5 (0.5;5.8)                         | 2.6 (1.3;5.7)                        | NS                                     |
|                   | Females      | 2.8 (0.8;6.5)                |            | 2.8 (1.1;6.1)                         | 2.8 (0.8;6.5)                        | NS                                     |
|                   | Total        | 2.7 (0.5;6.5)                |            | 2.7 (0.5;6.1)                         | 2.7 (0.8;6.5)                        | NS                                     |
| **HDL-cholesterol, mmol/L** | Males | 1.4 (0.6;2.7)               |            | 1.4 (0.8;2.7)                         | 1.3 (0.6;2.3)                        | < 0.001                                |
|                   | Females      | 1.5 (0.3;2.6)                |            | 1.5 (0.6;2.6)                         | 1.5 (0.3;2.6)                        | NS                                     |
|                   | Total        | 1.4 (0.3;2.7)                |            | 1.5 (0.6;2.7)                         | 1.4 (0.3;2.6)                        | 0.012                                  |
| **Triglycerides, mmol/L** | Males | 0.7 (0.1;5.3)               |            | 0.7 (0.1;5.3)                         | 0.9 (0.2;5.3)                        | < 0.001                                |
|                   | Females      | 0.8 (0.3;5.1)                |            | 0.8 (0.3;5.1)                         | 0.9 (0.3;3.3)                        | NS                                     |
|                   | Total        | 0.8 (0.1;5.3)                |            | 0.7 (0.1;5.3)                         | 0.9 (0.2;5.3)                        | < 0.001                                |
| **AER, mg/24 h**  | Males        | 7.5 (0.06;787)              |            | 7 (1.787)                             | 9 (0.06;533)                         | NS                                     |
|                   | Females      | 6.8 (0.08;667)              |            | 5.6 (0.5;180)                         | 9 (0.08;667)                         | < 0.001                                |
|                   | Total        | 7.2 (0.06;787)              |            | 6 (0.5;787)                           | 9 (0.06;667)                         | < 0.001                                |
| **eGFRcreat, ml/min/1.73 m²** | Males | 96 (51,194)                 |            | 92 (51,169)                           | 121 (68,194)                         | < 0.001                                |
|                   | Females      | 104 (59,154)                 |            | 98 (64,151)                           | 115 (59,154)                         | < 0.001                                |

BMI, body mass index; WtHR, weight to height ratio; HbA1c, glycosylated hemoglobin; LDL, low density lipoprotein; HDL, high density lipoprotein; AER, albumin excretion rate; eGFRcreat, estimated glomerular filtration rate using serum creatinine; eGFRcys, estimated glomerular filtration rate using serum cystatin C; NS, not significant.

* adjusted for gender, age and BMI Z-score;
BMI, body mass index; WHR, weight to height ratio; HbA1c, glycosylated hemoglobin; LDL, low density lipoprotein; HDL, high density lipoprotein; AER, albumin excretion rate; eGFRcreat, estimated glomerular filtration rate using serum creatinine; eGFRcys, estimated glomerular filtration rate using serum cystatin C; NS, not significant.

* adjusted for gender, age and BMI Z-score;

a – p \leq 0.001 comparing males vs. females in the whole cohort;

b – p < 0.001 comparing males vs. females in children group;

c – p < 0.001 comparing males vs. females in adults’ group.

### 3.2. Dyslipidemia and microvascular complications

There were 26.1% of patients with at least one microvascular complication. Retinopathy was diagnosed in 9% of patients in the whole cohort, neuropathy – in 10.8%. 13.5% were found to have elevated AER, 49.5% of them had microalbuminuria. Frequency of microvascular complications, dyslipidemia and obesity are presented in Table 2.

| Gender/Total | Frequency | Diabetes duration | p value (comparing diabetes duration groups) |
|--------------|-----------|-------------------|--------------------------------------------|
| Retinopathy, % (n) | | | |
| Males | 5 (19)\(^a\) | 0 | 10.4 (19) | < 0.001 |
| Females | 12.8 (51)\(^a\) | 1.7 (3) | 21.7 (48) | < 0.001 |
| Total | 9 (70) | 0.8 (3) | 16.6 (37) | < 0.001 |
| Neuropathy, % (n) | | | |
| Males | 8.9 (33) | 2.6 (5) | 16.1 (28) | < 0.001 |
| Females | 12.6 (49) | 2.3 (4) | 21.2 (45) | < 0.001 |
| Total | 10.8 (82) | 2.4 (9) | 18.9 (73) | < 0.001 |
| Elevated AER, % (n) | | | |
| Males | 13.2 (50) | 13.7 (27) | 12.6 (23) | NS |
| Females | 13.8 (55) | 10.1 (18) | 16.7 (37) | NS |
| Total | 13.5 (105) | 12 (45) | 14.9 (60) | NS |
| Dyslipidemia, % (n) | | | |
| Males | 60.3 (229) | 56.3 (111) | 64.5 (118) | NS |
| Females | 66.2 (264) | 61.8 (110) | 69.7 (154) | NS |
| Total | 63.3 (493) | 58.9 (221) | 67.3 (272) | 0.015 |
| Obesity, % (n) | | | |
| Males | 3.7 (14) | 4.7 (9) | 2.7 (5) | NS |
| Females | 3.5 (14) | 3.4 (6) | 3.6 (8) | NS |
| Total | 3.6 (28) | 4.1 (15) | 3.2 (13) | NS |

AER, albumin excretion rate; NS, not significant.

\(^a\) - p < 0.001 comparing males vs. females in the whole cohort.

Patients with diagnosed retinopathy, neuropathy and microalbuminuria had worse glycemic control than those without these complications, HbA1c 9.6% vs. 7.9%, 8.8% vs. 7.9%, and 8.6% vs. 8%, respectively, all p values < 0.05. Patients with dyslipidemia had higher HbA1c than patients with normal lipid profile, 8.3% vs. 7.6%, respectively, p < 0.001.

Duration of diabetes was significantly directly related to HbA1c, creatinine, AER, Tch, LDL and Tg concentrations. Negative linear correlations were found between cystatin C and HbA1c, r=-0.088, p < 0.05, as well as cystatin C and HDL, r=-0.097, p< 0.01. HbA1c correlated directly with Tch, LDL, Tg, eGFRcreat and eGFRcys, p < 0.001, and negatively with HDL, p < 0.05. All correlations are presented in Table 3.
Table 3
Correlations between continuous variables

| Duration of diabetes, years | 1.00 | 0.052 | -0.006 | 0.301*** | -0.035 | 0.288*** | 0.139*** | 0.089* | 0.085* | -0.057 | 0.241*** |
|-----------------------------|------|-------|--------|----------|--------|----------|----------|--------|--------|--------|----------|
| BMI Z-score                 | 1.00 | 0.661*** | 0.033 | 0.006 | 0.056 | 0.035 | 0.07 | 0.09* | -0.044 | 0.089* | - |
| WtHR                        | 1.00 | 0.084* | -0.096* | -0.131*** | -0.043 | 0.119** | 0.108** | -0.028 | 0.154*** | - |
| HbA1c, %                    | 1.00 | -0.088* | -0.049 | 0.067 | 0.227*** | 0.211*** | -0.083* | 0.418*** | - |
| Cystatin C, mg/L            | 1.00 | 0.228*** | 0.011 | -0.036 | -0.001 | -0.097** | 0.05 | - |
| Creatinine, µmol/L          | 1.00 | 0.131*** | -0.111** | -0.053 | -0.217*** | 0.149*** | - |
| AER, mg/24 h                | 1.00 | 0.024 | 0.04 | -0.036 | 0.108** | - |
| Total cholesterol, mmol/L   | 1.00 | 0.857*** | 0.357*** | 0.351*** | - |
| LDL, mmol/L                 | 1.00 | 0.003 | 0.368*** | - |
| HDL, mmol/L                 | 1.00 | -0.228*** | - |
| Triglycerides, mmol/L       | 1.00 | - |
| eGFRcreat, ml/min/1.73 m\textsuperscript{2} | - | - |
| eGFRcys, ml/min/1.73 m\textsuperscript{2} | - | - |

BMI, body mass index; WtHR, weight to height ratio; HbA1c, glycated hemoglobin; AER, albumin excretion rate; LDL, low density lipoprotein; HDL, high density lipoprotein; eGFRcreat, estimated glomerular filtration rate using serum creatinine; eGFRcys, estimated glomerular filtration rate using serum cystatin C.

* p value < 0.05;
** p value < 0.01;
*** p value < 0.001.

3.3. GFR and kidney function

The median of eGFRcys was lower in the whole cohort compared to eGFRcreat, 92 (57;201) vs. 101 (51;194) ml/min/1.73 m\textsuperscript{2}, respectively, p < 0.001. Statistically significant difference was found in children group comparing eGFRcreat vs. eGFRcys, 97 (51;169) vs. 87 (57;201) ml/min/1.73 m\textsuperscript{2}, p < 0.001. There were more patients classified with level 2 kidney function when using cystatin C vs. creatinine for eGFR calculations, p < 0.001. The frequency of eGFR levels based on eGFRcreat and eGFRcys are presented in Fig. 1.

After grouping patients according the equivalency of eGFR levels calculated by creatinine and cystatin C we found that 30.2% of patients had worse eGFR level calculated using cystatin C (Group 2 when eGFRcreat level < eGFRcys level), 61.1% had the same eGFR level using both biomarkers (Group 1) and 8.7% were classified as Group 3 (when eGFRcreat level > eGFRcys level).

4. Discussion

End-stage renal disease in diabetes patients is known to be the leading cause of increased risk for premature mortality [27]. Only having clear and reliable biomarkers for early diabetic kidney damage detection will lead to early interventions [26]. In the present study, we assessed the value of serum cystatin C as a biomarker for diabetic nephropathy in children and young adults with T1D diagnosis.

The main finding of our study was that using cystatin C helped to find those young T1D patients who may be suffering from early kidney damage, as one third of the whole cohort was classified with worse eGFR level when using cystatin C vs. creatinine. As demonstrated in Tsai et al. study with adult population using eGFR based on cystatin C helped to reclassify kidney function from preserved to reduced, especially in patients with diabetes [29], this means cystatin C could be used as earlier predictor of kidney damage compared to creatinine. There are few studies of prognostic value of serum cystatin C in children with diabetes, yet, most studies evaluated cystatin C in children with acute kidney injury. The 2017 meta-analysis of Nakhjavan-Shahraki et al. showed that serum cystatin C is potentially a more sensitive marker of acute kidney damage in children compared to serum creatinine [11].
Consistent with the Third National Health and Nutrition Examination Survey (NHANES III), our study showed that the levels of serum cystatin C were higher in children compared to adults [30] and were significantly higher in males compared to females in all age groups [31, 32]. On the other hand, Norlund et al. did not find any differences of cystatin C levels between genders [33]. These discrepancies might be explained by different age distribution of the studied cohorts: the median of age of our diabetic cohort was 16.2 years, while Norlund et al. included healthy adult patients above the age of 20 years.

A negative correlation between serum cystatin C and HbA1c was found in our investigation which is in agreement with the SEARCH for Diabetes in Youth Study and the results from Maahas et al. study [13, 32], however, the importance of these findings in clinical practice remains unclear. Furthermore, our study showed a linear negative correlation between cystatin C and HDL cholesterol. A low HDL level is used as one of the criteria for clinical diagnosis of metabolic syndrome [34]. The cross-sectional study with 925 dyslipidemic patients showed progressive increase in cystatin C, with the increasing number of metabolic syndrome components [35]. Patients with higher level of cystatin C have been shown to be at a twofold increased risk of cardiovascular events even after adjusting for a large variety of potential confounders [36]. The recent meta-analysis of over 22 000 participants from 14 studies showed, that higher cystatin C levels were strongly and independently associated with specific endpoints like stroke, myocardial infarction and heart failure [37].

One of the limitations of our study is that we did not measure GFR as it is considered to be the best assay of kidney function [38]. However, it would be clinically and ethically difficult to perform, especially in children, as it requires intravenous injection of the filtration marker. Therefore, most of the contemporary studies use estimated GFR levels for evaluation of kidney function either in children or in adults [38].

The other challenge that our study has faced was the lack of pediatric normative values for serum cystatin C. However, this is a worldwide issue, even for the most frequently used biomarkers, as neutrophil gelatinase-associated lipocalin, kidney injury molecule-1, and serum cystatin C, there are insufficient reference values in pediatric population [28].

5. Conclusion
Serum cystatin C adds significance for diagnosing early kidney damage, as our study showed it could be successfully used in young patients with T1D for estimating GFR. To assess the precise prognostic value of cystatin C we need further follow-up studies of these young T1D patients in order to identify those who will develop advanced diabetic nephropathy.

Abbreviations
T1D - Type 1 diabetes, CVD - cardiovascular disease, GFR - glomerular filtration rate, eGFR
estimated glomular filtration rate, eGFRcreat–estimated glomerular filtration rate based on serum creatinine, eGFRcys - estimated glomerular filtration rate based on serum cystatin C, HbA1c - glycated hemoglobin, BMI–body mass index, WHR–Weight-to-Height ratio, LDL–low density lipoprotein, HDL–high density lipoprotein, Tg–triglycerides, TCH–total cholesterol, ADA–American Diabetes Association, AER - albumin excretion rate.

Declarations

Competing interests
The authors declare that they have no competing interests.

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Authors’ contributions
Lina Radzeviciene: Investigation, Reviewing & Editing the Manuscript Ingrida Stankute: Investigation, Statistical analysis, Writing the Original Draft Ausra Monstaviciene: Statistical analysis Rimante Dobrovolskiene: Investigation Evalda Danyte: Investigation Rasa Verkauskiene: Conceptualization of the Research, Reviewing & Editing the Manuscript.

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