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Non-albuminuric reduced eGFR phenotype in children and adolescents with type 1 diabetes

Procolo Di Bonito [a,b], Enza Mozzillo [a,*], Maria Esposito [a], Francesco Maria Rosanio [a], Alberto Casertano [a], Valentina Fattorusso [a], Adriana Franzese [a]

[a] Pediatric Diabetes Unit, Department of Translational Medical Science, Section of Pediatrics, Federico II University of Naples, Naples, Italy
[b] Department of Internal Medicine "S. Maria delle Grazie" Hospital, Pozzuoli (Naples), Italy

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ABSTRACT

Aims: To analyze the factors associated with non-albuminuric reduced estimated glomerular filtration rate (NAeGFR$^-$) phenotype in young people with type 1 diabetes (T1DM).
Methods: In this cross-sectional study were enrolled 140 outpatient diabetic children (age 7–18 years), consecutively observed in the period 2016–2017. Eighteen subjects with microalbuminuria (defined as albumin excretion rate ≥ 30 mg/24 h) were excluded. Fasting $HbA1_c$, uric acid (UA), neutrophils and lymphocytes count were recorded. Estimated glomerular filtration rate (eGFR) was calculated using the Schwartz's bed-side formula and reduced eGFR was defined by a value <90 mL/min/1.73 m^2.
Results: Out of 122 subjects analyzed, 76 (62%) showed normal eGFR and 46 (38%) showed NAeGFR$^-$ phenotype. They were characterized by higher prevalence of male sex (57% vs 33%, $p = 0.010$), autoimmune diseases (26% vs 12%, $p = 0.043$), high UA levels (4.0 ± 0.9 vs 3.3 ± 0.9 mg/dl, $p < 0.0001$) and high Neutrophils/Lymphocytes ratio (1.5 [1.2–2.0] vs 1.3 [1.0–1.8], $p = 0.023$).
Conclusions: In our population, the prevalence on NAeGFR$^-$ phenotype is 38% and it is associated with male sex, high levels of UA, presence of other autoimmune diseases and low-grade inflammation. It should encourage pediatricians to monitor early both eGFR and UA in order to intercept diabetic youth more likely prone to develop progressive renal impairment.

1. Introduction

Diabetic kidney disease (DKD) is a frequent microvascular complication of type 1 diabetes (T1DM). Commonly, microalbuminuria is considered both the earliest manifestation of renal damage and a risk marker of chronic kidney disease (CKD) and cardiovascular disease [1]. In the last years, it has been argued that DKD is a heterogeneous entity characterized by non-albuminuric low eGFR phenotype (<60 mL/min/1.73 m^2) or microalbuminuric phenotype. The prevalence and the impact of these phenotypes on micro- or macrovascular outcomes has been mainly analyzed in type 2 diabetes (T2DM) [2,3], but only minimally in type 1 diabetic patients [4,5] and never in pediatric patients. In adults with T1DM, the Diabetes Control and Complication Trial (DCCT) primary cohort showed absence of MA and eGFR < 60 mL/min/1.73 m^2

* Corresponding author.
E-mail address: mozzilloenza@gmail.com (E. Mozzillo).
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in 50% of participants [6]. This result has been recently confirmed by Penno et al. [5]. A recent analysis from the Renal Insufficiency and Cardiovascular Events (RIACE) study demonstrated that subjects with T2DM and non-albuminuric low eGFR phenotype showed a higher mortality than those with microlbuminuric phenotype, which represents a higher risk of microvascular complications. This paper seems to confirm the existence of these two distinct phenotypes. Since the eGFR impairment starts before the appearance of microalbuminuria [4] and it is predominantly linear over time [7], it could be clinically relevant to explore whether the reduction of eGFR in the absence of microalbuminuria is detectable in pediatric age. Therefore, the aim of this study was to analyze the prevalence of non-albuminuric reduced eGFR phenotype (NAeGFR$^-$) and its associated factors in children and adolescents with type 1 diabetes.

1.1. Subjects, materials and methods

1.1.1. Study groups

Type 1 diabetes children and adolescents (age range 7–18 years) with disease duration 3–16 years, admitted consecutively at the outpatient pediatric diabetes unit of "Federico II University of Naples", were enrolled from January 2016 to January 2017. The diagnosis of T1DM was based on clinical criteria and the presence of islet cell antibodies. Subjects with diabetic ketoacidosis in the last year, or infections in the last 6 months were excluded. Ninety-one subjects were treated with multiple daily injection therapy, while 31 with continuous subcutaneous insulin infusion. None was on therapy with angiotensin-converting enzyme inhibitors or angiotensin-receptor blockers. Eleven out 140 subjects received celiac disease (CD) diagnosis (1 before T1DM diagnosis; 6 at T1DM diagnosis; 2 within the first year of T1DM; 2 after 5–8 years). Fifteen out 140 subjects received Hashimoto's Thyroiditis (TAI) diagnosis (8 at T1DM diagnosis; 4 within the first year of T1DM; 3 after 5–6 years). Two subjects out 140 received both CD and TAI diagnoses at T1DM onset. All procedures, involving human participants, were in accordance with the ethical standards of Helsinki and informed consent has been obtained by patients and caregivers.

1.1.2. Measurements

Height, weight and waist circumference were measured with standard methods. Body mass index (BMI) and BMI Z-score were calculated according to the Centers for Disease Control and Prevention 2000 standards. Blood pressure was measured on the right arm using standard procedures. Fasting biochemical data including lipids, uric acid (UA), glycosylated hemoglobin ($HbA1_c$), microalbuminuria, white blood cells count and subsets of neutrophils and lymphocytes were analyzed in centralized laboratory of "Federico II University". $HbA1_c$ was measured by HPLC. Microalbuminuria was calculated using 24-h urinary albumin excretion rate. Creatinine was measured by kinetic colorimetric Jaffe technique IDMs traceable (n. 3L81-32). eGFR was calculated by bed-side Schwartz's formula: 0.413 × height (cm)/serum creatinine (mg/dl) [8]. Neutrophils to lymphocytes ratio was calculated. The evaluation of complications was assessed according to the international guidelines [9]. In particular, the evaluation of retinopathy was performed every two years by an expert operator. The neuropathy was assessed by specific physical examination and clinical tests.

1.1.3. Definitions

Prepubertal status was defined as total absence of puberty signs according to the Tanner stage. Reduced eGFR was defined by a value < 90 mL/min/1.73 m^2 according to international guidelines [10]. The absence of MA was defined as 24 h urinary albumin excretion rate < 30 mg [10]. The presence of other autoimmune diseases (CD and TAI) was obtained from medical records.

1.1.4. Statistical analysis

Data are expressed as Mean ± SD, or proportions (%), or median and interquartile range (IQ) and 95% confidence interval (CI). Given the skewed distribution of triglycerides, neutrophils, lymphocytes and N/L ratio, these variables were analyzed after log-transformation, and expressed as median (IQ) in text and Table 1. Means were analyzed by Student's *t* test. Chi-square and Fisher's exact tests were used to compare proportions. Odds ratios and 95% CI were calculated by logistic regression analysis with backward procedure using NAeGFR$^-$ phenotype as dependent variable. Sex and autoimmunity were included separately as categorical covariates, UA and N/L ratio as continuous variables. A p value < 0.05 was considered statistically significant. Statistical analysis was performed with IBM SPSS Statistics, version 20.0.

1.2. Results

Eighteen MA-positive patients out of 140 participants were excluded from analysis. The presence of NAeGFR$^-$ phenotype was observed in 46 youth (38%). The characteristics of young people with and without NAeGFR$^-$ phenotype (n = 76, 62%) are expressed in Table 1. The two groups were similar for age, duration of diabetes, prepubertal status, $HbA1_c$, lipids, blood pressure, and daily insulin requirement. None showed values of eGFR < 60 mL/min/1.73 m^2 or signs of retinopathy or neuropathy. Subjects with NAeGFR$^-$ phenotype showed higher prevalence of male sex ($p = 0.010$), concomitant autoimmune diseases ($p = 0.048$), higher levels of UA ($p < 0.0001$), higher neutrophil count ($p = 0.028$) and higher N/L ratio ($p = 0.032$) in comparison to subjects with normal eGFR. The duration of diabetes did not correlate with anthropometric or biochemical variables such as lipids, HbA1c, eGFR, UA and N/L ratio. In the logistic regression analysis, subjects with NAeGFR$^-$ phenotype showed a high odds ratio for male gender and additional autoimmune diseases. Similarly, the Odds Ratio of the NAeGFR$^-$ phenotype was 2.5–2.8 folds greater for each increase of 1 mg/dl of UA or 1 unit of N/L ratio, independently of age, duration of diabetes, $HbA1_c$, prepubertal status and BMI Z-score (Table 2).

1.3. Discussion

This study provides the first evidence that one third of children and adolescents with T1DM shows a reduced eGFR in the absence of microalbuminuria. In our sample, this pheno-

Table 1 – Data of participants according to eGFR categories.

Clinical and biochemical variables	eGFR ≥ 90 mL/min/1.73 m^2 n = 76	eGFR < 90 mL/min/1.73 m^2 n = 46	*p* value
Age at examination (years)	14.2 ± 2.6	14.2 ± 3.4	0.974
Boys n (%)	25 (33)	26 (57)	0.010
Prepubertal status n (%)	6 (8)	7 (15)	0.204
Autoimmune diseases n (%)	9 (12)	12 (26)	0.043
Duration of diabetes (years)	6.8 ± 2.8	6.9 ± 3.4	0.968
BMI (kg/m^2)	21.3 ± 3.7	21.9 ± 4.2	0.401
BMI Z-score	0.44 ± 1.0	0.65 ± 0.9	0.275
Waist circumference (cm)	69.3 ± 12.1	70.2 ± 10.2	0.680
HbA1c (mmol/mol)	64.5 ± 10.3	64.2 ± 9.9	0.875
HDL-C (mg/dl)	56.8 ± 11.0	55.2 ± 10.6	0.440
Triglycerides (mg/dl)	63 (53–78)	65 (49–83)	0.767
Daily insulin dose IU/kg	0.79 ± 0.25	0.77 ± 0.27	0.707
Uric acid (mg/dl)	3.3 ± 0.9	4.0 ± 0.9	<0.0001
Neutrophils count ($\times 10^9$/l)	3.0 (2.6–4.2)	3.5 (2.9–4.2)	0.028
Lymphocytes count ($\times 10^9$/l)	2.4 (2.0–2.7)	2.2 (1.8–2.7)	0.451
Neutrophil/Lymphocyte ratio	1.26 (1.03–1.79)	1.49 (1.18–2.05)	0.032
Systolic blood pressure (mmHg)	110.8 ± 11.5	112.9 ± 12.6	0.349
Diastolic blood pressure (mmHg)	71.8 ± 8.2	72.2 ± 8.5	0.831
eGFR (1.73 m/min/1.73 m^2)	103.0 ± 16.6	82.3 ± 6.8	<0.0001

Data are expressed as mean ± SD, n (%), median (IQ range).

Table 2 – Odds ratio (95%CI)* of non-albuminuric low eGFR phenotype for male sex, additional autoimmune diseases and each increase of 1 unit of UA (mg/dl) or N/L ratio (Log).

	OR (95%CI)	*p* value
Male sex	2.65 (1.25–5.64)	0.011
Autoimmune diseases	2.80 (1.06–7.40)	0.038
Uric acid (per 1 mg increase)	2.29 (1.45–3.60)	<0.0001
Neutrophil/Lymphocyte ratio (per 1 unit increase)	2.80 (1.24–6.35)	0.013

* Adjusted for age, duration of diabetes, $HbA1_c$, prepubertal status, BMI z-score.

type clusters with high UA, autoimmunity, low-grade inflammation and male sex.

In adults, the presence of albuminuric and NAeGFR$^-$ phenotype has been demonstrated in T1DM [4,5] and in T2DM, both in cross-sectional [2] and longitudinal studies [3]. However the prevalence and the characteristics of NAeGFR$^-$ phenotype have never been analyzed in young people with T1DM. In our sample UA is the strongest predictor of such phenotype, confirming the observations obtained in adults [4,5]. Usually, high levels of UA are associated with more advanced stage of CKD, although the relationship between this metabolite and the glomerular function is still debated. In our study, the strong association between UA, even in the normal range, with a mild impairment of glomerular function in diabetes with short duration, is intriguing. This association needs further investigations; however, it suggests to perform a more frequent evaluation of UA in pediatric age, since it is inexpensive and simple to measure.

Furthermore, we demonstrate for the first time the association of NAeGFR$^-$ phenotype with low-grade inflammation and other autoimmune diseases. The association between high N/L ratio and KDN has been recently observed in T2DM [9], but never in young people with T1DM. N/L ratio is a marker of low-grade inflammation. This is in line with a previous study of Krolewsky et al. who reported an association between eGFR decline and high levels of tumor necrosis factor receptor [4]. Finally, it might be underlined that these alterations are independent by disease duration and $HbA1_c$.

The association between reduced eGFR and other autoimmune disease in young people with T1DM is a new finding. It is well known that T1DM shares an immuno-genetic background with other autoimmune diseases such as thyroiditis and celiac disease. It is possible that the inflammation associated with the autoimmunity may play a pathogenetic role [11], however the association between autoimmune diseases, T1DM and reduced eGFR is not easy to interpret, given the cross sectional nature of our study. Nevertheless, as a testimony of a possible relationship between T1DM, autoimmune diseases and glomerular impairment, it is worth mentioning an our recent study that reported a reduced eGFR in adults with T1DM and celiac disease as compared to those without [12].

Limitation of this study is the determination of eGFR based on levels of creatinine more than Cystatin-C. However, it should be considered that our observational data were derived from an unselected population using standard methods adopted in routine checks. Given the cross-sectional design of our study, we were not able to demonstrate the causative role of UA, other autoimmune diseases and low-grade inflammation in determining NAeGFR$^-$ phenotype. This

datum can be only a preliminary observation which needs to be confirmed by extensive prospective studies.

The strength of our observation is represented, first of all, by the unexpected high prevalence of NAeGFR$^-$ phenotype in a pediatric population with T1DM. Furthermore, in young people this particular phenotype can be observed without interference of drugs and other diabetes complications, as it is instead observed in adult population.

In conclusion, our study demonstrates that NAeGFR$^-$ phenotype is detectable in more than 30% of children and adolescents with T1DM and that it is associated with a cluster of conditions like higher UA, autoimmunity, and low-grade inflammation. Our study highlights the need for carefully monitoring eGFR and UA level in pediatric subjects with T1DM in order to early intercept those more likely prone to develop progressive renal impairment.

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Declaration of Competing Interest

The authors declare that they have no conflict of interest

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diabres.2019.07.005.

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