PROXIMAL TUBULE DYSFUNCTION IS ASSOCIATED WITH NEPHRIN AND URINARY VASCULAR ENDOTHELIAL GROWTH FACTOR EXCRETION IN NORMOALBUMINURIC TYPE 2 DIABETES MELLITUS PATIENTS: A CROSS-SECTIONAL STUDY

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Background

The classical concept concerning mechanisms of albuminuria in DN relies on defects in the glomerular filtration barrier.

Nephrin, a transmembrane protein of the immunoglobulin superfamily, is an important component of the slit diaphragm located between the foot processes of the podocytes. Its alterations lead to the limitation of the size-selectivity of the slit diaphragm.

Vascular endothelial growth factor (VEGF) is a pro-angiogenic factor, produced mainly by the podocytes, which acts upon these cells through an autocrine mechanism.

Urinary excretion of VEGF may increase even in the normoalbuminuria stage, a fact which suggests that urinary VEGF may be used as a sensitive biomarker in the diagnosis of early DN.

Currently, there is a debate as to whether early diabetic nephropathy (DN) in type 2 DM may be attributed to the glomerulus or to the proximal tubule (PT). It is assumed that albuminuria is caused primarily by impaired tubular uptake of intact albumin rather than by an increased leakiness of the glomerular filtration barrier.

In previous works performed by us in normoalbuminuric patients with type 2 DM we demonstrated that PT dysfunction precedes the occurrence of albuminuria.

Amongst other causative factors related to PT dysfunction, advanced glycation end products (AGE) have been involved in the pathogenesis of diabetic tubulopathy, an emerging entity.

Aim of study

to evaluate a potential relation of PT dysfunction with urinary nephrin and urinary VEGF excretion in patients with Type 2 DM
we queried if this association could be related to AGE intervention, which may impact both the PT and the podocytes

Methods

70 patients with type 2 DM attending the Department of Diabetes and Metabolic Diseases (38-normoalbuminuric; 32-microalbuminuric) and 21 healthy control subjects

a cross-sectional study

inclusion criteria

- long-standing DM (>5 years)
- normoalbuminuria (urine albumin-to-creatinine ratio (UACR) <30 mg/g) and microalbuminuria (UACR between 30 and 300 mg/g),
- patients were on oral antidiabetic medication, angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin II receptor blockers (ARBs), and statins

All p were assessed concerning:

GFR
C-reactive protein (CRP)
plasma advanced glycation end-products (pAGEs)
serum cystatin C
urine albumin/creatinine ratio (UACR)
urinary alpha1 microglobulin
urinary kidney injury molecule-1 (KIM-1)
urinary nephrin
urinary vascular endothelial growth factor (VEGF)
urinary advanced glycation end-products (uAGEs)

Statistical analysis

clinical, biological and cerebral haemodynamics indices are presented as means, Standard Deviations(SD) and proportions

one-way analysis of variance and Bonferroni's test for the differences among the groups was used to compare the means among the three groups.

simple and multiple linear regression analyses were carried out to evaluate the significance of the relation between continuous variables for all groups together (pooled data).

only significant variables yielded by univariate regression analysis were introduced in the models for multivariate regression analysis (Cox & Snell R square).

the P values for all hypothesis tests were two-sided, and statistical significance was set at $P<0.05$.

All analyses were conducted with Stata 9.2 (Statacorp, Texas, USA).

urinary KIM-1, urinary nephrin, urinary VEGF, plasma AGEs, and urinary AGEs were evaluated by the ELISA method

serum cystatin C, urinary alpha1 microglobulin and albuminuria were assessed by means of particle-enhanced immunonephelometry using the BN ProSpec System

CKD was defined and the stages(1-5) of CKD were established according to the KDIGO Guidelines 2012 (estimated GFR- CKD-EPI equation formula)

Results

UACR, urinary alpha$_1$-microglobulin, urinary KIM-1, urinary nephrin, urinary VEGF, plasma AGEs, and urinary AGEs

The demographic, clinical and laboratory data of the patients and control subjects are presented in Table 1.

Parameter	Group A	Group B	Group C	p
Number of subjects	38	32	21	–
Age (years)	56.27±7.87	58.13±9.01	56.18±4.91	0.635
DM duration (years)	9.93±4.09	10.63±5.20	–	0.545
BMI (kg/m^2)	31.75±4.68	32.52±6.49	23.97±0.50	<0.0001
SBP (mmHg)	130.73±11.44	130.68±7.28	119.54±6.10	0.0036
DBP (mmHg)	76.25±7.54	74.54±7.05	67.27±7.19	0.0022
Hb (g/dl)	13.06±1.23	12.97±0.80	14.22±0.72	0.0047
Serum creatinine (mg/dl)	0.97±0.20	1±0.17	0.82±0.09	0.034
GFR (ml/min/1.73m^2)	75.20±16.15	70.39±14.45	88.34± 5.98	0.005
Glycaemia (mg/dl)	149.93±40.41	156.63/-68.61	92.91±7.42	0.001
HbA_{1c} (%)	7.04±0.65	7.21±1.07	5.07±0.14	0.0003
HbA_{1c} (mmol/mol)	53.4±4.8	55.3±9.3	31.9±1.3	0.0003
Serum cholesterol (mg/dl)	221.75±50.81	228.77±53.25	150.27±22.18	<0.0001
Triglycerides (mg/dl)	150.79±52.75	158.77±49.49	94.54±18.38	0.0014
hsCRP(mg/dl)	6.83±4.29	17.79±5.85	0.98±0.19	<0.0001
UACR (mg/g)	22.03±6.02	75.61±34.02	21.13±3.91	<0.0001
Serum cystatin C (mg/l)	0.83±0.19	0.98±0.25	0.62±0.06	<0.0001
Urinary alpha1/creat (mg/g)	4.30±1.41	6.63±2.29	3.06±0.61	<0.0001
Urinary KIM-1 (ng/g)	74.14±32.93	102.30±37.05	27.35±5.24	<0.0001
Urinary VEGF (ng/g)	85.76±48.30	138.05±65.26	26.28±22.03	<0.0001
Urinary nephrin (mg/g)	0.118±0.035	0.968±0.498	0.046±0.028	<0.0001
Urinary AGEs (pg/ml)	44.96±18.02	72.34±39.11	32.18±1.69	<0.0001
Plasma AGEs (pg/ml)	445.77±178.61	662.49±154.37	275.39±25.24	<0.0001

Table 1. Clinical and biological data of the studied patients
DM: diabetes mellitus; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; Hb: haemoglobin; eGFR: estimated glomerular filtration rate; HbA_{1c}: glycated haemoglobin; hsCRP: high sensitive C reactive protein; UACR: urinary albumin:creatinine ratio; alpha$_1$/creat: alpha$_1$-microglobulin:creatinine ratio; KIM-1: kidney injury molecule-1; VEGF: vascular endothelial growth factor; AGEs: advanced glycation end-products

Variable	Parameter											
	KIM-1			Urinary alpha1/creat			Nephrin			VEGF		
	R-squared	Coef β	p	R-squared	Coef β	p	R-squared	Coef β	p	R-squared	Coef β	p
eGFR	0.166	-0.953	<0.001	0.167	-0.053	<0.001	0.087	-0.008	0.007	0.218	-1.836	<0.001
Cystatin C	0.128	57.336	0.001	0.130	3.215	0.001	0.157	0.809	<0.001	0.226	127.806	<0.001
Urinary AGEs	0.405	0.825	<0.001	0.578	0.054	<0.001	0.251	0.008	<0.001	0.308	1.211	<0.001
UACR	0.125	0.427	0.001	0.269	0.034	<0.001	0.498	0.010	<0.001	0.227	0.969	<0.001
KIM-1				0.531	0.040	<0.001	0.203	0.005	<0.001	0.360	1.008	<0.001
Urinary alpha1/creat	0.531	13.107	<0.001				0.529	0.167	<0.001	0.709	25.469	<0.001
Nephrin	0.203	35.316	<0.001	0.529	3.169	<0.001				0.527	95.648	<0.001
VEGF	0.360	0.356	<0.001	0.709	0.027	<0.001	0.527	0.005	<0.001			

Table 2. Univariate regression analysis for the urinary biomarkers
eGFR: estimated glomerular filtration rate; AGEs: advanced glycation end-products; UACR: urinary albumin:creatinine ratio;
KIM-1: kidney injury molecule-1; alpha1/creat: alpha$_1$-microglobulin:creatinine ratio; VEGF: vascular endothelial growth factor

Parameter	Variable						
		Coef β	p	95% CI	F	Prob > F	R-squared
Urinary alpha1/creat	Constant	1.222	0.004	0.401 to 2.043	96.12	<0.001	0.886
	eGFR	-1.584	0.008	- 0.425 to - 2.743			
	Cystatin C	1.969	<0.001	0.911 to 3.027			
	Urinary AGEs	0.020	<0.001	0.013 to 0.028			
	KIM-1	0.011	<0.001	0.005 to 0.017			
	Nephrin	0.912	0.001	0.402 to 1.422			
	VEGF	0.015	<0.001	0.011 to 0.020			
KIM-1	Constant	1.544	0.002	0.226 to 3.127	91.81	<0.001	0.741
	eGFR	-1.862	0.006	- 0.241 to - 3.228			
	Cystatin C	2.308	<0.001	1.105 to 3.754			
	Urinary AGEs	0.041	<0.001	0.007 to 0.036			
	Urinary alpha1/creat	0.009	<0.001	0.032 to 0.024			
	Nephrin	1.205	0.003	0.266 to 1.681			
	VEGF	0.009	<0.001	0.002 to 0.037			
VEGF	Constant	-36.780	0.032	-70.406 to -3.153	70.60	<0.001	0.788
	eGFR	- 64.355	0.006	- 109.997 to - 18.713			
	Cystatin C	92.757	<0.001	53.442 to 132.072			
	Urinary alpha1/creat	28.411	<0.001	23.441 to 33.381			
	Urinary AGEs	0.465	0.011	0.823 to 0.108			
Nephrin	Constant	-0.274	<0.001	-0.383 to -0.165	88.93	<0.001	0.695
	UACR	0.007	<0.001	0.005 to 0.009			
	VEGF	0.003	<0.001	0.002 to 0.004			

Table 3. Multivariate regression analysis for the urinary biomarkers
alpha1/creat: alpha$_1$-microglobulin:creatinine ratio; KIM-1: kidney injury molecule-1;
VEGF: vascular endothelial growth factor; eGFR: estimated glomerular filtration rate; AGEs: advanced glycation end-products;
UACR: urinary albumin:creatinine ratio

Discussion

in our study we found a significant relation of plasma and urinary AGEs with biomarkers of PT dysfunction

the levels of urinary alpha$_1$-microglobulin and urinary KIM-1 were increased in both normo- and microalbuminuric patients, but significantly higher in the microalbuminuric group

urinary alpha$_1$-microglobulin and urinary KIM-1 correlated with UACR even at high-to-normal levels, thus raising the possibility that the PT injury may precede the onset of microalbuminuria

the results of our study substantiate the fact that nephrinuria is increased in patients with type 2 DM, even in the normoalbuminuria stage.

we found elevated levels of nephrinuria which correlated with the biomarkers of PT dysfunction, urinary AGEs, UACR, cystatin C, and the eGFR.

urinary VEGF correlated with nephrinuria and UACR, but also with the biomarkers of PT dysfunction, urinary AGEs, cystatin C, and eGFR.

we assume that this observation documents an association of PT with urinary VEGF uptake and processing, a fact substantiated by the strong correlation of the PT dysfunction biomarkers with urinary VEGF, even in normoalbuminuric patients. Most likely, as was the case with urinary nephrin, the PT interferes with the expression of podocyte damage markers in early DN.

in our patients, increased levels of urinary VEGF were present in both normo- and microalbuminuric patients, leading to the assumption that an increased AGE-induced VEGF expression by the podocytes may occur in the early stages of DN.

Conclusion

in patients with Type 2 DM, there is an association of PT dysfunction with podocyte damage biomarkers, even in the normoalbuminuria stage.

this observation raises the possibility of a putative role of the PT in urinary nephrin and urinary VEGF excretion in early DN.

moreover, it forwards the hypothesis according to which podocyte damage and PT dysfunction may precede the onset of microalbuminuria.

AGEs could impact the PT and the glomerulus in the early stages of DN, thus explaining increased levels of urinary nephrin and urinary VEGF in normoalbuminuric patients with Type 2 DM.

presumably, the PT interferes with the expression of glomerular injury in early DN by coordinating albumin, as well as nephrin and VEGF uptake and processing.

it may be assumed that the PT delays the expression of glomerular involvement in early DN and explains development of normoalbuminuric renal insufficiency.

Acknowledgements

This research received funding from an Internal Grant of "Victor Babes" University of Medicine and Pharmacy Timisoara, PIII-C1-PCFI-2014/2015.

51ERA-EDTA
278--MP
I) Diabetes. I2) Clinical studies.
LIGIA PETRICA
DOI: 10.3252/pso.eu.51era.2014
Poster presented at: 51 ERA-EDTA CONGRESS AMSTERDAM 2014