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CLINICAL STUDY

Urinary megalin in association with progression factors of diabetic nephropathy

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ABSTRACT

AIM: The aim of this study is to evaluate the association between urinary megalin, renal function, blood pressure, lipid profile, vitamin D and glycemic control in patients with type 2 diabetes mellitus (T2DM).

METHODS:. This was a cross-sectional study which recruited 209 patients with T2DM. Urinary megalin was positively associated with systolic blood pressure (SBP) ($r=0.218$, $p=0.04$) but negatively with glomerular filtration rate (GFR) ($r=–0.16$, $p=0.023$). The levels of urinary albumin, triglycerides (TGs) and glycosylated hemoglobin (HbA1c) were higher in the "high-megalin" group, compared to those in "low-megalin" group. Moreover, there was a significant inverse association between vitamin D_3 levels and megalin levels in urine ($OR=0.281$, $p=0.047$).

CONCLUSION: Our study showed for the first time that megalin is associated with progression factors of diabetic nephropathy as well as vitamin D deficiency *(Tab. 3, Fig. 1, Ref. 15)*. Text in PDF *www.elis.sk.*

KEY WORDS: diabetic nephropathy, progression factors, biomarker, type 2 diabetes, megalin.

Introduction

Urinary albumin excretion has been used as an established diagnostic and progression marker for diabetic nephropathy (DN). (1, 2) Recently, urinary megalin excretion has been evaluated as a potential urinary marker of nephropathy(3). In fact, urinary full-length megalin was correlated with the severity of DN in patients with T2DM and its levels were significantly high even in normoalbuminuric patients (3). However, the possibility to use megalin as a marker for predicting therapeutic effects/interventions on the progression of diabetic nephropathy has not been studied. Therefore, this study is aimed at evaluating the association between urinary megalin, renal function, blood pressure, lipid profile, vitamin D and glycemic control in patients with T2DM who have any degree of chronic kidney disease (CKD) and/or albuminuria. This study will enable us to provide further validation of megalin as a urinary marker for DN as well as possibility to predict the development and/or progression of diabetic nephropathy (Fig. 1).

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Methods

Settings

This was a cross-sectional study that recruited patients with diagnosed T2DM (without attention to the duration of the diagnosis), or any degree of CKD and/or albuminuria who were treated at the Jordan University Hospital (JUH) in the period between September 2014 and September 2017. The study was approved by the JUH Institutional Review Board (IRB), while all patients and/or their guardians provided written informed consent to participate in the study. To enhance patients' participation, vitamin D supplements were provided to each patient.

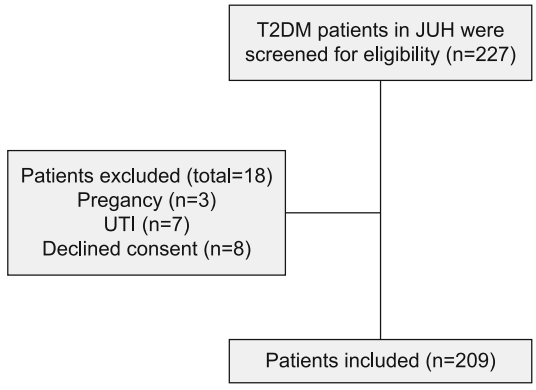

Fig. 1. T2DM: type 2 diabetes mellitus, JUH: Jordan University Hospital, UTI: urinary tract infection.

Tab. 1. Descriptive summary of patients' clinical parameters (n = 209).

	Mean	Median	SD	Percentiles	
				25	75
Age (years)	55.56	56.50	10.065	48.25	63.75
BMI (kg/m^2)	31.68	31.02	7.41	27.43	35.16
DBP (mm Hg)	80.03	80.00	10.33	75.00	88.00
SBP (mm Hg)	131.4	130.0	16.99	120.0	140.0
Waist Circumference (cm)	112.10	111.00	11.449	106.50	117.00
GFR (mL/min)	106.51	96.55	55.03	62.795	141.21
ACR (mg/g)	34.80	17.39	12.93	8.74	42.55
HbA1c %	9.12	7.35	13.28	6.52	8.87
FPG (mg/dL)	170.61	156.45	72.97	115.1	204.0
BUN (mg/dL)	34.11	29.00	28.428	22.13	36.90
Vitamin D_3 (ng/dL)	26.12	23.75	13.98	15.30	37.40
Total cholesterol (mg/dL)	180.01	147.0	182.37	130.0	184.0
LDL-C (mg/dL)	178.3	96.0	253.5	75.5	145.0
HDL-C (mgldL)	43.74	42.0	16.31	33.0	51.0
TG (mg/dL)	177.55	140.0	106.78	109.0	204.0
Calcium (mg/dL)	10.02	9.47	4.12	9.10	9.70
Phosphate (mg/dL)	3.63	3.40	1.25	2.96	3.87
PTH (pg/ml)	109.08	83.90	138.78	31.40	128.25
Megalin (pmol/g creatinine)	155.05	110.0	128.04	74.0	171.00

BMI – body mass index, TG – triglycerides, LDL-C – low-density lipoprotein, HDL-C – high-density lipoprotein cholesterol, TC – total cholesterol, GFR – glomerular filtration rate, ACR – Albumin-to-creatinine ratio, FPG – fasting plasma glucose, HbA1c – glycosylated hemoglobin, BUN – blood urea nitrogen, PTH – parathyroid hormone

Participants and Data collection

Patients were included if they met the following criteria: (1) Adult female or male patients who are 18 years old or older, (2) Patients with the history of T2DM with no or any degree of CKD and/or albuminuria. Patients were excluded in cases of pregnancy or breast-feeding, or other conditions that might cause albuminuria (e.g., urinary tract infection, glomerulonephritis or other causes of glomerulonephropathies), and systemic diseases that might involve the kidneys (systemic lupus erythematosus, Sjögren's syndrome, vasculitis, sarcoidosis, myeloma, HIV, syphilis, amyloidosis, congenital nephrotic syndrome).

Tab. 2. Correlation between urinary megalin (pmol/g of creatinine) and clinical parameters.

Clinical parameter	Correlation coefficient	P-value
BMI	–0.171	0.013
SBP	0.218	0.041
GFR	–0.157	0.023
Calcium	–0.199	0.004

BMI – body mass index; SBP – systolic blood pressure, GFR – glomerular filtration rate

Sample collection and determination of megalin urine levels

The method of S. Ogasawara was used with modifications (3). Urine was centrifuged and the pellet was assayed using the human megalin detection kit (Cusabio Technology Llc, USA). Megalin excretion rate was calculated in pmol/g of creatinine. Serum creatinine and vitamin D_3 levels, urinary creatinine, and urinary albumin were obtained from patients' data files.

Sample size

Sample size was calculated by using G*power 3.0.10, according to Ogasawara et al (3), to detect r^2 of 0.76 between megalin levels and eGFR; sample size of 200 patients is required to achieve a power of 0.8 and $\alpha = 0.05$

Statistical analysis

Patients' baseline variables were summarized as mean ±standard deviation and median with interquartile range. Indepen-

Tab. 3. Comparison of kidney function, vitamin D3 and FPG per megalin group.

	High megalin (n = 60)		Low megalin (n = 149)		p-Value
	Mean	SD	Mean	SD	
HbA1c	9.68	3.52	7.70	1.51	0.032
FPG	170	73.1	170	59.8	NS
SBP	130	4.60	131	7.06	NS
DBP	79.8	2.92	80.1	4.26	NS
LDL-C	113	62.0	110	55.8	NS
HDL-C	45.1	13.9	43.1	12.0	NS
TG	163	70.2	183	86.4	0.034
BMI	30.4	6.48	32.1	5.67	0.016
GFR	95.8	40.3	110	41.4	0.047
ACR	37.1	12.1	33.8	7.71	0.031

HbA1c – glycosylated hemoglobin, FPG – fasting plasma glucose, SBP – systolic blood pressure, DBP – diastolic blood pressure, TG – triglycerides LDL-C – low-density lipoprotein, HDL-C – high-density lipoprotein cholesterol, BMI – body mass index, GFR – glomerular filtration rate, ACR – albumin-to-creatinine ratio, NS – not significant

dent student's t-test was used to compare clinical parameters of patients with high megalin and those with low megalin. Pearson's correlation test was used to assess the relationship between urinary megalin levels and various clinical parameters; then a stepwise regression was used to assess the association between serum levels of vitamin D_3 and urinary megalin to adjust for various covariates. Statistical analysis was performed with SPSS 20 software.

Results

Two hundred and nine patients were included in the study. The description of clinical parameters is found in Table 1. Thereafter, we evaluated the correlation between urinary megalin levels and clinical parameters. Interestingly, pellet urinary megalin was positively associated with SBP ($r = 0.218$, $p = 0.04$) but negatively correlated with GFR as a measure of kidney function ($r = –0.16$, $p = 0.023$). In addition, when patients were divided according to megalin cut-off point level that qualifies failure defined by Ogasawara et al (3) of urinary megalin 523.5 fmol/g creatinine (which is equivalent to 62.8 pg/g creatinine), to two groups with high pellet megalin and low megalin levels: urinary albumin, and TGs were higher in the "high-megalin" group compared to those in low-megalin group. HbA1c was statistically and significantly higher in the group with high urinary megalin (Tabs 2 and 3). A stepwise forward logistic regression model was performed to study the effect of vitamin D deficiency on megalin groups, while taking into account covariates as follows: SBP, FPG, and calcium levels. The results in Table 4 show that there is a significant negative association between the levels of vitamin D and urine megalin (OR = 0.281, p = 0.047; 95 % CI: 0.08–0.98), meaning that high level of deficiency in vitamin D will increase the probability of having high urinary megalin levels.

Discussion and conclusions

This is the first study to evaluate pellet urinary megalin levels in Jordanian T2DM patients and to correlate these levels with kidney functions and progression factors of renal diseases, as well as vitamin D status. In this study, urinary megalin was positively associated with SBP ($r = 0.218$, $p = 0.04$) but negatively correlated with GFR ($r = –0.16$, $p = 0.023$). In addition, when patients were divided according to urinary megalin cutoff point level (which qualifies as failure), urinary albumin, and TGs were higher in the "high-megalin" group, compared to those in "low-megalin" group. HbA1c was statistically and significantly higher in the high-megalin group. Interestingly, this matches with the fact that aforementioned parameters (HbA1c, BP, and TGs), are associated with poorer progression of renal dysfunction. Moreover, there was a significant inverse association between vitamin D levels and urinary megalin levels (OR = 0.281, p = 0.047). Megalin is involved in the reabsorption of various low molecular weight proteins that are filtered by glomeruli, such as albumin (4). Accordingly, megalin dysfunction may be implicated in the development of albuminuria in patients with diabetes mellitus, especially those with T2DM (5, 6). In patients with type 1 diabetes mellitus, microalbuminuria is found to be associated with enhanced excretion of megalin and cubilin (3, 7, 8) Ogasawara et al (3) assessed the clinical significance of different forms of megalin as novel biomarkers for diabetic nephropathy in patients with type 2 diabetes mellitus. They found that the urinary full-length (C-) megalin was correlated with the severity of DN in patients with type 2 diabetes. Interestingly, C-megalin urine levels were significantly high in normoalbuminuric patients. Their elevation was in line with increased albuminuria and showed a better association with eGFR than with albuminuria. The upper normal cut-off points of urinary full-length megalin was determined in 160 normal controls as 523.5 fmol/g of urinary creatinine. (3) Based on the above, we can hypothesize that interventions that can affect megalin expression, could have an ameliorating effect on diabetic nephropathy.

In addition, the relationship between diabetic nephropathy and vitamin D deficiency has been studied (9–11). Similar to our study, the study of Diaz et al. (2009) (12) found a significant association between vitamin D deficiency/insufficiency and nephropathy in patients with diabetes mellitus. In addition, vitamin D deficiency (< 30 ng/mL) was detected in 86 % of CKD patients.(9) Moreover, in a cohort study by De Boer et al (2007), a strong association was found between the albuminuria rates and the decrease in vitamin D absorption (13). Although this may reflect the inability of a failing kidney to activate the vitamin D to calcitriol, it is also possible to explain this finding by the loss of megalin occurring in CKD patients.

Recently, a randomized controlled trial showed that the daily administration of 2 μg paricalcitol, an analogue of the active form of vitamin D3, in patients receiving renin-angiotensin-aldosterone inhibtors, lowered the residual albuminuria in patients with diabetic nephropathy (14). A high dose of paricalcitol may have been needed to generate such a beneficial effect in patients with diabetic nephropathy because of the reduced renal functions of megalin in these patients. The mechanisms of paricalcitol action on the kidney remain unknown. Therefore, it would be of interest to investigate whether it acts on the renal megalin excretion. While the effect of paricalcitol add-on therapy on diabetic nephropathy was previously evaluated (14, 15) there is no study that would evaluate the association between vitamin D levels and diabetic nephropathy as measured by urinary megalin as a novel marker of DN.

The weakness of our study is inherent to cross-sectional character of our study, because rather than causation, it assesses merely the association. Many co-variates and medical intervention should be taken into consideration before we can confirm the true relationship.

Therefore, our study concluded that megalin is associated positively with progression factors of DN and negatively with vitamin D_3 levels. In order to validate this protein as a prognostic marker, future studies should prospectively evaluate the change in megalin levels in patients with nephropathy, and assess the effect of interventions to slow down the progression of DN such as control of blood pressure, glycemic status, vitamin D, etc. on the levels or urinary megalin.

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