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NEPHROLOGY - ORIGINAL PAPER

Renalase: Another puzzle piece between hypertension and simple renal cysts?

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Abstract
Background and aim Since renalase is mostly expressed in kidney tubules, simple renal cyst (SRC) originates from the kidney tubules, and both conditions are related to hypertension, it may be possible that SRC is associated with increased renalase levels. Therefore, in the current study we aimed to confirm the relation between renalase and epinephrine levels, the association between SRC and renalase levels and the association between renalase, blood pressure levels and endothelial dysfunction.

Materials and methods We made a cross-sectional study including 75 patients with SRC, and 51 controls were included to the study. Flow-mediated dilatation (FMD) was assessed, and serum renalase and epinephrine levels were determined.

Results Patient with SRC had lower renalase, higher epinephrine and lower FMD levels when compared to patients without SRC ($p < 0.05$). Log renalase was correlated with log epinephrine ($r = -0.302$, $p = 0.001$) and log FMD ($r = 0.642$, $p < 0.0001$). There was no correlation between renalase and urine albumin/creatinine ratio and glomerular filtration rate. In univariate analysis, age, glomerular filtration rate, renalase and FMD were associated with the presence of SRC. Multivariate regression analysis of factors which are statistically significant in univariate analysis showed that age and renalase was associated with the presence of SRC.

Conclusion We have demonstrated that renalase levels were associated with the presence of SRC and endothelial dysfunction. Further research is necessary to highlight underlying mechanisms.

Keywords Simple renal cyst · Renalase · Epinephrine · Endothelial dysfunction

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Introduction

Renalase, a recently discovered flavoprotein, is strongly expressed in the kidney and less in heart and skeletal muscle. After its finding, research on renalase exploded, with recent advances leading to more detailed knowledge of its biology, structure, enzymatic activity, mechanisms of action, and associations with human disease states. The main action of this hormone is to metabolize

catecholamines. It was shown that renalase metabolizes plasma epinephrine, L-DOPA and dopamine by 82, 63 and 31 %, respectively [1]. Thus, it was suggested that renalase deficiency may be related to excess catecholamine states and accordingly to elevated blood pressure and hypertension. Indeed, some previous studies have shown that renalase is associated with hypertension [2, 3].

Simple renal cysts (SRCs) are commonly observed in human kidneys with increasing age. They are different from hereditary polycystic kidney disease and usually considered a harmless anomaly [4]. However, recent evidence suggests that, apart from possible complications such as flank pain, hematuria, infection and obstruction, SRCs are also associated with arterial hypertension [4–6].

Since renalase is mostly expressed in kidney tubules, SRCs originate from kidney tubules, and both conditions are related to hypertension, it may be possible that SRC is associated with increased renalase levels. Therefore, in the current study we aimed to confirm.

1. The relation between renalase and epinephrine levels.
2. The association between SRC and renalase levels.
3. The association between renalase and endothelial dysfunction.

Materials and methods

Study design and participants

This was a cross-sectional study conducted at the Istanbul Medeniyet University Goztepe Research and Training Hospital that included patients attending the outpatient clinic for a general routine medical investigation. The local ethical committee approved the study protocol. Patients with hypertension, diabetes mellitus, chronic kidney disease, coronary artery disease, cerebrovascular and peripheral arterial disease were excluded from the study. Additionally, subjects using any kind of medication of which could influence endothelial function (such as polyvitamins, statins) were not included. Subjects with a medical history, family history, or echo image of polycystic kidney disease, von Hippel–Lindau disease, tuberous sclerosis, medullary sponge kidney, medullary cystic kidney disease, renal ectopia, duplex kidney, any cause of hydronephrosis, renal tumor, renal transplantation, or partial nephrectomy, and a cyst number ≥ 2 were also excluded.

Biochemical analysis

All blood samples were obtained from patients in the morning, after 12 h of fasting. Serum glucose and albumin were analyzed by a Roche Cobas autoanalyzer. Estimated glomerular filtration rate (eGFR) values determined using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [7]. Concentrations of human renalase and epinephrine (EPI) were analyzed by ELISA using commercial kits (Sunred Biological Technology Co. Ltd), in accordance with manufacturers' instructions. Intra-assay and inter-assay coefficient of variations for renalase and EPI assays were <10 and <12 %, respectively. The sensitivity of renalase and EPI assays was 156 and 0.218 ng/mL. Assay range of renalase was 3–700 and 0.3–60 ng/mL for EPI. Measurements were taken using Thermo Scientific plate washer and enzyme-linked immunosorbent assay plate reader Thermo Multiscan Go (Thermo Fisher Scientific Inc).

Renal ultrasound

After an overnight fast for 8 h at least, all individuals underwent an abdominal echo examination, which was performed by an experienced physician using static grayscale and real-time B-mode units with a 3.5-MHz transducer. The diagnostic criteria for simple renal cysts were defined as echo lucent, round or oval in shape with a thin wall, well-defined and smooth contours with sharply demarcated posterior wall, no calcification, no Doppler signals from within the cyst, as well as sound wave amplification behind the cystic renal mass, which was not suggestive of a renal malignancy [8].

Assessment of endothelial function

Endothelium-dependent vasodilatation [flow-mediated dilatation (FMD)] of the brachial artery was assessed noninvasively, using high-resolution ultrasound as described by Celermajer et al. [9]. The vascular assessment method was in agreement with the criteria set forth by the International Brachial Artery Reactivity Task Force [10]. All vasoactive medications were withheld for 24 h before the procedure. The subjects remained at rest in the supine position for at least 15 min before the examination started. Each subject's right arm was comfortably immobilized in the extended position to allow consistent recording of the brachial artery 2–4 cm above the antecubital fossa. Three adjacent measurements of end-diastolic brachial artery diameter were taken from single 2D frames. All ultrasound images were recorded on super video home system (S-VHS) videotape for subsequent blinded analysis. The FMD was then calculated as the percent change in diameter compared with baseline resting diameters. A single observer who was blinded to the status of the subjects performed all endothelial function studies measurements.

Statistical analysis

All calculations were performed using the statistical software package SPSS 16.0 for Windows (SPSS Inc. Chicago,

IL). Descriptive statistics were used to summarize the data. Categorical variables were expressed as percentages and continuous variables as mean ± standard deviation. Comparisons between two groups were performed using Pearson Chi-square for categorical variables or the Student's *t* test or Mann–Whitney *U* test for continuous variables. For the analysis of correlation coefficients, Pearson correlation analysis was used. For non-normally distributed variables such as CKD-EPI, renalase, epinephrine, hs-CRP and FMD, logarithmic transformation was used. Variables that showed an association with the presence of SRC in univariate logistic regression analysis plus gender was used in multivariate logistic regression analysis. We performed a backward stepwise multivariate logistic regression analysis to identify independent predictors of renal cyst formation; *p* values <0.05 in the final multivariable model were considered statistically significant. Model fit was assessed with the Hosmer–Lemeshow goodness-of-fit test.

Results

In total, 126 patients were included in the study. A total of 75 patients had SRC, whereas 51 patients had no cysts. The comparative demographic and laboratory variables for the patients with and without cyst are shown in Table 1. Patient with SRC had lower renalase (Table 2; $p = 0.002$), higher epinephrine and lower FMD levels when compared to patients without SRC (Table 2).

Correlations between renalase and clinical and laboratory parameters

Log renalase was correlated with log epinephrine ($r = -0.302$, $p = 0.001$) and log FMD ($r = 0.642$, $p < 0.0001$) (Fig. 1). There was no correlation between renalase and urine albumin/creatinine ratio and CKD-EPI eGFR.

A univariate and multivariate regression analysis were performed to determine independent factors related to the presence of SRC. In univariate analysis, age, CKD-EPI eGFR, renalase and FMD were associated with the presence of SRC (Table 3).

Multivariate regression analysis of factors which are statistically significant in univariate analysis showed that age and renalase were associated with the presence of SRC (Table 4).

Discussion

In the present study, we evaluated the relationships between SRC, renalase, epinephrine and FMD in patients without

Table 1 Comparative demographic and laboratory variables of the patients with simple renal cyst and control group

	All ($n = 126$)	Simple cyst (+) ($n = 75$)	Control group (−) ($n = 51$)	*p* value
Age [years, (mean ± SD)]	52.0 ± 9.9	54.8 ± 9.8	47.9 ± 8.6	<0.0001*
Gender [*n*/*n*, (female/male)]	45/81	26/49	19/32	0.766**
Smoking [*n* (%)]	20, 15.9 %	12, 16 %	8, 15.6 %	0.962**
Body mass index [kg/m^2, (mean ± SD)]	29.3 ± 3.70	29.2 ± 3.61	29.4 ± 3.78	0.850***
Systolic blood pressure [mmHg, (mean ± SD)]	122.5 ± 10.6	123.4 ± 10.5	121.3 ± 10.7	0.269***
Diastolic blood pressure [mmHg, (mean ± SD)]	77.6 ± 5.5	78.4 ± 4.9	76.5 ± 6.2	0.067***
Fasting blood glucose [mg/dL, (mean ± SD)]	94.0 ± 8.7	94.5 ± 8.9	93.2 ± 8.4	0.287*
CKD-EPI [mL/min/1.73 m^2, (mean ± SD)]	95.8 ± 15.5	91.8 ± 15.7	101.7 ± 14.9	<0.0001*
Albumin [g/dL, (mean ± SD)]	4.49 ± 0.26	4.51 ± 0.27	4.46 ± 0.24	0.292***
Sodium [mEq/L, (mean ± SD)]	139.1 ± 2.18	139.3 ± 2.15	138.8 ± 2.21	0.194***
Potassium [mEq/L, (mean ± SD)]	4.49 ± 0.36	4.51 ± 0.34	4.44 ± 0.39	0.359***
Hemoglobin [g/mL, (mean ± SD)]	14.17 ± 1.25	14.27 ± 1.42	14.0 ± 0.86 14.0 ± 0.9	0.286***
Uric acid [mg/dL, (mean ± SD)]	4.93 ± 1.22	5.02 ± 1.27	4.70 ± 1.04	0.151*
C-reactive protein [mg/dL, (mean ± SD)]	0.55 ± 0.31	0.53 ± 0.29	0.58 ± 0.32	0.156*
Urine albumin/creatinine [$\times 10^{-3}$, (mean ± SD)]	64.8 ± 129.0	81.9 ± 142.6	34.3 ± 94.0	0.002*

* *p* value is based on Mann–Whitney *U* test; ** *p* value is based on Chi-square test; *** *p* value is based on Student's *t* test

Table 2 Comparision of flow-mediated dilatation, renalase and epinephrine in patients with simple renal cyst and control group

	All ($n = 131$)	Simple renal cyst group (+) ($n = 80$)	Control group ($n = 51$)	p value
FMD (mean ± SD)	8.11 ± 1.22	7.91 ± 1.04	8.39 ± 1.42	0.034*
Renalase [ng/mL, (mean ± SD)]	142.5 ± 61.0	130.3 ± 60.6	160.4 ± 57.6	0.002*
Epinephrine [ng/mL, mean ± SD]	13.70 ± 4.66	14.14 ± 4.70	13.06 ± 4.58	0.261*

* p value is based on Mann–Whitney U test

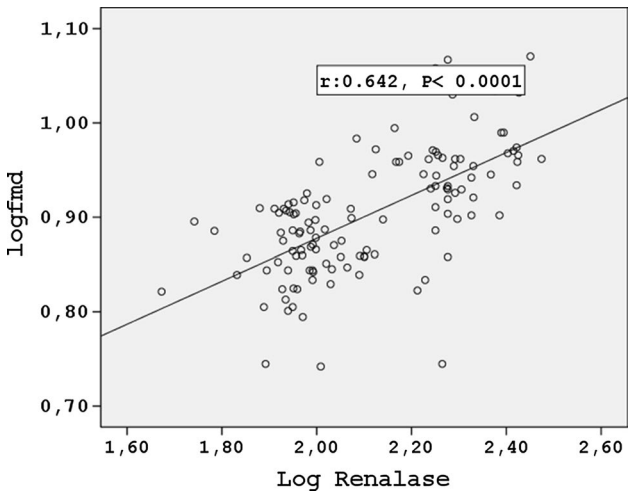

Fig. 1 Correlation analysis of flow-mediated dilatation (FMD) and renalase

Table 3 Univariate regression analysis to determine independent factors for the presence of simple renal cyst

	Adjusted odds ratio	p value	95 % CI for OR	
			Lower	Upper
Age (years)	1.081	<0.0001	1.037	1.127
FMD	0.724	0.038	0.534	0.982
Renalase (ng/mL)	0.992	0.008	0.986	0.998
CKD-EPI (mL/min/1.73 m^2)	0.953	0.001	0.926	0.980

any chronic disease (including hypertension, diabetes and heart disease) and without any kind of treatment. As a result, we have demonstrated the following: (i) renalase levels are lower in patients with SRC compared to patients without SRC, and renalase levels were independently and negatively associated with the presence of SRC; (ii) renalase levels were positively associated with endothelial dysfunction as evaluated by FMD. To the best of our knowledge, our findings are not demonstrated before.

As a major finding, we have shown that renalase was negatively associated with the presence of SRC. Currently, we have no clear explanation underlying this

Table 4 Multivariate regression analysis to determine independent factors for the presence of simple renal cyst

	Adjusted odds ratio	p value	95 % CI for OR	
			Lower	Upper
Age (years)	1.054	0.027	1.006	1.104
Renalase (ng/mL)	0.993	0.032	0.984	0.997

phenomenon; however, some speculations can be made. As well known, the major secretion site of renalase is from kidneys. SRC is also known to originate from kidney tubule, and the cells lining the cyst lose normal anatomy. Thus, SRC can potentially be considered as non-functional with respect to secretion of renalase. This assumption suggests that renalase is associated with functional kidney mass and thus kidney function. However, in the present study we did not demonstrate any relationship between renalase and kidney function as measured by CKD-EPI. Additionally, there was no relationship between renalase and urine albumin/creatinine. This lack of association may be explained in the context of patient population. Our population has normal renal function, which may explain the lack of association.

It is known that SRC may be related to elevated BP and hypertension [4–6]. The reason for this association was not clear although increases in RAS activity were thought to be responsible [11, 12]. We did not demonstrate any relationship between systolic BP, diastolic BP and renalase levels because of inclusion of normotensive individuals and a small study population. One should also bear in mind that while some studies showed a relation between renalase and BP [2, 13], others failed to demonstrate such relationship [14–16]. Thus, it is needed to assess these relationships in larger patient population with hypertension.

As an interesting finding, we have demonstrated that renalase levels are associated with FMD in a positive manner. There is only scarce data in the literature regarding this issue. In a recent study, the relationship between renalase and endothelial injury markers was investigated among prevalent heart allograft recipients. The authors demonstrated that although renalase was correlated with endothelial cell injury and inflammation, the only predictor

of renalase was serum creatinine on multiple regression analysis [17]. In another study, in kidney allograft recipients, renalase has been related upon univariate analysis to markers of endothelial cell injury, such as vWF, thrombomodulin and vascular cell adhesion molecule. However, multivariate analysis revealed the only predictor of renalase values to be renal function [3].

In situations where catecholamine excess occurs, elevated markers of endothelial dysfunction such as ADMA and sVCAM-1 also increase leading to endothelial dysfunction. Thus, one can speculate that lower renalase levels may lead to increased catecholamine levels, elevated secretion of adhesion molecules and lead to endothelial dysfunction. Since endothelial dysfunction is closely related to hypertension, low renalase levels may lead to endothelial dysfunction and hypertension.

We found that renalase levels were reversely associated with epinephrine levels. This in fact is an expected finding since renalase metabolizes epinephrine, and global renalase knockout (KO) mouse model has normal renal function and a ~threefold increase in serum and catecholamine levels [18]. Furthermore, renalase causes a sustained (24 h), large (2.6-fold) decrease in epinephrine levels [19].

We admit that the current study has some limitations. First, the causal and effect relationship cannot be suggested in the current study. Second, the measurements were taken only once and temporal suggestions cannot be suggested. Third, we include only patients with solitary cyst. The associations between cyst number and cyst size with renalase levels were not specifically investigated. It is known that urinary phosphorus and sodium excretion may modulate renalase levels [20, 21]. However, we did not measure these parameters in the current study. Lastly, our study population is relatively small. Nevertheless, the patients were free of medications and any kind of chronic disease ruling out the effect of medications and disease on renalase levels and its relationship with SRC. It is needed to confirm these relationships in other populations such as patients with hypertension and chronic kidney disease.

In conclusion, we have demonstrated that renalase levels were associated with the presence of SRC and endothelial dysfunction. Further research is necessary to highlight underlying mechanisms.

Conflict of interest The authors declare that they have no conflict of interest.

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