The Relationship Between Proteinuria and Urinary Angiotensinogen Levels in Patients with Nephrotic Syndrome

Nefrotik Sendromlu Hastalarda Proteinüri ve İdrar Anjiyotensinojen Düzeyleri Arasındaki İlişki

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ABSTRACT

OBJECTIVE: Recently, urinary angiotensinogen (AGT) is used frequently as a marker of intrarenal renin-angiotensin-aldosterone system (RAAS) activity. In this study, we aimed to investigate the relationship between urinary AGT and proteinuria in patients with nephrotic syndrome.

MATERIAL and METHODS: Twenty-four patients followed up with nephrotic syndrome were included. The patients diagnosed with vasculitis, lupus nephritis, diabetes mellitus or amyloidosis, those with a glomerular filtration rate (GFR) <90 ml/min/1.73 m2, and patients receiving RAAS inhibitors, aldosterone receptor antagonists, diuretics or immunosuppressive drugs were excluded. Urinary AGT was calculated as AGT-to-creatinine ratio and proteinuria was calculated as protein-to-creatinine ratio (UP/Ucre) (mg/mg). Logarithmic transformations of UAGT/Ucre and UP/Ucre values were calculated to obtain the normal distributions.

RESULTS: Log(UP/Ucre) was 2.9±0.9, and log(UAGT/Ucre) was 0.7±0.2. There was a strong positive correlation between log(UP/Ucre) and log(UAGT/Ucre) (r=0.783, p<0.001). The strong correlation between log(UP/Ucre) and log(UAGT/Ucre) remained when controlled for the effects of 24-hour mean systolic and diastolic blood pressures (r=0.752, p=0.001).

CONCLUSION: We found a strong correlation between proteinuria and urinary AGT levels. Different from the previous studies, GFR was >90 ml/min in all patients. Therefore, high urinary AGT levels in earlier stages of nephrotic syndrome may reflect intrarenal RAAS activity that is supposed to play a role in pathogenesis of the disease.

KEY WORDS: Urinary angiotensinogen, Proteinuria, Nephrotic syndrome

ÖZ

AMAÇ: Son yıllarda idrar anjiyotensinojen (AGT) düzeyi, intrarenal renin-anjiyotensin-aldosteron sistemi (RAAS) aktivitesinin göstergelerinden birisi olarak sıkça kullanılmaktadır. Biz çalışmada nefrotik sendromlu hastalarda idrar AGT ve proteinüri düzeyi arasındaki ilişkiyi incelemeyi amaçladık.

GEREÇ ve YÖNTEMLER: Çalışmaya nefrotik sendromlu 24 hasta dahil edildi. Vaskülit, lupus nefriti, diyabetes mellitus veya amiloidoz tanılı, glomerüler filtrasyon hızı (GFH)<90 ml/dk/1,73 m2 olan, RAAS inhibitörü, aldosteron reseptör antagonisti, diüretik veya immünsupresif ilaç kullanan hastalar çalışma dışı bırakıldı. AGT atılımı, AGT/kreatinin oranı (UAGT/Ucre) (μg/gr); proteinüri, protein/kreatinin oranı (UP/Ucre) (mg/mg) olarak hesaplandı. Normal dağılımı sağlamak amacıyla UAGT/Ucre ve UP/Ucre parametreleri logaritmik dönüşümleri yapılarak değerlendirmeye alındı.

BULGULAR: Log(UP/Ucre):2,9±0,9, log(UAGT/Ucre): 0,7±0,2 saptandı. 24 saatlik ortalama sistolik ve diyastolik kan basınçlarının etkisi kontrol altında tutulduğunda log(UP/Ucre) ile log(UAGT/Ucre) arasındaki korelasyonun güçlü bir şekilde pozitif yönde devam ettiği görüldü (r:0,752, p=0,001).

SONUÇ: Çalışmamızda, proteinüri ve idrar AGT düzeyleri arasında güçlü korelasyon saptanmıştır. Önceki çalışmalardan farklı olarak hastaların tümünde GFH≥90 ml/dk/1,73 m2 idi. Bu durum nefrotik sendromda erken dönemde de yüksek saptanabilen idrar AGT düzeyinin hastalık patogenezinde yer aldığı öngörülen intrarenal RAAS aktivitesini yansıtabileceğini düşündürmektedir.

ANAHTAR SÖZCÜKLER: İdrar anjiyotensinojen, Proteinüri, Nefrotik sendrom

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INTRODUCTION

The renin-angiotensin-aldosterone system (RAAS) is a hormone system regulating blood pressure and fluid-electrolyte balance (1, 2). Angiotensin II (Ang-II) is synthesized from angiotensinogen (AGT), which is the only known renin substrate, and Ang-II's mechanism of action is the most extensively studied one among the peptides involved in RAAS (3-5). Increased AGT production results in increased Ang-II synthesis, and consequently increased RAAS activity (3). Recently, local tissue RAAS has been mentioned in the organs such as kidney, brain, vessels, heart, eyes, and adipose tissue, independently of systemic RAAS (6-12). AGT is excreted in urine in very small amounts since it is a large protein similar to albumin (AGT: 65 kD, albumin: 67kD). In addition, AGT that passes to glomerular ultrafiltrate is reabsorbed by endocytosis (13, 14). Therefore, it was thought that AGT found in urine was produced locally rather than originating from the systemic circulation, and both clinical studies and animal models supported this hypothesis (15-17). In the light of present data, it was suggested that urinary AGT level could be used as a marker of intrarenal RAAS activity (18, 19). It has been reported that increased intrarenal Ang-II levels play role in the pathophysiology of primary hypertension as well as other diseases such as chronic allograft injury, amyloidosis and IgA nephropathy (IgAN), independent of hypertension (20-25).

In this study, we aimed to investigate the relation between urinary AGT and proteinuria in patients with nephrotic syndrome.

MATERIALS and METHODS

Study Population

Twenty- four consecutive patients who were followed up in Ankara Numune Education and Research Hospital with the diagnosis of nephrotic syndrome were included prospectively in the study. The patients with a glomerular filtration rate (GFR)<90 ml/min/1.73 m^2, those with a primary diagnosis of vasculitis, amyloidosis, lupus nephritis, and diabetes mellitus (DM), and patients who were already receiving RAAS inhibitors, aldosterone receptor antagonists, diuretics or immunosuppressive drugs were excluded. The study was conducted in accordance with Declaration of Helsinki, and approved by the Local Ethics Research Committee. All subjects provided their written informed consents prior to the study.

Laboratory Procedures

The patients were evaluated with the urine and blood samples obtained when they were included in the study. Blood samples were obtained at 8-10 am, after an overnight fasting. Photometric analysis of hemoglobin was carried out with Sysmex XE 2100 (Roche Diagnostics Corp. Indiana, USA) hematology auto-analyzer. The albumin colorimetric method was used to measure creatinine and total protein; and fasting glucose, triglyceride and total cholesterol levels were measured with enzymatic colorimetric method. High- density lipoprotein cholesterol was measured with homogenous enzymatic colorimetric method using Hitachi Modular P800 (Roche Diagnostics Corp. Indiana, USA) auto-analyzer. Low- density lipoprotein cholesterol (LDL) was calculated with Friedewald method.

GFR was calculated according to The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation formula: $141 \times \min(S_{cr}/\varkappa, 1)^{\alpha} \times \max(S_{cr}/\varkappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018$ [if female] × 1.159 [if black] (26).

Urinary Angiotensinogen and Protein Measurements

Spot urine samples were collected from all patients in the morning to measure urinary angiotensinogen (UAGT), urinary creatinine (Ucre) and protein (UP) levels. Within 30 minutes after collection, the samples were centrifuged at 1000 rpm for 20 minutes, and stored at -80°C for a maximum period of 3 months. UAGT measurements were performed via an angiotensinogen ELISA kit (enzyme-linked immunosorbent assay Kit, USCN Life Science Inc. Houston). Ucre values were also measured in the same samples (mg/dL) using spectrophotometric method. UAGT/Ucre ratio (μg/g) and UP/Ucre ratio (mg/mg) were used in the statistical analysis to eliminate the effect of urine volume and density on UAGT levels.

Blood Pressure Measurements

All subjects had 24-hour arterial blood pressure monitoring via WatchBP 03 device (Microlife WatchBP AG, Switzerland). The patients were informed about the procedure and the device. They were asked to maintain normal daily activities, but to stay immobile during measurements. Devices were programmed to obtain measurements at 20-min intervals during all day (24-h).

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) for Windows 20 (IBM SPSS Inc., Chicago, USA) program was used for statistical assessments. Kolmogorov-Smirnov test was utilized to determine the distribution of data. Continuous variables with normal distribution were expressed in mean±standard deviation, and continuous variables without normal distribution were expressed in median [minimum-maximum]. Logarithmic transformations of UAGT/Ucre (log UAGT/Ucre) and UP/cre (log(UP/cre) were done to obtain the normal distributions of these parameters. Categorical variables were presented in numbers and percentage. Continuous variables with normal distribution were compared with independent sample t-test where appropriate. The relationship between the numeric parameters was analyzed by Pearson and Spearman correlation analysis. A stepwise multiple linear regression analysis was performed to identify independent determinants of log UAGT/Ucre and log(UP/cre). $P < 0.05$ is considered significant for statistical analyses.

RESULTS

There were 13 male and 11 female patients. Their mean age was 37.9±15.4 years. 24-hour mean systolic blood pressure (SBP) was 116.5±12.8 mmHg, 24- hour mean diastolic blood

pressure (DBP) was 73.8±10.3 mmHg, mean serum creatinine level was 0.93±0.3 mg/dL, log(UP/Ucre) was 2.9±0.9, and log(UAGT/Ucre) was 0.7±0.2 (μg/g). There were no differences between urinary AGT levels and, age and gender. There were 8 patients with focal segmental glomerulosclerosis, 5 patients with IgAN, 8 patients with minimal change disease and 3 patients with membranous glomerulopathy. Demographic and clinical characteristics and laboratory findings of the subjects were presented in Table I.

Log(UAGT/Ucre) had positive correlations with cholesterol and LDL levels (r=0.644, p=0.002 and r=0.605, p=0.004, respectively). It had positive correlations with 24- hour mean SBP and 24- hour mean DBP (r=0.462, p=0,023, and r=0.423, p=0.039, respectively) and a negative correlation with serum albumin levels (r= -0.522, p=0.009) (Table II).

Log(UP/Ucre) had positive correlations with cholesterol and LDL levels (r=0.590, p=0.005, and r=0.582, p=0.006), and a strong negative correlation with serum albumin levels (r= -0.752, p=0.001) (Table II).

There was a strong positive correlation between Log(UP/cre) and log(UAGT/Ucre) (r=0.783, p<0.001). The strong correlation between log(UP/Ucre) and log(UAGT/Ucre) remained when controlled for the effects of 24- hour mean systolic and diastolic blood pressures (r=0.752, p=0.001) (Table II, Figure 1).

The age, gender (ref: female), body mass index, drugs, 24- hour mean SBP, 24- hour mean DBP, serum albumin, cholesterol, and LDL levels were analyzed in the stepwise regression model. According to the results of multivariable regression analysis, SBP (B±SE=0.370±0.150, p=0.021), cholesterol (B±SE=0.200±0.100, p=0.039) and serum albumin

Table I: Baseline characteristics and laboratory findings of the study population.

Variables	Values	Variables	Values
Patient (n)	24	Creatinine (mg/dL)	0.93±0.3
Male (n)	13	GFR (mL/min/1.73 m^2)	98.5±7.3
Age, years	37.9±15.4	Sodium (mEq/L)	139.3±3.3
Body mass index, kg/m^2	24.7±4.1	Potassium (mEq/L)	4.4±0.4
Primary diagnosis (n)		Calcium (mg/dL)	9.3±0.4
FSGS	8	Phosphorus (mg/dL)	3.6±0.6
IGAN	3	Albumin (mg/dL)	3.7±1.0
MCD	8	Glucose (mg/dL)	90.2±8.2
MGN	3	Total cholesterol (mg/dL)	228.8±80.6
MPGN	2	LDL cholesterol (mg/dL)	140.5±30.3
Follow-up period (months)	10 (3-76)	HDL cholesterol (mg/dL)	65.2±25.7
Comorbid disease (n)		Triglyceride (mg/dL)	116 (49-261)
Hypertension	6	CRP (mg/L)	2.0 (0.2-6)
Hyperlipidemia	6	WBC ($x10^3$)	7.8±2.8
Drugs		Hemoglobin (g/dL)	13.9±1.8
CCB	4	Platelet ($x10^3$)	257.8±99.3
Beta-blokers	2	Log(UP/Ucre) (mg/mg)	2.9±0.9
Systolic BP (mmHg)	116.5±12.8	Log (UAGT/Ucre) (mcg/g)	0.7±0.2
Diastolic BP (mmHg)	73.8±10.3		

FSGS: Focal segmental glomerulosclerosis, **IGAN:** IgA nephropathy, **MCD:** Minimal change disease, **MGN:** Membranous glomerulopathy, **MPGN:** Membranoproliferative glomerulonephritis, **BP:** Blood pressure, **GFR:** Glomerular filtration rate, **LDL:** Low-density lipoprotein, **HDL:** High-density lipoprotein, **CRP:** C-reactive protein, **WBC:** White blood cell, **UP/Ucre:** Urine protein-to-creatinine ratio, **UAGT/Ucre:** Urine angiotensinogen-to-creatinine ratio.

Data were presented as mean±SD, median (min-max) or number (percentiles).

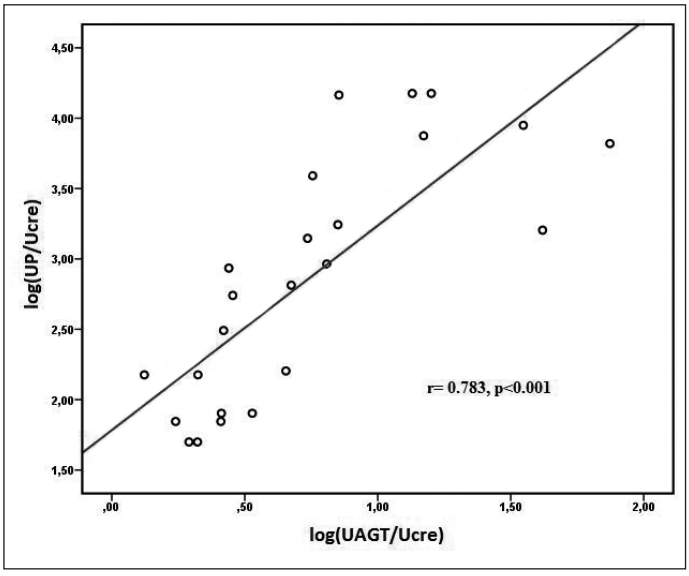

Figure 1: *The correlation between UAGT/Ucre and UP/Ucre.*
*** Correlation is significant at the 0.01 level (2-tailed).*
UAGT/Ucre: *urine angiotensinogen-to-creatinine ratio,*
UP/Ucre: *urine protein-to-creatinine ratio*

level (B±SE=-0.253±0.095, p=0.017) were identified as independent predictors for log(UAGT/Ucre). The log(UAGT/Ucre) (B±SE=1.129±0.229, p<0.001) and serum albumin level (B±SE=-0.401±0.098, p =0.001) were identified as independent predictors for log(UP/Ucre) (Table III).

DISCUSSION

In this study, a positive correlation was found between proteinuria and urine AGT levels. This finding is in concordance with the results of previous studies.

Systemic RAAS exerts its effects via endocrine pathways, and here AGT is synthesized by liver and converted to angiotensin I by renin synthesized in juxtaglomerular apparatus (2, 27). Local production of RAAS elements in tissues and their effects were mentioned in recent years in addition to systemic RAAS (28). Recently, determination of renin and other RAAS peptides and their receptors in different tissues (kidney, vascular endothelium, heart, brain, eye, adipose tissue etc.) have been recognized as indicators of local RAAS presence (7-12, 28-30). AGT is one of these markers and its level is closed to the Michaelis-Menten constant for renin. Therefore, the levels of AGT can determine the activity of the RAAS, as renin. AGT, which is known to be predominantly synthesized in the liver, is a big molecule and it has a similar molecular weight to albumin (AGT: 65 kD, albumin: 67kD), and therefore it is excreted in the urine in very small amounts. On the other hand, the small amount of AGT that passes to the glomerular ultrafiltrate is reabsorbed by megalin-mediated endocytosis. Therefore, AGT found in the urine was thought to have a renal origin rather than originating from the systemic circulation (12-14). AGT production was also shown in epithelial cells of proximal tubules (16, 31).

Table II: The correlations of parameters with log(UP/Ucre) and log(UAGT/Ucre)

Variables	Log(UP/Ucre)		Log(UAGT/Ucre)	
	R	P	R	P
Age	0.079	0.713	0.063	0.770
Body mass index	0.174	0.415	0.231	0.276
Systolic BP	0.278	0.188	0.462	0.023*
Diastolic BP	0.359	0.085	0.423	0.039*
Log(UP/Ucre)	-	-	0.783	<0.001**
Log(UAGT/Ucre)	0.783	<0.001**	-	-
Total cholesterol	0.590	0.005*	0.644	0.002*
LDL cholesterol	0.582	0.006*	0.605	0.004*
HDL cholesterol	0.215	0.061	0.262	0.107
Triglyceride	0.216	0.163	0.166	0.280
Albumin	-0.752	0.001*	-0.522	0.009*
Creatinine	-0.111	0.606	-0.099	0.645
GFR	0.128	0.552	0.054	0.802
CRP	-0.121	0.601	-0.171	0.459

* Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed).

BP: Blood pressure, **UP/Ucre:** Urine protein-to-creatinine ratio, **UAGT/Ucre:** Urine angiotensinogen-to-creatinine ratio, **LDL:** Low-density lipoprotein, **HDL:** High-density lipoprotein, **GFR:** Glomerular filtration rate, **CRP:** C-reactive protein.

In an animal study, human AGT was infused into rats but circulating human AGT was not detected in urine samples. The same study showed that Ang II infusions to normal rats increased urinary AGT in a time-and dose-dependent manner and urinary AGT level was correlated with renal Ang II level but not with plasma Ang II concentration. Additionally, there was a dissociation between urinary AGT levels and proteinuria (15). Another study on rats reported significantly higher AGT mRNA and AGT protein levels were detected in proximal tubule cells after Ang II infusion (17).

Many clinical studies also showed that urinary AGT level and prognosis might be correlated in patients with amyloidosis, DM, hypertension, renal transplantation, preeclampsia, IgAN, and chronic kidney disease (20, 22, 32-38)

A study on renal transplant patients with a mean proteinuria less than 1 g/day reported that basal urine AGT level was

Table III: Independent predictors for UAGT/cre and UP/cre by multivariate regression analysis.

Characteristic	B±SE	95 C.I.		p value
Log(UAGT/cre)§				
Systolic BP	0.370±0.150	0.060	0.680	0.021*
Total cholesterol	0.200±0.100	0.100	0.300	0.039*
Albumin	-0.253±0.095	-0.454	-0.053	0.017*
R^2=0.633, p=0.002				
Log(UP/Ucre)‡				
Log (UAGT/Ucre)	1.129±0.229	0.646	1.611	<0.001*
Albumin	-0.401±0.098	-0.608	-0.194	0.001*
R^2=0.833, p<0.001				

§: The stepwise regression model included age, gender, body mass index, drugs, systolic BP, diastolic BP, albumin, total cholesterol and low-density lipoprotein cholesterol, as possible independent variables.

‡: The stepwise regression model included age, gender, body mass index, drugs, systolic BP, diastolic BP, albumin, total cholesterol and low-density lipoprotein cholesterol and log(UAGT/cre), as possible independent variables.

***** P<0.05 is considered significant for statistical analyses.

higher in renal transplant patients compared to the controls, and proteinuria level and basal urine AGT levels showed a positive correlation (21). In another study including AA amyloidosis patients, urine AGT level showed a positive correlation with proteinuria, and negative correlation with serum albumin level in patients with renal amyloidosis. Mean serum albumin level was similar (3.8 gr/day), but mean serum creatinine level was higher (1.3 mg/dl) in that study compared to our study (22).

A study on chronic kidney disease reported that urinary AGT level was significantly higher than filtered AGT in patients with subnephrotic proteinuria. On the other hand, positive correlation between urinary AGT and serum AGT levels, and most of urinary AGT seemed to be originated from systemic circulation in patients with nephrotic-range proteinuria. Although the authors indicated that AGT is produced even by kidney, this correlation between serum and urinary AGT levels in the presence of nephrotic-range proteinuria was interpreted in favor of glomerular injury. Therefore AGT could be non-specifically excreted in the urine as albumin, since those two molecules had similar molecular weights (39). However, a similar study with IgAN patients, reporting a positive correlation between urinary AGT levels and proteinuria, showed that AGT mRNA level in renal tissue was high in harmony with urinary AGT. In the same study, a negative correlation between urinary AGT level and GFR was shown to be correlated with disease progression after 3 years of follow up (38).

Another study demonstrated high urinary AGT levels in diabetic nephropathy and membranous nephropathy subgroups; however urine AGT level was not high despite presence of more severe proteinuria in the minimal change disease group. Those results indicate that high urinary AGT, as an indicator of RAAS, can be related to the pathogenesis of the disease and a distinct entity from nonspecific proteinuria (37). In another study, urinary AGT levels were significantly higher in normoalbuminuric patients with type 1 DM when compared to healthy controls (32).

Our patients were normotensive and their GFR were within normal limits, and we found a strong correlation between proteinuria and urinary AGT. This result is in concordance with the results of the previous studies. In contrast to most of the previous studies, we did not have any patients with a GFR <90 ml/min. Based on the correlation between urinary AGT level and proteinuria, we suppose that urine AGT may be an indicator of renal injury also in early stage. We found positive correlations between systolic and diastolic blood pressures and urinary AGT level, and showed that systolic blood pressure was an independent predictor for high urinary AGT level. On the other hand, it was seen that the positive correlation between urinary AGT level and proteinuria strongly remained even after controlled for the effects of the blood pressure. Thus, the positive correlation between proteinuria and urinary AGT level is independent of the positive correlation between urinary AGT level and blood pressure in our normotensive patient group. Based on those data, it may be suggested that intrarenal RAAS activity is an independent determinant for the severity of proteinuria and urinary AGT is a convenient marker of intrarenal RAAS activity.

The small and heterogeneous patient group is the limitation of our study. Although we did not use the additional methods for differentiation of systemic and intrarenal RAAS, the source of urinary AGT was presumably the kidney, when we consider the results of previous studies in this area.

In conclusion, high urinary AGT levels in earlier stages of nephrotic syndrome correlated with proteinuria may reflect intrarenal RAAS activity that is supposed to play a role in pathogenesis of the disease. In order to more clearly determine the link between intrarenal RAAS and the pathogenesis of renal diseases, studies with larger patient groups including the simultaneous use of different markers and measurement methods are needed.

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