

The relationship between serum and urinary Fetuin-A levels and kidney stone formation among kidney stone patients

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Introduction Mineralization inhibitors are required to prevent the precipitation of minerals and inhibit the formation of kidney stones and other ectopic calcifications. In laboratory studies, Fetuin-A as a glycoprotein has inhibited hydroxyapatite precipitation in calcium and phosphate supersaturated solutions; however, information about patients with kidney stones is limited. The aim of this study was to investigate the association of serum and urinary Fetuin-A levels with calcium oxalate kidney stones.

Material and methods In this case-control study, 30 patients with kidney stones and 30 healthy individuals without any history of urolithiasis who were referred to the urology ward of Sina Hospital of Tehran, Iran, in 2015 were entered into the study. All patients underwent computerized tomography scans. After collecting demographic information, serum and urine levels of Fetuin-A and some other calcification inhibitors and promoters, were measured and compared using T-test, Mann-Whitney and logistic regression between the two study groups.

Results Patients with kidney stones, on average, had lower levels of Serum Fetuin-A (1522.27 ± 755.39 vs. 1914.64 ± 733.76 $\mu\text{g/ml}$; $P = 0.046$) as well as lower levels of Urine Fetuin-A (944.62 ± 188.5 vs. 1409.68 ± 295.26 $\mu\text{g/ml}$; $P < 0.001$). Multivariate logistic analysis showed that urinary calcium and serum creatinine are the risk factors and Fetuin-A is a urinary protective factor for kidney stones.

Conclusions PFC Our study showed that patients with kidney stones had lower serum and urinary levels of Fetuin-A. In the logistic regression model, urinary Fetuin-A was reported as a protective factor for kidney stones.

Key Words: fetuin-A ↔ kidney stones ↔ crystallization

INTRODUCTION

Calcification as a physiological process is essential for the process of bone formation. At the same time misplaced calcification is potentially pathogenic [1]. The urinary stone, which is most commonly composed of calcium oxalate, is an example of misplaced calcification [2]. Misplaced calcification inhibitors have recently attracted attention [3, 4]. These inhibitors increase the concentrations of calcium and oxalate required for spontaneous formation of new crystals and reduce the growth, accumulation and attachment of crystals to kidney cells [5].

Fetuin-A, also known as alpha 2-Heremans-Schmid glycoprotein, is a systemic misplaced calcification inhibitor, which is produced in the liver and is present in high levels in blood and bones. Fetuin-A has been shown to inhibit the precipitation of hydroxyapatite from a supersaturated solution of calcium and phosphorus in vitro [6, 7]. Fetuin-A can prevent the precipitation and enlargement of calcium phosphate crystals from mineral supersaturated solutions due to its high binding affinity to calcium and phosphorus [8] and can remove the extra load of calcium and phosphorus by creating sustainable calciprotein complex solutions [9, 10]. Previous studies have shown that patients with kidney stones have lower

levels of both serum and urinary Fetuin-A than the control group. However so far the role of Fetuin-A has not been clearly identified in the formation of urinary stones, and the results are contradictory [7, 11]. A few studies have evaluated the Fetuin-A levels in urine [12, 13]; but serum levels also need to be checked due to inaccurate urine levels of Fetuin-A for example as a result of tissue damage in an affected kidney [5]. The present study was conducted to investigate the relationship between urinary and serum Fetuin-A levels with the risk of kidney stone formation and to find an independent predicting factor for developing kidney stones.

MATERIAL AND METHODS

Study design

This case-control study was performed on patients hospitalized at the urology ward of Sina Hospital in 2015.

Study population

Inclusion criteria for the case group were definite diagnoses of kidney or ureter stones as diagnosed by spiral computerized tomography scan without injection of contrast material.

The control group was selected from among transplant donors who had no history of renal or ureteral stones and no evidence of stone on imaging (spiral computerized tomography scan without injection of contrast material). Patients with a history of hypertension, coronary artery disease, diabetes, liver or kidney dysfunction, any malignancy and also patients with other types of stone composition except calcium oxalate monohydrate and calcium oxalate dehydrate, were excluded from study.

Sample determination

30 non-randomized samples were considered for each of the groups.

Studied variables

24-hour urine volume (ml/24h), urinary levels of Fetuin-A ($\mu\text{g/ml}$), oxalate (mg/24h), citrate (mg/24h), magnesium (mg/24h), phosphate (mg/24h), calcium (mg/24h), uric acid (mg/24h), creatinine (mg/24h) and serum levels of Fetuin-A ($\mu\text{g/ml}$), magnesium (mEq/l), phosphate (mg/dl), calcium (mg/dl), uric acid (mg/dl), and creatinine (mg/dl) were measured by recording the sex, age and Body Mass Index (BMI) of subjects in the two groups. Urinary

and serum levels of Fetuin-A were measured using a special kit (Human FETU-A Elisa Kit/ SHANGHAI CRYSTAL DAY BIOTECH CO, .LTD) and the enzyme-linked immunosorbent assay (ELISA) method.

Ethics

This study was approved by the ethics committee of University of Medical Sciences Urology Research, Center Sina Hospital (Code: IR.TUMS.REC.1394.1080). All subjects were informed of the objectives and methodology of the study and signed a written consent form to participate in the project.

Statistical analysis

Data obtained from the study was statistically analyzed using the SPSS version 16 (SPSS Inc, Chicago, IL, USA). Quantitative data was compared between groups using the independent t-test, and in case of non-normally distributed data using the Mann-Whitney test. Multivariate logistic regression was also used to evaluate the effect of variables on the risk of kidney stones.

RESULTS

A total of 30 patients and 30 controls were included and further evaluated. Baseline variables of the two groups are summarized in Table 1.

The difference between the mean age of case (36.7 ± 10.8) and control (28.4 ± 4.97) groups was statistically significant ($p < 0.001$). The case group also had a higher body mass index (29.18 ± 6.98 vs. $25.26 \pm 1.6 \text{ kg/m}^2$, $P = 0.005$).

The subjects were not significantly different in terms of 24-hour urine volume.

It should be mentioned that in the case group, the Hounsfield unit (HU) densities of the stones as determined by non-contrast helical computed tomography ranged from 1193 to 2261. Moreover, considering the stone composition, all the stones were composed of calcium oxalate monohydrate or calcium oxalate dihydrate.

There were significantly lower serum Fetuin-A levels in the case group compared with the control group (1522.27 ± 755.39 vs. $1914.64 \pm 733.76 \mu\text{g/ml}$; $P = 0.046$). Urinary Fetuin-A was significantly lower in the case group (944.62 ± 188.5 vs. $1409.68 \pm 295.26 \mu\text{g/ml}$; $P < 0.001$).

Considering the remaining serum parameters (Table 2), patients with kidney stones had, on average, lower levels of calcium (8.78 ± 0.69 vs. $9.25 \pm 0.77 \text{ mg/dl}$; $P = 0.016$), phosphate (3.51 ± 0.59 vs. $3.87 \pm 0.45 \text{ mg/dl}$; $P = 0.011$), but higher levels

of creatinine (1.22 ± 0.14 vs. 1.15 ± 0.11 mg/dl; $P = 0.026$). Also, in the urine samples of patients with kidney stones, there were, on average, lower levels of creatinine (1189.57 ± 325.57 vs. 1561.33 ± 345.91 mg/24h; $P < 0.001$), magnesium (68.43 ± 33.41 vs. 106.3 ± 30.81 mg/24h; $P < 0.001$), citrate (280.43 ± 47.77 vs. 338.67 ± 102.23 mg/24h; $P = 0.007$), but higher levels of calcium (436.7 ± 191.58 vs. 208.33 ± 55.96 mg/24h; $P < 0.001$) and oxalate (49.33 ± 13.33 vs. 31.13 ± 8.85 mg/24h; $P < 0.001$) (Table 3).

Based on multivariate logistic regression analysis, urinary Fetuin-A (Adjusted Odds Ratio = 0.664) was a protective factor, and urinary calcium (AOR = 1.89) and serum creatinine (AOR = 17.9) were considered as risk factors for developing kidney stones.

DISCUSSION

This study was conducted in order to investigate the relationship between serum and urinary levels of Fetuin-A and the formation of kidney stones. Fetuin-A is a glycoprotein with systemic inhibitory properties for misplaced calcification.

Previous studies suggest that Fetuin-A has a high affinity for calcium ions and therefore is considered as one of the most important non-collagenous proteins involved in osteogenesis [14, 15]. Fetuin-A can block creation and development of hydroxyapatite crystals [10, 16, 17]. This protein seems to be absorbed by calcium phosphate particles [18] and prevents them from sedimentation, and in fact inhibits non-bone calcification by creating colloidal particles and solution from Fetuin-A and calcium phosphate [19, 20]. Fetuin-A has also been introduced as a predicting factor of mortality in patients undergoing hemodialysis or with severe atherosclerosis [21]. In studies on rats, the low serum level of Fetuin-A has been associated with extensive calcification in different tissues such as kidney, heart, lung and skin [4]. This protein can also reduce the calcification process in vascular smooth muscle cells [20].

Cai stated in a review study that Fetuin-A has no role in preventing mineralization outside of bones in physiological conditions and the importance of this protein can be observed in mineral metabolism dysfunction or in other mechanisms dealing with misplaced mineralization [22].

Contradictory reports also are available. For example, Umekawa and Nishio demonstrated that Fetuin-A is unable to prevent the formation and growth of calcium oxalate crystals [23, 24]. However, in both studies, crystallization had been induced in vitro.

Table 1. Baseline variables in two groups

Variable	Group	Mean	Std.D	P
Age (years)	Cases (n = 30)	36.70	10.80	<0.001
	Controls (n = 30)	28.40	4.97	
Body Mass Index (kg/m ²)	Cases	29.18	6.98	0.005
	Controls	25.26	1.6	
Urine volume (ml/24h)	Cases	1485.17	309.16	0.520
	Controls	1531.33	241.21	

Table 2. Serum variables

Variable	Group	Mean	Std.D	P
Serum Fetuin-A (µg/ml)	Cases (n = 30)	1522.27	755.39	0.046*
	Controls (n = 30)	1914.64	733.76	
Serum Creatinine (mg/dl)	Cases	1.22	0.14	0.026*
	Controls	1.15	0.11	
Serum Calcium (mg/dl)	Cases	8.78	0.69	0.016*
	Controls	9.25	0.77	
Serum Phosphate, (mg/dl)	Cases	3.51	0.59	0.011*
	Controls	3.87	0.45	
Serum uric acid (mg/dl)	Cases	5.88	1.72	0.918
	Controls	5.93	1.77	
Serum Magnesium (mEq/l)	Cases	1.73	0.21	0.260
	Controls	1.68	0.18	

Table 3. Urine variables

Variable	Group	Mean	Std.D	P
Urine Fetuin-A (µg/ml)	Cases (n = 30)	944.62	188.50	<0.001*
	Controls (n = 30)	1409.68	295.26	
Urine Creatinine (mg/24h)	Cases	1189.57	325.57	<0.001*
	Controls	1561.33	345.91	
Urine Calcium (mg/24h)	Cases	436.70	191.58	<0.001*
	Controls	208.33	55.96	
Urine Phosphate (mg/24h)	Cases	616.63	243.69	0.355
	Controls	667.63	174.16	
Urine uric acid (mg/24h)	Cases	549.17	165.29	0.373
	Controls	515.33	123.28	
Urine Magnesium (mg/24h)	Cases	68.43	33.41	<0.001*
	Controls	106.30	30.81	
Urine citrate (mg/24h)	Cases	280.43	47.77	0.007*
	Controls	338.67	102.23	
Urine oxalate (mg/24h)	Cases	49.33	13.33	<0.001*
	Controls	31.13	8.85	

Based on the present study results, mean levels of serum and urinary Fetuin-A in the case group were significantly lower than in the control group.

In examining the risk of kidney stones, urinary Fetuin-A was a protective factor and urinary calcium and serum creatinine were the risk factors for the development of kidney stones.

Stejskal et al. examined the association between urinary Fetuin-A and kidney stones for the first time and, similarly to the results of our study, lower urinary Fetuin-A levels were observed in patients with kidney stones. The difference between the two groups in terms of factors such as citrate, calcium and oxalate in the urine was similar to the present study [11].

Hypercalciuria is the most common cause of renal calcium stone formation and in the present study both its presence and its role as a strong risk factor have been proven.

In the final model, in addition to low urinary Fetuin-A levels and hypercalciuria, higher serum creatinine is another risk factor. Although there were significant differences between the two groups in terms of serum creatinine levels, the measures were all within the normal range and do not imply any specific clinical condition. This is also true about the lower serum levels of calcium and phosphate in the case group, which are not in the final model but there are differences between the two groups. Again, like the serum creatinine levels, serum calcium and serum phosphate levels are within the normal range and cannot be considered a hypocalcemic or hypophosphatemic state and therefore these differences may not be clinically interpretable.

Subjects in both groups had significant differences in age (36.7 vs. 28.4 years). The serum levels of Fetuin-A have a significant and inverse correlation with age [26, 27, 28]. Hence, decreased serum levels of Fetuin-A in this study may be because of the higher age of patients compared with the control group; however, in the final regression model, age had no independent effect on the risk of kidney stones formation. It should be noted, however, that in the study of Aksoy, despite matched in age in the two groups, significant differences were reported in Fetuin-A levels. Aksoy et al. introduced the polymorphism at position 766C >G as a risk factor for calcium oxalate in kidney stones and suggested that these patients should be followed for rejecting recurrent nephrolithiasis; furthermore, serum levels of Fetuin-A were significantly lower in patients than in the control group [6]. Gurbuz et al. found no significant difference between serum levels or urinary Fetuin-A, in patients with or without urolithiasis; and also no differences were reported between the two groups in terms

of other factors such as inducers or inhibitors of urolithiasis, and this could undermine the validity of the results [25]. Also, in the study by Prezioso et al., no significant results were observed in the relationship between serum levels of Fetuin-A and recurrent urolithiasis in young people [5]. The latest study similar to the present research was conducted by Wu et al., in which urinary Fetuin-A levels in patients with kidney stones were lower than in healthy individuals, but there was no significant difference in serum levels between the two groups [7].

The results of the present study concerning Fetuin-A serum and urinary levels are similar to some previous studies' results but in contrast to other similar studies. Irrespective of differences in kits, laboratory errors and the lack of a gold standard for measuring Fetuin-A, sample size and other various factors such as gene polymorphism of Fetuin-A [6], mineral structure of Fetuin-A [9, 29], nanobacteria as nanoparticles of inducing calcification [30, 31] and finally racial differences could be reason of differences in the results of this study and other similar works.

Previous studies have shown that Fetuin-A, in addition to liver cells, is also secreted by renal tubular cells [23, 32, 33]. This mechanism has also been demonstrated in patients with metabolic syndrome [34]. In contrast, Wu stated that tubular damage caused by kidney stones and subsequent oxidative stress as well as increased urinary calcium gradually lead to decreased levels of urinary Fetuin-A [7]. This issue could also potentially make a difference in the results, since by the time the process of stone formation in the kidney has started, urinary levels of Fetuin-A may be subject to increase or decrease. Therefore, to determine certain levels of Fetuin-A, which is associated with increased risk of kidney stones, there is a need for prospective studies with large sample sizes of healthy subjects.

Non-matched cases and controls based on demographic variables were the limitations of this study. In addition, regardless of their differences in terms of mentioned calcification factors levels; there are other proteins that make individuals prone to kidney stone formation, such as Matrix Gla protein [35], Osteopontin [36] and Tamm-Horsfall [1] which were not measured but may have affected the results. So, further studies enrolling well matched groups should be conducted to evaluate the exact role of Fetuin-A by considering the role of many other inhibitors/promoters of renal calcium stone formation.

CONCLUSIONS

According to the results of the study, serum and urinary Fetuin-A levels of patients with kidney

stones were significantly lower than in healthy individuals and based on multivariate logistic regression analysis, urinary Fetuin-A levels are inversely associated with the risk of kidney stones. Thus, the routine checking of Fetuin-A levels in stone formers in order to identify high risk groups

for calcium stone formation is suggested and also, if available in the future, its replacement is recommended in these patients.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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