Clinical Research

Urinary calcium and matrix Gla protein levels in the kidney stones: a case-control study

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ABSTRACT

BACKGROUND Kidney stones are a global issue with varying prevalence. The most common metabolic risk factor is hypercalciuria, a condition where excess calcium in the urine promotes stone formation. Matrix Gla protein (MGP) inhibits stone formation by preventing crystal growth. This study aimed to analyze the correlation between urinary calcium and MGP levels in kidney stone formation.

METHODS A case-control study at Kardinah Hospital, Indonesia, included 64 patients with kidney stone and 64 healthy controls. Exclusion criteria included renal failure, stroke, kidney tumor, heart failure, and hemodialysis. Urinary calcium and MGP were measured using the 5'-nitro-5'-methyl-BAPTA method and enzyme-linked immunosorbent assay. Cut-off values were determined via receiver operating characteristic analysis.

RESULTS Among 128 participants (mean age: 51.6), the optimal cut-off for urinary MGP was 1,405 ng/l (p = 0.00024) with 62.5% sensitivity and 72% specificity. Urinary calcium cut-off was 72.5 mg/24 hours with 81.3% sensitivity and 62.5% specificity. Higher urinary calcium and MGP levels were linked to kidney stones (OR: 7.22; 95% CI: 3.23–16.18 and OR: 4.26; 95% CI: 2.03–8.96, respectively). A significant association was found between urinary calcium and MGP (OR: 5.11; 95% CI: 2.31–11.29, p = 0.00006) that hypercalciuria and increased MGP levels are predictors of kidney stone formation.

CONCLUSIONS Urinary calcium and MGP levels are associated with kidney stones. Elevated urinary calcium (>1,405 ng/l) increases MGP levels more than 5-fold. Depending on their levels, urinary calcium and MGP act as both promoters and inhibitors of stone formation.

KEYWORDS calcium, kidney stones, matrix Gla protein, nephrolithiasis

Kidney stones are a worldwide health issue, with prevalence rates ranging from 1–5% in Asia.[1,2] Stone formation may occur because of various factors, including age, sex, body mass index, daily intake, anatomical abnormalities of the urinary tract, systemic and hormonal/metabolic abnormalities, genetic factors, climate, and chronic exposure to environmental contaminants.[3,4] The inability to remove kidney stones can lead to obstruction and subsequent acute renal failure. They can also serve as a nidus for infection, which can be fatal in severe cases. Other complications include abscess formation, urosepsis, scarring, ureteral narrowing, ureteral perforation, and loss of renal function because of prolonged obstruction.[5]

An imbalance between the promoters and inhibitors of the urinary system is a key factor in kidney stone formation. Stone inhibitors increase the supersaturation required to initiate nucleation, decrease the rates of crystal growth and

aggregation, and prevent secondary nucleation. These substances include inorganic chemicals, proteins, and glycosaminoglycans. In contrast, the promoters increase the likelihood of stone formation in supersaturated solutions. Deviations in the function and/or concentration of these substances can alter urinary physiochemical circumstances, leading to the production of stones.[6] Previous studies have stated that calcium is a urinary stone promoter and hypercalciuria is the most common metabolic risk factor for stone formation. Hypercalciuria is observed in 5–10% of adults and approximately one-third of individuals with calcium stones.[2]

The matrix Gla protein (MGP) is a large molecule that inhibits kidney stone formation. *In vitro* research has suggested that MGP suppresses crystal formation, specifically during the first stages of nucleation and stone deposition. It functions as an inhibitor of renal stone development in cell cultures, with a higher MGP level inhibiting calcium salt accumulation and a lower level promoting mineralization.[7,8] Prior research has identified a correlation between serum MGP levels and kidney stone occurrence, although the findings were inconsistent.[9] However, few studies have investigated the association between urinary calcium and MGP levels in patients with kidney stones. Proving this correlation may require routine urine samples collected from patients suspected of having kidney stones in a clinical setting. Both tests can be performed quickly and may provide high sensitivity or specificity compared with other urine markers of kidney stones. Therefore, this study aimed to analyze the role of urinary calcium and MGP levels in kidney stone formation, and the association between these two factors.

METHODS

This case-control study was conducted at Kardinah Hospital, Tegal, Central Java, Indonesia, from January 2024 to March 2024. The minimum sample formula for unpaired categorical analysis was applied. Based on a previous study reporting hypercalciuria in 10% of the adult population,[10] and using an alpha deviation of 1.96 and beta deviation of 0.84, the minimum required sample was calculated to be 62 samples in each group. This study divided 128 participants into two groups: one group composed of individuals diagnosed with kidney stones and a control group comprising healthy individuals without kidney stones. Adults (age ≥18 years) diagnosed with kidney stones and were willing to participate in the study were included. The study excluded individuals with a prior or current diagnosis of renal failure, a history of stroke, kidney tumors, or heart failure, having undergone or currently undergoing hemodialysis, and refusal to participate in the study. Diagnosis in the kidney stone group was based on patient history and radiological examination, including computed tomography (CT), intravenous urography (IVU), or ultrasonography. The inclusion criterion for the control group was adults (age ≥18 years old) with no prior history of kidney stones. All controls also underwent history-taking, plain radiology, and urinalysis. If the results were within normal limits, further imaging techniques, such as CT, IVU, and ultrasonography, were not performed.

Data were collected from the medical records of Kardinah Hospital between January and August 2023. Urinary calcium levels were measured using the 5'-nitro-5'-methyl-BAPTA method in a 24-hour urine sample at the CITO Laboratory, Semarang, Central Java. Urinary MGP levels were measured at the GAKI Laboratory, Universitas Diponegoro, Semarang, Central Java. Urine samples for MGP analysis were collected in sterile tubes and centrifuged at 2,000–3,000 rpm for 20 min. The liquid fraction obtained after centrifugation, known as the supernatant, was collected. The enzyme-linked immunosorbent assay reagent (MGP BT-Lab kit, Cat No. E1248Hu; BT LAB, China) was added to the supernatant and incubated for 1 hour at 37°C, followed by five rinses of the plate. Solutions A and B were incubated for 10 min at 37°C. Subsequently, a stop solution was introduced and the mixture was incubated until the desired color appeared. The optical density (OD) was measured within 10 min. A standard curve was constructed by plotting the average OD values on the y-axis and the concentration on the x-axis. Participant data were collected and analyzed using SPSS software version 25.0 (IBM Corp., USA). Data distribution was examined using the Kolmogorov–Smirnov test.

Receiver operating characteristic (ROC) curves were used to determine the optimal cut-off values for urinary calcium and MGP levels. The association between urinary calcium and MGP levels was analyzed using the chi-square test with a significance level of $p<0.05$, using the cut-off level from the ROC curve analysis. Ethical approval for the study was originally obtained from the Ethics Committee of

Kardinah Hospital, Tegal (No: 01/KEPK/RSUK/I/2023). The ethical prolongation was also obtained a year after sample collection and title adjustment (No: 01/KEPK/RSUK/I/2024). All participants provided written informed consent before participating in the study.

RESULTS

A total of 128 participants were included in the study: 64 with kidney stones in the case group and 64 healthy participants without kidney stones in the control group. None of the data were normally distributed. The mean age of the participants was 51.59 (13.34) years. Among the participants, 49% were women and 51% were men. A history of diabetes mellitus was reported in 11% of the participants, whereas 13% had a history of hypertension. The urinary calcium was 91.50 (14–338) mg/24 hours. The mean urinary MGP level was 1,347.4 (498.38) ng/l. Details regarding the subjects' characteristics are shown in Table 1.

Based on the ROC curve in Figure 1a, the optimal cut-off for urinary calcium level was 72.5 mg/24 hours, with a sensitivity of 81.3% and specificity of

Table 1. Characteristic of subjects included in the study

Variables	Subjects with kidney stones (n = 64)	Subjects without kidney stones (n = 64)	Total (n = 128)	*p**
Age (years), mean (SD)	52.34 (11.71)	50.58 (17.64)	51.59 (14.34)	0.05
Female sex, n (%)	29 (45)	28 (44)	63 (49)	-
BMI (kg/m²), mean (SD)	26.30 (4.21)	22.83 (3.86)	24.25 (4.39)	0.2
Body weight (kg), mean (SD)/ median (min–max)	66.50 (11.83)	58.50 (38–92)	62.00 (38–100)	0.2
Body height (cm), mean (SD)	158.72 (7.29)	161.83 (8.22)	160.23 (7.91)	**0.001**
History, n (%)				-
DM	10 (16)	4 (6)	14 (11)	
Hypertension	14 (22)	2 (3)	16 (13)	
Laboratory examination				
Blood urea (mg/dl)	27.35 (14.60–42.00)	25.30 (12.1–74.1)	26.8 (12.06–74.08)	0.2
Blood creatinine (mg/dl)	1.10 (0.51–16.0)	0.90 (0.04–103.00)	1.01 (0.50–1.90)	0.07
Urinary calcium (mg/24 hours), median (min–max)	115 (15.0–280.0)	72.50 (13.0–338.0)	91.50 (14–338)	**<0.001**
Urinary MGP (ng/l), mean (SD)	1,498 (490.47)	1,195.9 (460.8)	1,347.4 (498.38)	0.08
Water intake, median (min–max)	2,000 (1,000–3,000)	2,000 (1,500–3,000)	2,000 (1,000–3,000)	**<0.001**

BMI=body mass index; DM=diabetes mellitus; MGP=matrix Gla protein; SD=standard deviation
*Kolmogorov–Smirnov test

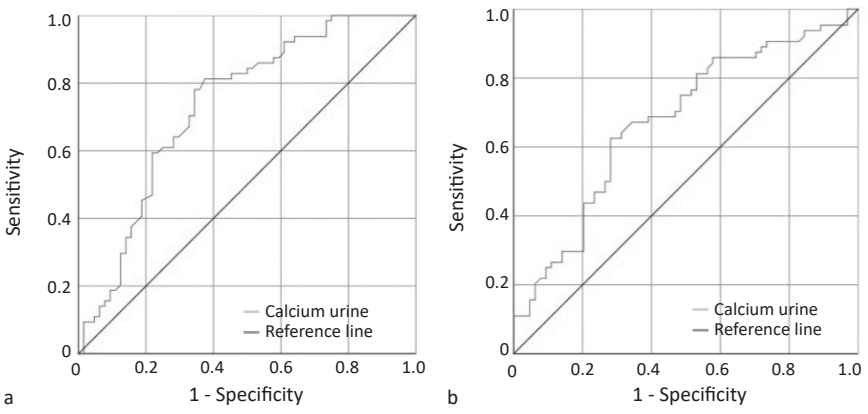

Figure 1. The ROC curve of urinary calcium (a) and urinary MGP (b). MGP=matrix Gla protein; ROC=receiver operating characteristic

Table 2. Association between urinary calcium and MGP levels and kidney stones

	Subject		Total	OR (95% CI)	*p*
	Case, n (%) (N = 64)	Control, n (%) (N = 64)			
Urinary calcium level					**0.0002**
High	52 (81)	24 (38)	76	7.22 (3.23–16.18)	
Low	12 (19)	40 (63)	52		
Urinary MGP level					**0.00001**
High	40 (63)	18 (28)	58	4.26 (2.03–8.96)	
Low	24 (38)	46 (72)	70		

CI=confidence interval; MGP=matrix Gla protein; OR=odds ratio

62.5% (95% confidence interval [CI]: 0.65–0.82). The observed area under the curve (AUC) was 0.734, with a significance level of $p<0.001$. The ROC curve analysis in Figure 1b revealed that the optimal cut-off point for urinary MGP level was 1,405 ng/l, with a sensitivity of 62.5% and specificity of 72% (95% CI: 0.58–0.77). The observed AUC was 0.675, and the significance level was $p<0.001$.

The association between urinary calcium and MGP levels was analyzed in patients with kidney stones. A significant association was observed between urinary calcium and MGP levels in patients with kidney stones (p = 0.000001; odds ratio [OR]: 7.22; 95% CI: 3.23–16.18 and p = 0.0002; OR: 4.26; 95% CI: 2.03–8.96, respectively), as shown in Table 2. Additionally, the association between urinary calcium and MGP levels was analyzed. High urinary calcium was found in 79% (n = 46/58) patient with high urinary MGP, meanwhile it was found in 43% (n = 30/40) patient with low urinary MGP. The OR was 5.11 (95% CI: 2.31–11.29, p = 0.00006).

DISCUSSION

This study found that urinary calcium levels were significantly associated with the development of kidney stones. Most kidney stones (approximately 75%) are composed of calcium, primarily in combination with oxalate, and less commonly phosphate. Uric acid stones account for approximately 10% of cases, while mixed stones, such as those containing both calcium oxalate and uric acid, are also common.[11] The formation of calcium-based stones follows a process that begins with nucleation, followed by crystal aggregation and growth.[12] Several contributing factors influence stone formation, including low urine volume, decreased urinary magnesium and citrate levels, abnormal urine pH (either too acidic or too alkaline), and increased excretion of calcium, oxalate, and uric acid.[13–15] The role of urinary calcium in facilitating kidney stone growth aligns with prior research findings, demonstrating a strong link between urinary calcium levels and stone formation.[16,17]

According to our findings, hypercalciuria increased urinary MGP levels by more than 5 times in participants with kidney stones. Wei et al[18] found a direct link between elevated levels of inactive dephosphorylated uncarboxylated MGP and kidney stone formation in a Flemish population. This confirms that MGP is a calcium ion ligand and an inhibitor of kidney stone pathogenesis.[19] A previous study on cultivated cells showed elevated MGP expression upon exposure to calcium oxalate monohydrate crystals. Similarly, immunohistochemical analysis of the kidneys of experimental mice revealed higher MGP synthesis around the peritubular blood vessels when exposed to hyperoxaluria and calcium oxalate crystal deposition.[20]

This study has some limitations. First, the kidney stones were not classified by type for each participant. Second, the sample size was relatively small. Third, other urinary metabolic factors influencing stone formation were not assessed. Finally, the dietary intake was not evaluated. Future research should include a more comprehensive analysis, a larger sample size, and an improved study design. Despite these limitations, this study explored the relationship between urinary calcium levels and MGP in patients with kidney stones. These findings contribute to existing knowledge and provide a foundation for further research on MGP's role in kidney stone formation.

In conclusion, an imbalance between the promoters and inhibitors of the urinary system, such as urinary calcium and MGP, is a key factor in kidney stone formation. The optimal cut-off for urinary calcium level was 72.5 mg/24 hours, with a sensitivity of 81.3% and specificity of 62.5%. The optimal cut-off point for urinary MGP level was 1,405 ng/l, with a sensitivity of 62.5% and specificity of 72%. A significant association was observed between urinary calcium and MGP levels in patients with kidney stones. Our study confirmed

that hypercalciuria and increased MGP levels are predictors of kidney stone formation.

Conflict of Interest
The authors affirm no conflict of interest in this study.

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