Comparison of urinary citrate between patients with nephrolithiasis and healthy controls

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Objectives : *Low urinary citrate excretion is a frequent metabolic abnormality found in kidney stone patients. The currently used definition of hypocitraturia (urinary citrate $\leq$ 320 mg/day) may not be applicable for Thai population since a large proportion of healthy controls are classified as hypocitraturic. The present study was aimed to determine urinary citrate level in nephrolithiasis patients compared to healthy controls, to evaluate diagnostic power of urinary citrate determination as well as to establish a hypocitraturic cutoff for Thais.*

Methods : *One - hundred and fourteen patients with nephrolithiasis and 90 healthy subjects were recruited. Urinary citrate was measured by citrate lyase method. Receiver operating characteristic (ROC) curve analysis was performed to assess the diagnostic power of the test and fine an appropriate cutoff point. Logistic regression was carried out to quantify magnitude of association between hypocitraturia and nephrolithiasis.*

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Results : *Nephrolithiasis patients excreted urinary citrate significantly lower than the controls. ROC analysis revealed an area under curve of 0.806 (95%CI; 0.744 - 0.864). The cutoff point at $\leq$ 200 mg/day was chosen to define hypocitraturic state. Sensitivity and specificity of the test were 84.21% and 64.44%, respectively. Hypocitrauria in healthy and nephrolithiasis groups were accounted for 35.56% and 84.21%, respectively. A significant association between hypocitraturia and nephrolithiasis was observed with adjusted odds ratio of 9.62 (95%CI; 4.92 - 18.78). Patients with uric acid stone trended to excrete urinary citrate lower than those with other types, although there was no statistical significance.*

Conclusion : *Hypocitraturia was a significant metabolic disturbance in Thai nephrolithiasis patients. Its definition for the Thais should be re-set to $\leq$ 200 mg/day in order to increase specificity and reduce false positive rate. Combination of urinary citrate with other metabolic risk factors would improve the power of diagnosis and identification of individuals at risk of kidney stone formation.*

Keywords : *Urinary citrate, hypocitrauria, nephrolithiasis, kidney stone, diagnostic values.*

ศราวุฒิ แซ่ภู่, ดุสิต อัษฎมงคลกูล, ปิยะรัตน์ โตสุโขวงศ์, จักรพันธ์ ปรีดานนท์, วรศักดิ์ โชติเลอศักดิ์, ชาญชัย บุญหล้า. การเปรียบเทียบระดับซิเทรตในคนปกติและการมีนิ่ว. จุฬาลงกรณ์เวชสาร 2552 ม.ค. – ก.พ.; 53(1): 51 – 65

วัตถุประสงค์ : *การขับออกซิเทรตในปัสสาวะต่ำเป็นความผิดปกติทางเมแทบอลิกที่พบได้บ่อยในผู้ป่วยโรคนิ่วไต นิยามของภาวะซิเทรตในปัสสาวะต่ำที่ใช้ในปัจจุบัน (ระดับซิเทรต ในปัสสาวะ ≤320 มิลลิกรัมต่อวัน) อาจจะไม่เหมาะสมกับประชากรไทย เนื่องจากมีสัดส่วนของคนปกติจำนวนมากถูกจัดว่ามีภาวะซิเทรตในปัสสาวะต่ำ การศึกษานี้มีวัตถุประสงค์เพื่อตรวจวัดระดับซิเทรตในปัสสาวะผู้ป่วยโรคนิ่วไต เทียบกับคนปกติสุขภาพดี ประเมินความสามารถในการวินิจฉัยของการวิเคราะห์ระดับซิเทรตในปัสสาวะ และกำหนดนิยามใหม่ของภาวะซิเทรตในปัสสาวะต่ำสำหรับคนไทย*

วิธีการ : *กลุ่มตัวอย่างได้จากผู้ป่วยโรคนิ่วไต จำนวน 114 ราย และคนปกติ จำนวน 90 ราย วัดระดับซิเทรตในปัสสาวะด้วยวิธีซิเทรตไลเอส ใช้ receiver operating characteristic (ROC) curve เพื่อทดสอบค่าความสามารถในการวินิจฉัยโรค และวิเคราะห์หาค่าอ้างอิง (cutoff) ที่เหมาะสม logistic regression ใช้สำหรับวิเคราะห์ระดับความสำคัญของความสัมพันธ์ระหว่างภาวะซิเทรตในปัสสาวะต่ำกับการเกิด โรคนิ่วไต*

ผลการทดลอง : *ผู้ป่วยโรคนิ่วไตขับซิเทรตออกมาในปัสสาวะต่ำกว่าคนปกติอย่างมีนัยสำคัญ การวิเคราะห์ค่าการวินิจฉัยโดยใช้ ROC curve ได้ค่าพื้นที่ใต้กราฟเท่ากับ 0.806 (95%CI; 0.744 - 0.864) เลือกค่าอ้างอิงของระดับซิเทรตในปัสสาวะที่ ≤200 มิลลิกรัมต่อวัน เป็นค่าที่ใช้นิยามภาวะซิเทรตในปัสสาวะต่ำ ณ ค่าอ้างอิงนี้ คำนวณค่าความไวและค่าความจำเพาะได้เท่ากับ 84.21% และ 64.44% ตามลำดับ ความชุกของภาวะซิเทรตในปัสสาวะต่ำในกลุ่มคนปกติและกลุ่มผู้ป่วยโรคนิ่วไตเท่ากับ 35.56% และ 84.21% ตามลำดับ พบนัยสำคัญในความสัมพันธ์ระหว่างภาวะซิเทรตในปัสสาวะต่ำกับการเกิดโรคนิ่วไต (OR = 9.62, 95%CI;4.92 - 18.78) ผู้ป่วยที่เป็นนิ่วกรดยูริกมีแนวโน้มว่ามีการขับออกของซิเทรตต่ำกว่านิ่วชนิดอื่น ๆ แต่ยังไม่พบนัยสำคัญ*

สรุปผล   :   ภาวะซิเทรตในปัสสาวะต่ำเป็นปัจจัยเสี่ยงทางเมแทบอลิกที่สำคัญของในผู้ป่วย
โรคนิ่วไตไทย นิยามของภาวะซิเทรตในปัสสาวะต่ำสำหรับคนไทยควรเปลี่ยน
เป็น ≤200 มิลลิกรัมต่อวัน เพื่อเพิ่มค่าความจำเพาะและช่วยลดอัตราความผิด
พลาดในการวินิจฉัย การวิเคราะห์ระดับซิเทรตในปัสสาวะร่วมกับความเสี่ยง
ทางเมแทบอลิกอื่น ๆ จะช่วยเพิ่มความถูกต้องในการวินิจฉัย และสามารถ
นำมาใช้ประเมินความเสี่ยงต่อการเกิดโรคนิ่วไตในบุคคลทั่วไปได้

คำสำคัญ   :   ซิเทรตในปัสสาวะ, ภาวะซิทเรตในปัสสาวะต่ำ, โรคนิ่วไต, ค่าการวินิจฉัย

Kidney stone formation is a complex process of development involving interplays of environmental, anatomical and genetic factors. [1] The prevalence of kidney stone is increasing worldwide. This is considered an impact of a major change in lifestyle and dietary habits during the past decade. [2] Climatic change is believed to have contributed to the increasing prevalence of stone disease. A recent study showed that the US stone belt is expanding north and westward from the southeastern region in response to the global warming. [3] In Thailand, nephrolithiasis is endemic; its prevalence varies up to 16.9 % depending on geographical regions. [4-5] The Thai stone belt is considered to be spanned from the Northeast toward the North.

The likelihood of stone formation increases with age, body mass index and positive family history. [6-8] However, the most important stone risk factor is metabolic abnormality that causes urine to be supersaturated, leading to crystal formation. Increased urinary excretion of stone promoters, e.g., hypercalciuria and hyperoxaluria is a major risk factor in the West. [9-11] In contrast, our study in Thai stone cases demonstrated that the decrease in stone inhibitor excretion, particularly hypocitraturia and hypokaliuria, is the principal cause. [12, 13] Hypocitraturia is also prevalent in stone patients in the West and it is considered as a significant risk for stone recurrence. [14] Therefore, the drug of choice for preventing stone relapse is potassium citrate salt that provides alkalinizing and citraturic effects. [15-16] Renal stone patients are also frequently presented with high oxidative stress and enhanced renal tubular damage. [17-18] Recently, we studied the medicinal quality of our lime powder regimen in nephrolithiasis

patients and found that it not only increases urine pH and citrate excretion, our regimen also exerts an antioxidative action and attenuates renal tubular injury in the patients, suggesting a better alternative for kidney stone prophylaxis. [19] Excretory levels of urinary citrate reported in western stone cases are usually greater than that in Thai patients. [9, 12, 20, 21] A study in Japanese population also documented a higher level of urinary citrate compared to the Thai population. [22, 23] This may be due to the differences in dietary habit, genetic background or even laboratory technique. Thus, it is necessary to establish an appropriate cutoff of urinary citrate in Thai stone patients. The definition of hypocitraturia that currently uses is urinary citrate $\leq$ 320 mg/day. [9, 23, 24] To our experience, this cutoff point is not suitable (too high) for Thai kidney stone patients as it provides a low specificity as well as a huge fraction of false positive rate.

In this study, we aimed to determine urinary citrate levels in nephrolithiasis patients compared to healthy controls, and to set up an appropriate cutoff point for hypocitraturia that provided a more accuracy in discrimination of stone patients from the controls.

Subjects and Methods

Ninety healthy subjects (aged between 21 and 61 years old) and 114 nephrolithiasis patients (aged between 18 and 60 years old) were recruited for the study. The mean age of the healthy controls and stone patients were 41±10 and 43 ±10 years old, respectively. The male-to-female ratios were 39:51 (0.76) and 56:58 (0.97) in the healthy and patient groups. The nephrolithiasis patients were admitted to King Chulalongkorn Memorial Hospital, Khon Kaen

Hospital or Rajavithi Hospital between 2005 and 2008. All admitted patients underwent surgical removal of stone, either by open surgery, percutaneous nephrolithomy, or shock wave lithotripsy. Patients associated with anatomical anomaly of kidneys and malignancies were excluded. Healthy volunteers with no history of urinary stone disease evaluated were analyzed by urine strip to warrant the normal urinary profile. Informed consents were obtained from all participants and the research protocol was approved by the Ethics Committee, Faculty of Medicine, Chulalongkorn University.

24-hour urine and stone specimens collection

Thymol was used as preservative in the collection of 24-hr urine. All subjects were well instructed for collecting urine specimen by themselves. In the patients group, urine samples were collected preoperatively. The urine samples were kept at -70°C prior to analysis. In adequate collection of urine specimens indicated by urine creatinine that was less than 0.5 mg/day were excluded.

Stone specimens obtained from nephrolithiasis patients were thoroughly washed three times with distilled water, dried in oven at 60 - 65°C for 16 hours (overnight), and grinded into powder using pestle and mortar. Stone powder samples were sent to the Scientific and Technological Research Equipment Centre, Chulalongkorn University for mineral composition analysis using Fourier-transform infrared spectroscopy. Stone types were classified according to the main mineral component into four types, i.e., calcium oxalate (CaOx), calcium phosphate (CaP), uric acid (UA) and magnesium ammonium phosphate (MAP). Of 114 nephrolithiasis

patients, 88 had enough stone materials for crystalline analysis. CaOx, CaP, UA and MAP stones were accounted for 60 (68%), 8 (9%), 11 (13%) and 9 (10%), respectively.

Determination of urinary citrate

Level of citrate in 24-hr urine samples was determined by citrate lyase method.[25-27] In brief, urine (200 µl) was added to Glycine-glycine buffer containing nicotinamide reduced form (NADH) and lactate dehydrogenase. The mixture was thoroughly mixed and stood at ambient temperature for 5 minutes. Absorbance at 340 nm was measured (A1). Citrate lyase (Sigma, St. Louis, MO, USA) was added to the mixture. After mixing, the solution was left at ambient temperature for another 8 minutes and was measured for a second absorbance (A2).

Concentration of citrate (g/L) was computed from $(V \times MW \times \Delta A)/(\varepsilon \times d \times v \times 1000)$, where V is final volume (3.14 ml), MW is molecular weight of citrate (192.1 Da), ΔA is A1-A2, ε is absorption coefficient of NADH at 340nm (6.3 mM^{-1} cm^{-1}), d is cuvette pathlength (1 cm), v is sample volume (0.2 ml).

Statistical analysis

Mean $\pm$ standard deviation (SD) was used as representative of normally distributed data. Data with skewed distribution were presented as median and interquatile range (IQR). Difference between two independent groups was tested by two-sample t-test or Mann-Whitney test as appropriated. Comparison between more than two independent groups was carried out by one-way ANOVA or Kruskal-Wallis test.

To evaluate the diagnostic power of urinary citrate determination, receiver operating characteristic

(ROC) curve analysis was carried out. How efficient the test can distinguish between diseased and non-diseased status is indicated by an area under curve (AUC) of ROC. An AUC of 0.5 indicates that the test can not separate diseased individuals from non-diseased individuals (having 50% sensitivity and 50% specificity). An AUC of 1.0 indicates an ideal test that can perfectly differentiate between the two groups (achieving 100% sensitivity and 100% specificity). Thus, a greater AUC indicates a better diagnostic test. In addition, a good diagnostic test should have small false positive and false negative rates. An appropriate cutoff was chosen from the ROC curve to compute the diagnostic values of urinary citrate determination.

Logistic regression was used to quantify the association (odds ratio, OR) between hypocitraturia and nephrolithiasis. All statistical analyses were performed by STATA version 8.0 (College Station, TX, USA). Two-side analysis at a significance level of $\alpha = 0.05$ was set for all calculations.

Results

Of 204 subjects, 114 subjects were renal stone patients and the remainder (n = 90) was healthy controls as shown in Table 1. Mean age between the two groups was not significantly different (43 ± 10 vs. 41 ± 10 years; P = 0.105). The patient group comprised of 56 (49%) males and 58 (51%) females whereas the healthy group contained 39 (43%) males and 51 (57%) females. Gender distribution between these two groups was not statistically different (P = 0.410). This indicates the equivalence of demographic figure between the controls and the patients.

Urinary level of citrate in nephrolithiasis group was significantly lower than that in the control group (median (IQR); 80.9 (120.9) vs. 262.5 (233.2) mg/day; P < 0.001) (Fig. 1). Figure 2 shows the distribution of urinary citrate excretions compared between healthy controls (Fig. 2a) and nephrolithiasis patients (Fig. 2b). An obvious difference of urinary citrate distribution between the two groups was found. The majority of stone patients had low urinary citrate levels while most of healthy individuals excreted citrate at higher levels. However, a minute fraction of healthy controls showed a decreased urinary citrate excretion resembling stone patients.

Table 1. Demographic data of subjects

Characteristics	Healthy controls	Nephrolithiasis patients	P value
Number of subjects (n)	90	114	
Age (years)			0.105[a]
- Mean ± SD	41 ± 10	43 ± 10	
- Median (IQR)	41 (15)	46 (15)	
Gender (%)			0.410[b]
- Male	39 (43)	56 (49)	
- Female	51 (57)	58 (51)	

[a]: Two-sample t-test, [b]: chi-square test
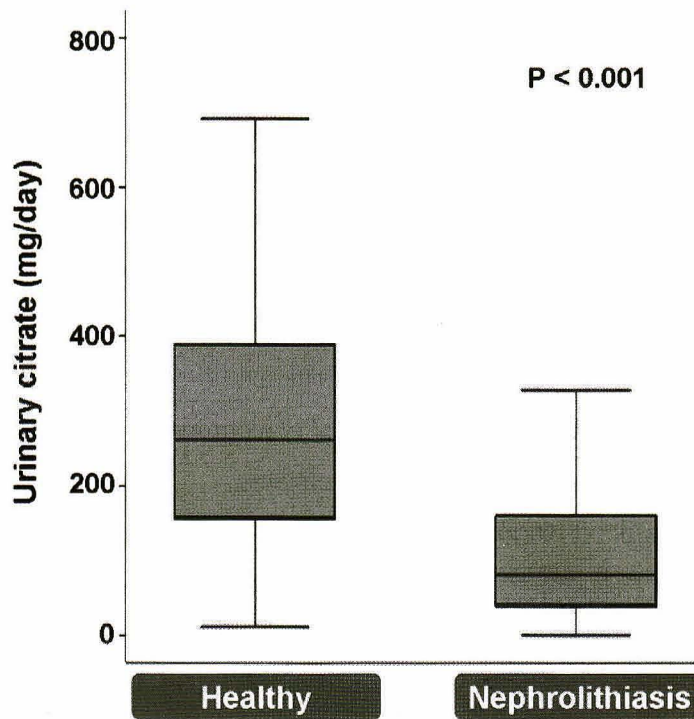
Figure 1. Box-Whiskers plot shows comparison of urinary citrate excretion between healthy control (n = 90) and nephrolithiasis patients (n = 114). Level of urinary citrate in nephrolithiasis patients was significantly lower than that in the controls (Mann-Whitney test, P < 0.001). Outliers are omitted.

To evaluate the clinical value of urinary citrate in separating nephrolithiasis from healthy subjects, an ROC curve was generated and the cutoff point was chosen to calculate the diagnostic values. An area under ROC curve of urinary citrate determination was 0.806 (95%CI; 0.744 - 0.868) (Fig. 3). At a commonly accepted cutoff point of 320 mg/day, the test provided accuracy, sensitivity, false positive rate and false negative rate of 69.12%, 94.74%, 36.67%, 63.33% and 5.26%, respectively (Table 2). A higher accuracy (75.49%) and lower false positive rate (35.56%) were obtained at a cutoff point of 200 mg/day. Sensitivity, specificity, and false negative rate at this cutoff were 84.21%, 64.44%, 15.79%, respectively (Table 2). Thus, we chose a cutoff at 200 mg/day for defining hypocitraturic trait in Thai

population in stead of the currently accepted definition at 320 mg/day. Individuals with urinary citrate ≤ 200 mg/day were defined as hypocitraturia whereas those with a greater urinary citrate levels were identified normocitraturia.

Based on the new cutoff point, hypocitraturia was found 96 out of 114 (84.21%) in nephrolithiasis patients and it was significantly associated with nephrolithiasis (P < 0.001) (Table 3). In the control group hypocitraturia was accounted for 35.56% (32/90).

To assess the magnitude of association between hypocitraturia and kidney stone disease, logistic regression was carried out. Crude OR of hypocitraturia for kidney stone disease was 6.67 (95%CI; 4.98 - 18.76, P < 0.001). After screening for
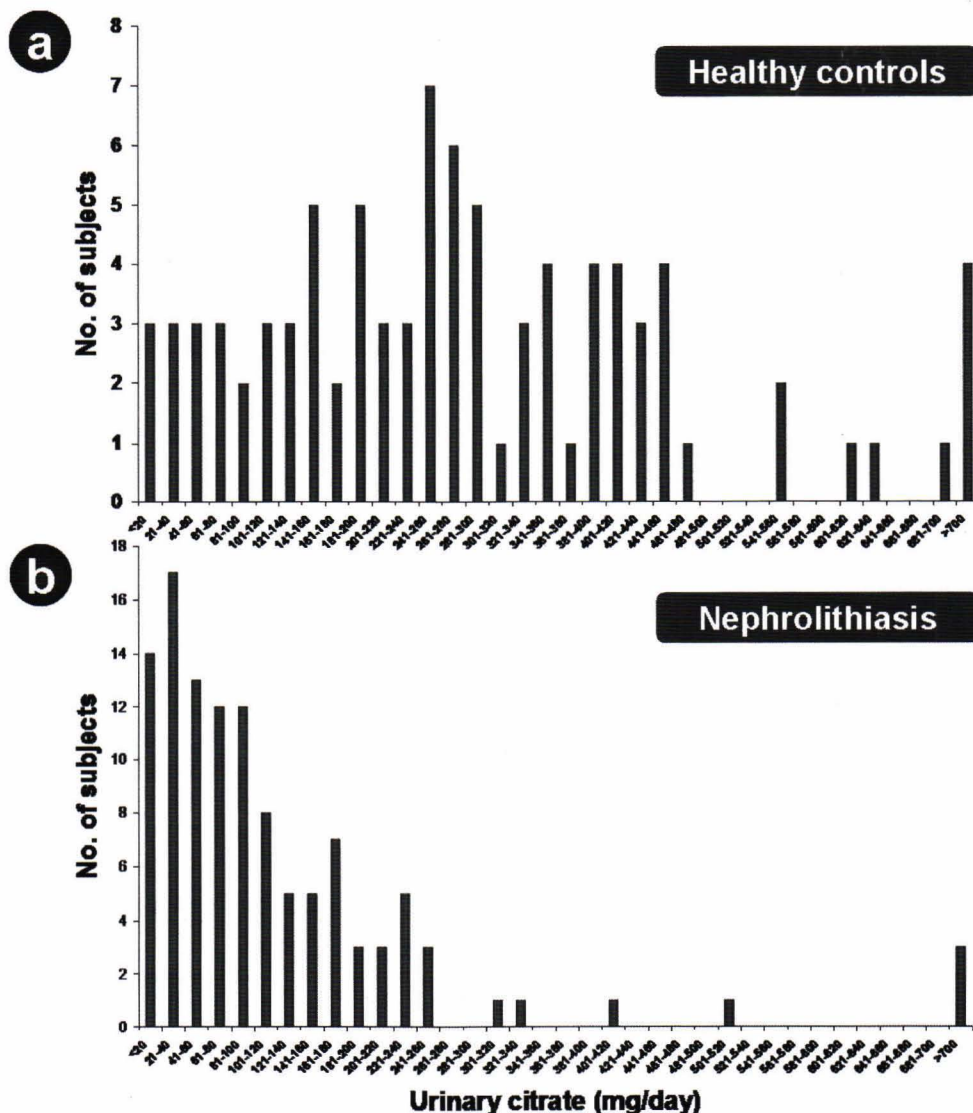
Figure 2. Distribution of urinary citrate levels in healthy controls (a) and nephrolithiasis patients (b). The majority of nephrolithiasis patients excreted urinary citrate less than 200 mg/day.

age and sex, the adjusted OR of 9.62 (95%CI; 4.92-18.78, P < 0.001) was revealed (Table 3). This means that individuals with hypocitraturia had 9.62 times higher probability to develop kidney stone than those without it. Our finding confirmed that hypocitraturia increased risk for kidney stone formation.

To evaluate whether excretion of urinary citrate was associated with the type of stones, urinary citrate levels were compared among stone types. Of 144 cases, 88 had known stone types which were classified as CaOx (n = 60), CaP (n = 8), UA (n = 11) and MAP (n = 9) stones. Median (IQR) of urinary levels in CaOx, CaP, UA and MAP were 72.15 (88.39), 114.24 (203.27), 27.64 (79.83) and 83.73 (104.26), respectively. Levels of urinary citrate among these four groups of stones were not significantly different (P = 0.102) (Fig. 4). However, UA stone seemed to have lowest excretion of urinary citrate compared to the others.
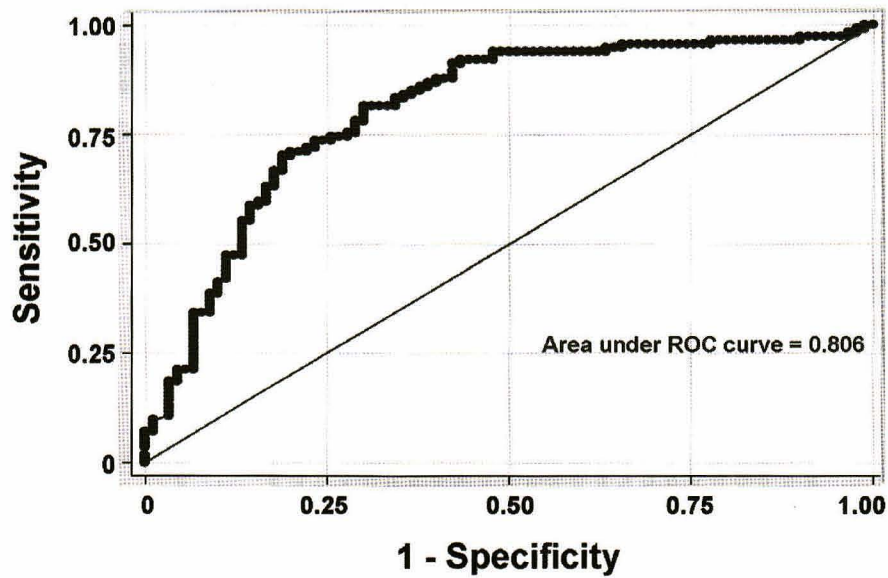
Figure 3. An ROC curve of urinary citrate determination. Area under ROC curve of 0.806 (95%CI: 0.744 - 0.864) was obtained.

Table 2. Diagnostic values of urinary citrate determination at various cutoff values for classifying nephrolithiasis patients from healthy subjects

Diagnostic values (%)	Urinary citrate cutoff (mg/day) for positive result	
	≤ 320	≤ 200
Sensitivity	94.74	84.21
Specificity	36.67	64.44
Positive predictive value	65.45	75.00
Negative predictive value	84.62	76.32
False positive rate	63.33	35.56
False negative rate	5.26	15.79
Accuracy of test	69.12	75.49

**Table 3.** Association of hypocitrauria with nephrolithiasis

	Healthy (n=90)	Nephrolithiasis (n=114)	P value
Citraturic status (%)			< 0.001
- Normocitraturia (> 200mg/day)	58 (64.44)	18 (15.79)	
- Hypocitraturia (≤ 200mg/day)	32 (35.56)	96 (84.21)	

P value from chi-square test

Table 4. Odds ratios for nephrolithiasis regarding to hypocitraturia

	Crude OR (95%CI)	P value	Adjusted OR* (95%CI)	P value
Hypocitraturia				
- No	1		1	
- Yes	9.67 (4.98-18.76)	< 0.001	9.62 (4.92-18.78)	< 0.001

* Controlled for age and sex.
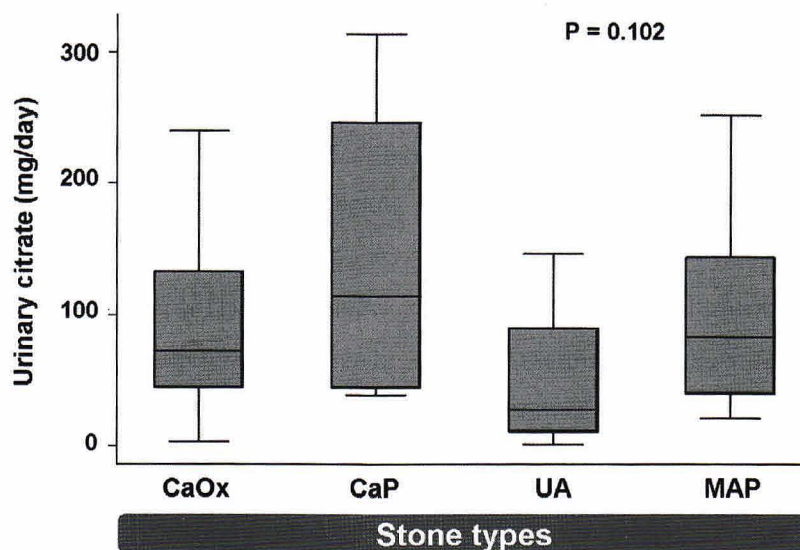
Figure 4. Box-Whiskers plot shows comparison of urinary citrate excretion among patients with different stone types. Levels of urinary citrate in patients with CaOx (n = 60), CaP (n = 8), UA (n = 11) and MAP (n = 9) were not significantly different (Kruskal-Wallis test, P = 0.102). Patients with uric acid stone trended to have the lowest urinary citrate excretion. Outliers are omitted. CaOx; calcium oxalate, CaP; calcium phosphate, UA; uric acid, MAP; magnesium ammonium phosphate.

Discussion

Decreased urinary excretion of citrate is frequently found in nephrolithiasis patients and is associated with the recurrence of calculi. [12,13,19, 28 – 30] Citrate is a potent urinary stone inhibitor. It has a higher affinity than oxalate to bind calcium and form a soluble complex, in stead of an insoluble calcium oxalate salt. Citrate can also adsorb on the crystals' surface to inhibit crystal growth and agglomeration. Thus, determination of urinary citrate is clinically useful for assessing the risk of stone formation. [31]

In this study, we found that nephrolithiasis patients excreted urinary citrate significantly lower than the healthy controls. An appropriate cutoff point of urinary citrate for Thai population should be at 200 mg/day. Hypocitraturia was significantly associated with nephrolthiasis with an adjusted OR of 9.62 (95% CI; 4.92 - 18.78). Among various stone types, urinary citrate levels were not statistically different.

Common metabolic risk factors for kidney stone include hypercalciuria, hypocitraturia, hyper-oxaluria, hyperuricosuria and abnormally low urinary

pH. [32] Hypocitraturia is a leading metabolic abnormality found in Thai patients. [12, 21, 33] According to the literature, hypocitraturia may be secondary to potassium depletion, metabolic acidosis or having an idiopathic cause. [10] Our previous data found that hypocitraturia and hypokaliuria was frequently coincided suggesting potassium deficiency as a responsible etiology. [12] Low intake of dietary citrate is also associated with hypocitraturia. Our recent finding showed that patients with kidney stone consumed low amount of citrus-fruits and vegetables. This is suggestive of an additional reason for low urinary citrate excretion. [7] However, genetic susceptibility to hypocitraturia cannot be totally ruled out. A study in Japanese stone formers found an association of *sodium-dicarboxylate cotransporter-1 (NaDC-1)* polymorphism with low excretion of urinary citrate. [22] Genetic risk of hypocitraturia in Thai nephrolithiasis patients is waiting to be investigated.

Urinary citrate excretion in our patients was obviously lower than that reported in the West [9, 24, 29] and Japanese cases [22, 23] For instance, mean ± SD of urinary citrate in recurrent kidney stone formers and controls in the Italian population were 2.06 ± 1.04 mmol/day (395.77 ± 199.80 mg/day) and 3.42 ± 1.33 mmol/day (657.05 ± 255.52 mg/day), respectively. [29] Although reasons for this phenomenon have not been described, it may be due to the difference in dietary habit, genetic basis or even laboratory variation. Thus, the traditionally accepted definition of hypocitraturia at ≤ 320 mg/day may not be applicable for the ethnically-different populations such as the Thais.

Based on ROC curve analysis, an AUC of 0.806 (95%CI; 0.744 - 0.864) for urinary citrate

determination in our populations were obtained. This indicated that the test is adequate for diagnostic purpose. At the cutoff of ≤ 320 mg/day, the test provided a high false positive rate (63.33%) and low specificity (36.67%) with a probability of correctively classified of 69.12%. This means that a huge proportion of healthy subjects would be defined as hypocitraturic. To increase the specificity and accuracy, we chose the cutoff point at ≤ 200 mg/day. At this point, it imparted adequate sensitivity (84.21%) and specificity (64.44%). Additionally, the false positive rate went down from 63.33% to 35.56%. A combination with other metabolic abnormalities would improve the diagnostic power for kidney stone disease. Based on our finding, we concluded that a more appropriate cutoff point for defining hypocitraturic state in Thai populations is ≤ 200 mg/day.

Hypocitraturia in nephrolithiasis and control groups were accounted for 84.21% and 35.56%, respectively. It was strongly associated with nephrolithiasis with adjusted OR of 9.62 (P < 0.001). These data confirm that hypocitraturia is an important risk factor for kidney stone disease. A recent study of metabolic risk factors in Thai stone formers reported that hypocitraturia in stone formers and normal controls were found in 69.6% and 55.9%. [21] Since their definition for hypocitraturia was urinary citrate ≤ 2 mmol/day (384.24 mg/day), a substantial proportion of normal subjects were classified as hypocitrauria (55.9%) indicating a high false positive rate. Again, we suggest that a cutoff at ≤ 200 mg/day would be more applicable for Thai population.

We also investigated whether urinary citrate excretion varied by stone types. Levels of urinary citrate among patients with CaOx, CaP, UA and MAP

stones were not significantly different. However, UA stone patients trended to have lowest urinary citrate excretion. A significant reduction of urinary citrate in UA stone patients compared to calcium stone patients has been demonstrated. [23] Uric acid stone is associated with abnormally acidic urine (pH < 5.5). [34] At low pH, citrate^{3-} is protonated to form citrate^{2-} which is further uptake into renal proximal tubular cells by a dicarboxylate cotransporter, NaDC-1, leading low excretion of urinary citrate. [35] An insignificant association of UA stone with decreased urinary citrate excretion found in this study may be due to the small sample size.

In conclusion, the present study confirms that nephrolithiasis patients excrete urinary citrate significantly lower than healthy controls. Cutoff point for defining hypocitraturia in Thai people should be made lower from $\leq$ 320 mg/day to $\leq$ 200 mg/day in order to obtain a higher diagnostic power for separating the healthy individuals from kidney stone patients. A strong association between hypocitraturia and nephrolithiasis is found emphasizing an important metabolic risk factor for kidney stone formation. Urinary citrate determination is recommended to perform in all stone patients as well as in healthy individuals who are at risk of stone formation to order to provide an appropriate mean for preventing the development of calculi.

Acknowledgements

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