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Original article

Electrical conductivity of 24-hour urine is decreased in patients with calcium oxalate urolithiasis

Kamonchat Boonkam[a], Natcha Madared[a], Pakpum Somboon[b], Alongkorn Pimpin[c], Chanchai Boonla[a, *]

[a]*Departments of Biochemistry, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*
[b]*Biomedical Engineering program, Faculty of Engineering, Chulalongkorn University, Bangkok, Thailand*
[c]*Department of Mechanical Engineering, Faculty of Engineering, Chulalongkorn University, Bangkok, Thailand*

Abstract

Background: Electrical conductivity (EC) of urine depends on the ionic substance-to-water ratio. Calcium oxalate (CaOx) is the most common type of urinary stone, and its formation is driven by decreased urine volume, increased lithogenic substances, and decreased stone inhibitory compounds (specifically citrate). Low urinary citrate excretion (hypocitraturia) is a prominent risk factor in Thai urolithiasis patients.

Objective: To measure the urinary EC, urinary calcium, and urine specific gravity in CaOx stone patients compared with non-stone forming (NSF) subjects.

Methods: The urinary EC was measured in 24-hour urine samples obtained from 42 CaOx stone patients and 121 NSF subjects. Urinary calcium and urine specific gravity were measured to evaluate whether they were associated with the urinary EC.

Results: The urinary EC of CaOx stone patients was significantly lower than the NSF subjects. The urinary EC level was positively correlated with urine specific gravity, but not urinary calcium. At the selected cutoff of 14.3 mS/cm, the sensitivity, specificity, and accuracy of the urinary EC for diagnosing CaOx urolithiasis were 74.0%, 50.0%, and 56.0%, respectively. In CaOx stone group, patients who had low urinary citrate had lower urinary EC than patients who had high urinary citrate. Experimentally, we demonstrated in artificial urine that citrate concentrations actively influenced the EC values. Decreased citrate level directly caused decreased EC value.

Conclusion: The EC of 24-hour urine in CaOx stone patients was decreased relative to the NSF individuals. The urinary EC was linearly correlated with urine specific gravity. Low urinary EC observed in CaOx stone patients possibly resulted from a low urinary citrate excretion that was highly prevalent in the stone patients. In addition, gradual decrease in citrate level caused a gradual decrease in EC level.

Keywords: Calcium oxalate, electrical conductivity, kidney stone, urine specific gravity, 24-hour urine.

Urolithiasis is a common urological condition in all countries, especially in countries with tropical climates like Thailand.[1] Calcium oxalate (CaOx) is the most common type of urinary stone, and it is highly recurrent. [2, 3] The formation of CaOx stone is primarily driven by urinary supersaturation of calcium and oxalate that subsequently initiates CaOx crystallization. However, the crystallization of CaOx is also moderated by citrate, a potent stone inhibitor. Citrate competitively binds calcium to yield a soluble calcium citrate compound, hence reducing the risk of CaOx stone formation. Low urinary excretion of citrate (hypocitraturia) is a well-known risk factor of CaOx stone formation and recurrence [4, 5], and it is the main metabolic risk factor found in Thai kidney stone patients. [6, 7]

Human body fluids are relatively good conductors. [8] The conductivity of biological fluid is greatly influenced by concentrations of electrolytes, ions, and charged substances.[9] In urinary stone patients, urinary excretion of substances is frequently altered. [10] Therefore, we hypothesized that the urinary electrical conductivity (EC) of urine from stone patients differed from non-stone forming individuals.

Fundamentally, EC indicates the ability of materials or media to allow the electric current to flow through it. A study by Fazil Marickar YM. measured the EC in 2,000 spot urine samples (early morning urine and random urine) obtained from patients attending the urinary stone clinic and reported that the urinary EC values were ranged between 1.1 and 33.9 mS/cm with an average of 21.5 mS/cm. [11] There are only a few studies that reported the urinary

***Correspondence to: Chanchai Boonla,** Department of Biochemistry, Chulalongkorn University 10330, Thailand.
E-mail: Chanchai.B@chula.ac.th
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EC levels in urolithiasis patients. Chhabra HL. and Manocha KK. measured the EC in first and fasting mid-stream morning urine samples and showed that the urinary EC of stone formers (idiopathic renal/ ureteric radio-opaque stone) was significantly lower than non-stone formers.[12] Their study later in 24- hour urine samples also showed that the urinary EC of idiopathic kidney stone formers was significantly lower than non-stone formers. [13] Later, they measured the EC in both serum and first morning urine samples of the same subjects and demonstrated that the urinary EC of idiopathic kidney stone formers was significantly lower than non-stone formers, but the serum EC between these two groups were not significantly different. [14] To our knowledge, the level of urinary EC in CaOx stone formers has not been explored.

In this study, we aimed to determine the EC values in 24-hour urine samples obtained from CaOx stone patients compared with those from non-stone forming (NSF) subjects. Urinary calcium and urine specific gravity were also determined to assess their correlation with urinary EC. Experimentally, we tested whether gradual decrease in citrate level caused gradual decrease in EC value in artificial urine.

Materials and methods

Subjects and 24-hour urine specimens

A total of 163 subjects divided into CaOx kidney stone patients (n = 42) and NSF subjects (n =121) were recruited for the study. The 24-hour urine samples were collected between 2017 and 2018 from 42 CaOx urolithiasis patients who were admitted to Mahasarakham Hospital and 121 NSF subjects who lived in Mahasarakham Province, Thailand. The presence of CaOx stone was confirmed by computerized tomography (CT) scan and fourier transformed infrared spectroscopy (FTIR). The urine samples were kept at -20 °C. Urine samples were thawed, centrifuged, and filtered through the 0.2 μm membrane before testing. The research proposal was presented to the research ethics review committee, the Faculty of Medicine, Chulalongkorn University for approval (IRB no. 1036/64).

Measurement of urinary EC

The filtered urines samples were diluted (1 : 20) before measuring the EC values. Diluted urine samples were placed into clean 50 mL tubes. The EC values were measured by the portable EC meter (HI98331, Hanna, USA) at room temperature. The conductivity probe was immersed in the urine samples, and the appeared EC value was recorded.

Urinary calcium determination

The urinary calcium was measured in 24-hour urine samples using the Arsenazo III method. Arsenazo III reagent used for the reaction was 100 mM Arsenazo III (Sigma-Aldrich, USA) in 75 mM imidazole (Sigma-Aldrich, USA) buffer, pH 6.5. Standard calcium chloride was prepared at concentrations of 0, 0.25, 1 and 2 mM. The reaction was performed by placing 10 μL of diluted urine sample (1 : 2), water (blank) and standard calcium chloride solution into wells of 96 well plate. Then, 250 μL of Arsenazo III reagent was added, mixed thoroughly, and incubated at room temperature for 5 minutes. Absorbance at 603 nm was measured. The concentrations of calcium in urine samples were calculated from the calcium chloride standard curve.

Urine specific gravity measurement

The urine specific gravity was measured in 24-hour urine samples using the analog refractometer. Basically, refractometer measures the specific gravity by comparing the gravity of urine sample with that of water (ratio of density of urine-to-density of water). After calibrating with distilled water, 100 μL of urine sample was dropped on the prism, and the urine specific gravity was read and recorded.

Effect of citrate on EC in artificial urine

The artificial urine was prepared according to the earlier study [15] with minor modification. It contained 200 mM urea, 1 mM uric acid, 4 mM creatinine, 54 mM NaCl, 30 mM KCl, 15 mM NH_4Cl, 3 mM $CaCl_2$, 2 mM $MgSO_4$, 2 mM $NaHCO_3$, 9 mM Na_2SO_4, 3.6 mM KH_2PO_4, and 0.4 mM Na_2HPO_4, pH 6.2. Various concentrations of sodium citrate (Merck Millipore, USA) solution (5,000, 2,500, 1,500, 1,000, 500, 400, 200, 100, 50, 25, 12.5, and 0 mg/L) were added to artificial urine samples. The EC level of each citrate-added artificial urine sample was measured to see whether a gradual decrease in citrate concentration caused a gradual decrease in EC level.

Statistical analysis

Data was presented as mean ± standard deviation (SD) or median (interquartile range; IQR), as appropriate. Difference of urinary EC levels

between stone and non-stone groups were tested by two-sample unpaired *t* - test and Mann-Whitney test. Receiver operating characteristic (ROC) analysis was performed to evaluate how well the urinary EC level could distinguish CaOx urolithiasis patients from NSF subjects. Diagnostic values including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated. The correlation between two variables was evaluated by the Pearson correlation test. Statistical analysis was calculated by GraphPad Prism 10 (GraphPad Software, MA, USA) and Stata/SE 12 (StataCorp LLC, TX, USA). $P < 0.05$ was considered statistically significant.

Results

The urinary EC decreased in CaOx stone patients

The demographic data of the studied subjects are shown in (**Table 1**). Average age of the stone group was higher than the NSF group, and the stone group had more proportion of males than the NSF group. However, the 24-hour urine volume and urinary excretion of creatinine were not significantly different between the two groups **(Table 1).**

The level of urinary EC in CaOx stone group was significantly lower than that in the NSF group (median (Q1 - Q3): 10.7 (6.8 - 14.7) *vs*. 14.0 (9.7 - 18.4) mS/cm) **(Figure 1A)**. Urinary calcium and urine specific gravity were also measured. The results showed that urinary calcium excretion in CaOx stone patients was significantly lower than the NSF controls [median (Q1 - Q3): 12.7 (4.4 - 35.9) *vs.* 26.4 (13.3 - 55.7) mg/day] **(Figure 1B)**. Likewise, urine specific gravity in the CaOx stone group was significantly lower than the NSF group [median (Q1 - Q3): 1.008 (1.006 - 1.013) *vs.* 1.016 (1.012 - 1.022)] **(Figure 1C).**

Table 1. Demographic data of the studied cohorts.

Variables	NSF subjects	CaOx stone patients	*P* - values
Number of subjects	121	42	
Age (years)	50.3 ± 9.2	56.1 ± 11.5	0.001
Sex			< 0.001
Males	34 (28.1%)	31 (73.8%)	
Females	87 (71.9%)	11 (26.2%)	
Urine volume (mL)	1334.0 ± 641.0	1530.0 ± 512.0	0.075
Urine creatinine (mg/day)	577.0 ± 383.0	622.0 ± 633.0	0.589

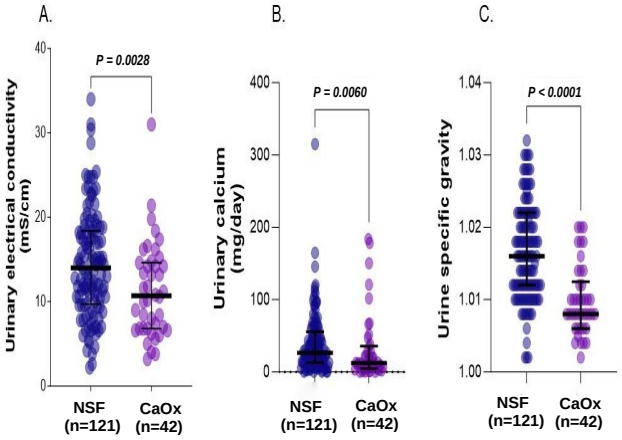

Figure 1. Comparison of urinary EC levels, urinary calcium excretion, and urine specific gravity between CaOx stone and NSF groups. **(A)** The urinary EC of 24-hour urine from CaOx stone patients was significantly lower than the NSF subjects. Bar indicates median. Error bars indicate IQR; **(B)** The urinary calcium level of CaOx stone patients was significantly lower than NSF subjects; **(C)** Urine specific gravity of CaOx stone patients was significantly lower than NSF subjects. Bar indicates median. Error bars indicate IQR.

The urinary EC was correlated with urine specific gravity, but not urinary calcium

We evaluated whether urinary EC levels were correlated with urinary calcium and urine specific gravity or not. In all cases, urinary EC value showed a good positive correlation with urine specific gravity ($r = 0.579$, $P < 0.001$), but it was not correlated with the urinary calcium levels ($r = -0.072$, $P = 0.368$). These correlations were further analyzed separately in CaOx stone and NSF groups. The same correlation patterns were observed. The positive correlation between urinary EC and urine specific gravity was found in both CaOx stone and NSF groups **(Figure 2A).** In contrast, the significant association between urinary EC and urinary calcium levels were not observed in both CaOx stone and NSF groups **(Figure 2B).**

The performance of urinary EC to distinguish CaOx stone patients from NSF subjects

We further performed an ROC analysis to evaluate how well the urinary EC could discriminate the CaOx stone patients from the NSF individuals. Based on the ROC analysis result, the urinary EC showed an area under ROC curve of 0.654 (95% CI: 0.560 - 0.748) **(Figure 3).** We selected the cutoff value of urinary EC at 14.3 mS/cm in order to yield

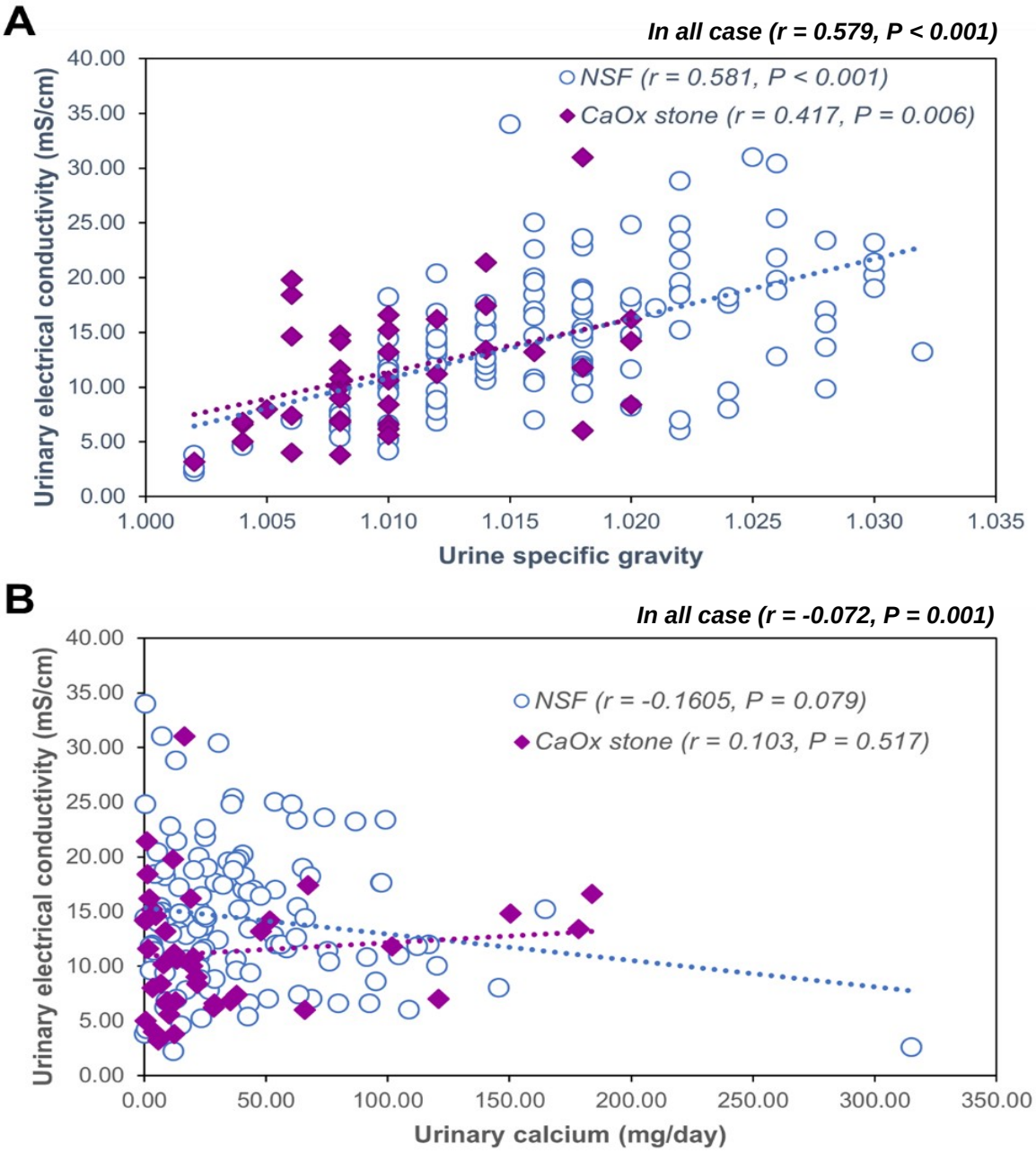

Figure 2. Association of urinary EC with urinary calcium and urine specific gravity in CaOx stone and NSF groups. **(A)** The urinary EC levels were positively correlated with urine specific gravity both in CaOx stone and NSF groups; **(B)** The significant correlation between urinary EC and urinary calcium levels were not found both in CaOx stone and NSF groups.

the best diagnostic accuracy. The urinary EC values of 14.3 mS/cm were counted as positive results, and those of > 14.3 mS/cm were negative results. According to this cutoff value, the urinary EC gave the sensitivity, specificity, PPV, NPV and accuracy of 73.8%, 49.6%, 33.7%, 84.5% and 55.8%, respectively. This indicated that the diagnostic performance of urinary EC for CaOx nephrolithiasis was not very good, but acceptable.

The urinary EC was lower in CaOx stone patients who had lower citrate in urine

Hypocitraturia is the main cause of CaOx stone formation, and it is the main risk factor found in Thai kidney stone patients. [6,7,16] Fundamentally, calcium oxalate monohydrate (COM) crystals are key lithogenic crystals responsible for the CaOx lithogenesis whereas calcium oxalate dihydrate (COD) crystals are normally found in the healthy urine. [17]

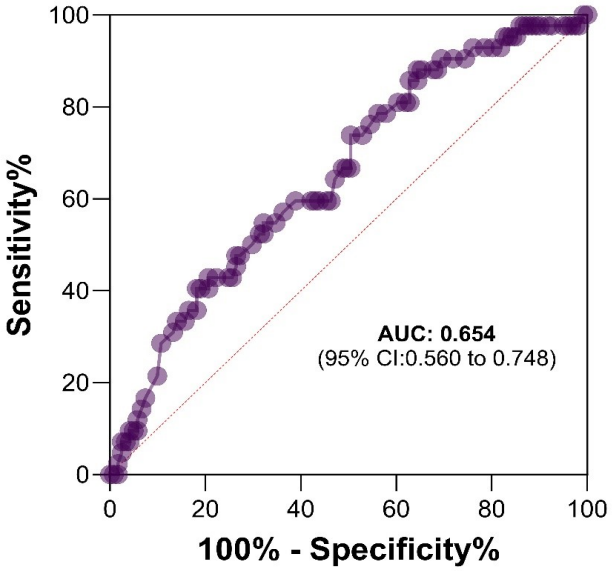

Figure 3. The ROC curve of the urinary EC for separating the CaOx stone patients from NSF subjects. An area under curve (AUC) of 0.654 ($P = 0.003$) appeared.

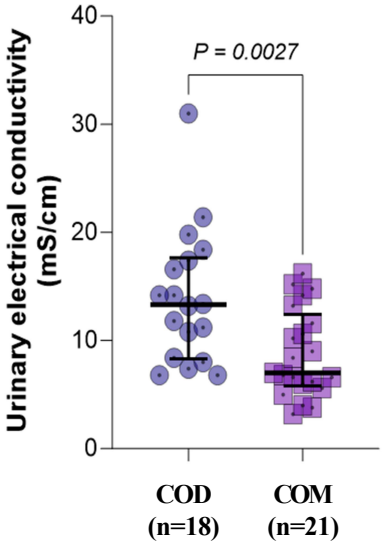

Figure 4. Comparison of urinary EC levels between CaOx patients with COD in urine and patients with COM in urine (n = 21). The urinary EC of patients with COD in urine was significantly greater than those with COM in urine. Bar indicates median. Error bars indicate IQR.

The formation of COD in urine greatly depends on the concentration of urinary citrate. The results demonstrated that a higher concentration of citrate added a higher amount of COD crystals formed. [18, 19] Since citrate is one of the main anions in urine, it could have a considerable contribution to the urinary EC. We hypothesized that urine with a lower citrate concentration would have a lower urinary EC value. We, therefore, recategorized the CaOx stone patients into two groups according to the formation of the main CaOx crystals (either COM or COD) in their urine after challenging with oxalate and calcium chloride. We confirmed that patients who had COD crystals in their urine had higher urinary citate level than those who had COM crystals in their urine. The urinary EC levels between these two groups were compared. The result showed that the urinary EC level of CaOx patients who had COD in urine (n = 18) was significantly higher than those who had COM in urine (n = 21) (median (Q1 - Q3): 13.3 (8.3 - 17.7) *vs.* 7.0 (5.8 - 12.4) mS/cm) **(Figure 4)**. This finding suggested urinary citrate greatly influenced the urinary EC.

Decrease in citrate concentration caused decrease in EC value in artificial urine

We performed an experiment in artificial urine to prove that citrate greatly influenced the EC level. The average EC value of our artificial urine was 16.0 mS/cm. We further added citrate to the artificial urine with various concentrations starting from 5,000 mg/L to 12.5 mg/L and measured the EC of the citrated-added artificial urine. Notably, the EC was linearly correlated with the concentrations of added citrate ($r = 0.9998$, $P < 0.001$, **Figure 5.** This finding indicated that a gradual decrease in citrate concentrations directly caused a gradual decrease in EC values.

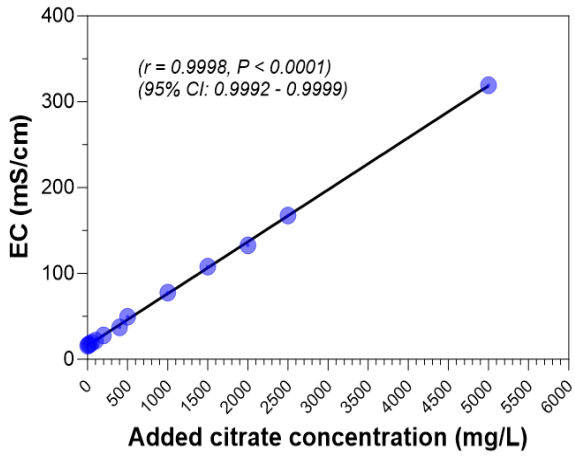

Figure 5. Effect of citrate on EC level in artificial urine.

Discussion

CaOx stone formation involves an altered excretion of urinary substances that favor CaOx crystallization. Citrate is the main organic anion in mammalian urine [20] , and low urinary excretion of citrate is a prominent risk for CaOx stone development. [5, 6, 10, 16] We previously demonstrated that urinary citrate in Thai kidney stone patients was significantly lower than the healthy controls. [7, 21] Fundamentally, the EC of solution depends upon the presence of ionic species in the solution. We hypothesized that change in urinary excretion of substances in CaOx stone patients would alter their urinary EC level. In this study, we found that the urinary EC of CaOx stone patients was significantly lower than the NSF controls. Also, the urinary calcium and urine specific gravity in CaOx stone patients were significantly lower than the NSF controls. The urinary EC was positively correlated with the urine specific gravity, but not urinary calcium. We experimentally demonstrated in artificial urine that a decreased EC was straightforwardly caused by a decrease in citrate concentration.

A decreased urinary EC (measured in the first morning urine samples) in idiopathic kidney stone patients relative to NSF controls was documented. [12, 14] Also, study in 24-hour urine showed that the urinary EC of idiopathic kidney stone patients was about 2 - 3 times lower than the NSF controls. [13] Our present data corresponded well with these previous reports. It was underlined that the urinary EC of stone patients was decreased compared with the NSF individuals. However, the urinary EC of only CaOx stone patients has not been reported. To our knowledge, this is the first study demonstrating that the urinary EC of 24-hour urine samples obtained from CaOx stone patients was significantly lower than the NSF controls.

We further evaluated if urinary EC was correlated with urinary calcium and urine specific gravity. In this study, we found that both urinary calcium and urine specific gravity in CaOx stone patients were significantly lower than the NSF controls, but only urine specific gravity was significantly correlated with urinary EC. The positive correlation of urinary EC and urine specific gravity has been reported. [22] We confirmed herein that urinary EC level was positively correlated with urine specific gravity both in patients and control groups. Since the level of urinary citrate is awfully low in Thai kidney stone patients [6, 7, 21] we speculate that the decreased levels of urinary EC and urine specific gravity seen in our CaOx stone patients might be caused by the low urinary excretion of citrate. Further experiment is required to warrant this. However, we did a pilot experiment to explore this, and we demonstrated that the EC level directly depends on the citrate level in artificial urine. Decreasing citrate concentrations proportionally caused decreasing EC values. Although it was not direct evidence, this experimental finding suggested that a decreased urinary EC observed in the CaOx stone patients might be, at least in part, explained by the low concentration of urinary citrate in the patients.

Hypercalciuria is a primary risk factor for kidney stone formation in the western countries. [23, 24] Based on our research experience, hypercalciuria is rarely found in Thai kidney stone patients [7] , but hypocitraturia is the most frequent one. [6, 21] In this study, we found that urinary calcium was significantly decreased in CaOx stone patients relative to the NSF controls. This discrepancy figure of hypercalciuria might be a reflective of different dietary patterns between Thai and western population.

We evaluated whether a simple measurement of urinary EC in a 24-hour urine sample could provide a good diagnostic performance for CaOx urolithiasis. In general, a test with AUC of 0.5 has no discrimination power to diagnose the disease condition, whereas a test with AUC over 0.9 is considered as an outstanding diagnostic test. [25] Based on ROC analysis, the urinary EC gave an AUC of 0.654 (95% CI: 0.560 - 0.748) for discriminating CaOx stone formers from NSF individuals. We concluded that the urinary EC test was not a very good test to be used for diagnosing CaOx urolithiasis by itself. However, the EC test was easy, simple, and inexpensive, and its diagnostic accuracy was acceptable. It could be clinically used to support other diagnostic tests for urinary stone disease.

Limitations of the study should be mentioned. The 24-hour urine specimens used in this study were leftover specimens from our previous study. Although the urine samples were stored in the proper place and condition (at - 20 ^{o}C), the EC values might be different with the freshly collected urine samples. The urinary citrate was not measured in this study. It might limit the extrapolation of the statement that stated that the decreased EC in CaOx stone patients was possibly caused by the low urinary citrate concentration in the patients.

Conclusion

We demonstrated that the urinary EC of CaOx stone patients was significantly lower than the NSF controls. Urinary calcium and urine specific gravity in CaOx stone patients were also decreased relative to the NSF controls. The urinary EC was positively correlated with the urine specific gravity both in CaOx stone and NSF groups. The diagnostic performance of urinary EC for CaOx nephrolithiasis was acceptable with sensitivity, specificity, and accuracy of 74.0%, 50.0%, and 56.0%, respectively. In addition, we experimentally showed that citrate level actively influenced the EC value of artificial urine. This finding implied that the low urinary EC in CaOx stone patients might be caused by the low excretion of urinary citrate in the patients.

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Conflict of interest statement

Each of the authors has completed an ICMJE disclosure form. None of the authors declare any potential or actual relationship, activity, or interest related to the content of this article.

Data sharing statement

The present review is based on the reference cited. Further details, opinions, and interpretation are available from the corresponding authors on reasonable request.

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