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Hypercalciuria, hyperoxaluria, and hypocitraturia screening from random urine samples in patients with calcium lithiasis

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Abstract Calcium lithiasis is the most frequently diagnosed renal lithiasis and is associated with a high percentage of patients with metabolic disorders, such as hypercalciuria, hypocitraturia, and hyperoxaluria. The present study included 50 patients with recurrent calcium lithiasis. We conducted a random urine test during nocturnal fasting and a 24-h urine test, and examined calcium, oxalate, and citrate. A study of the linear correlation between the metabolites was performed, and the receiver operator characteristic (ROC) curves were analyzed in the random urine samples to determine the cutoff values for hypercalciuria (excretion greater than 200 mg), hyperoxaluria (excretion greater than 40 mg), and hypocitraturia (excretion less than 320 mg) in the 24-h urine. Linear relationships were observed between the calcium levels in the random and 24-h urine samples ($R = 0.717$, $p = 0.0001$), the oxalate levels in the random and 24-h urine samples ($R = 0.838$, $p = 0.0001$), and the citrate levels in the random and 24-h urine samples ($R = 0.799$, $p = 0.0001$). After obtaining the ROC curves, we observed that more than 10.15 mg/dl of random calcium and more than 16.45 mg/l of random oxalate were indicative of hypercalciuria and hyperoxaluria, respectively, in the 24-h urine. In addition, we found that the presence of less than 183 mg/l of random citrate was indicative of the presence of hypocitraturia in the 24-h urine. Using the proposed values, screening for hypercalciuria, hyperoxaluria, and hypocitraturia can be performed with a random urine sample during fasting with an overall sensitivity greater than 86 %.

Keywords Screening · Hypercalciuria · Hyperoxaluria · Hypocitraturia

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Introduction

Renal lithiasis with a calcium composition is the most frequent form of lithiasis (a calcium composition is present in up to 75–80 % of patients diagnosed with lithiasis). Because calcium lithiasis has a high rate of recurrence in patients, metabolic analyses are recommended. Interestingly, studies have shown that up to 90 % of metabolic and mineral disorders can be discovered after performing a complete evaluation in patients with calcium lithiasis [1, 2]. Evaluations of patients with calcium lithiasis should include an adequate case history with imaging tests to study the composition of the calculus. Indeed, mineralogical analyses can help in the detection of metabolic disorders [3]. Undeniably, the most reliable diagnosis of metabolic disorders and risk factors responsible for the formation of lithiasis is found in metabolic and mineral studies [4]. In addition, metabolic studies must be performed correctly [5], and a series of guidelines must be followed to obtain reliable data and diagnose the metabolic and mineral disorders that are present. Common metabolic and mineral disorders include hypercalciuria and hypocitraturia, but hyperoxaluria and hyperuricosuria may also be

present in patients with calcium renal lithiasis [6–10]. In general, metabolic studies are usually performed from the random collection of two samples from a 24-h urine test, and the different minerals and metabolites involved in the formation of lithiasis are analyzed [1]. To avoid having to use 24-h urine samples, the metabolic outcomes of testing with random urine samples have been compared with 24-h urine samples; however, the outcomes have been variable [9].

The objective of the present study was to analyze the urinary calcium, oxalate, and citrate from fasting nocturnal random urine samples and correlate the results with the corresponding values from 24-h urine samples. In addition, the present study established the cutoff values for the diagnosis of hypercalciuria, hyperoxaluria, and hypocitraturia in a random fasting sample.

Methods

Study subjects

The present study, which included a total of 50 patients who were diagnosed lithiasis with a calcium composition, involved a metabolic study of random urine samples after 8 h of fasting nocturnal of solids and liquids and 24-h urine samples. The patients with calcium stones were diagnosed based on the clinical findings (medical history, physical examination, radiography, and ultrasound) and stone composition (only patients with oxalate and phosphate calcium stones were selected).

Inclusion criteria were men and women between 25 and 60 years of age with oxalate or phosphate calcium renal lithiasis. Patients between 25 and 60 years were selected because in this range of age the renal stone disease is more common [11].

Exclusion criteria were patients greater than 60 years of age or less than 25 years of age, congenital bone disease, congenital renal disease, hyperparathyroidism, inflammatory bowel disease, renal tubular acidosis, treatment with bisphosphonates, hormone replacement therapy, treatment with thiazides, treatment with potassium citrate, treatment with corticosteroids, and/or treatment with calcium and vitamin D.

24-h urine samples were collected after the patient had been on a normal calcium diet during 7 days (ingestion of approximately 1,000 mg of calcium per day). Patients were given a dietary recommendations with food tables in which the amount of calcium contained in each food was indicated.

Random urine samples with nocturnal fasting was collected after a minimum of 8 h of fasting by the patient, discarding the first morning urine that is produced during the night and collecting the next one.

Study variables

In the random urine sample with nocturnal fasting, we measured creatinine (mg/dl), calcium (mg/dl), oxalate (mg/l), and citrate (mg/l). The recommended urine volume ranged from 40 to 60 ml.

In the 24-h urine sample, we measured diuresis (ml), creatinine (mg/dl), calcium (mg/dl), oxalate (mg/l), and citrate (mg/l).

Hypercalciuria was considered when the urinary excretion of calcium was greater than 200 mg/day, hyperoxaluria was defined as a urinary excretion of oxalate that was greater than 40 mg/day, and hypocitraturia as a urinary excretion of citrate that was less than 320 mg/day [2].

Statistical study

Pearson's correlation analysis was used to assess the correlation between the variables of the fasting random and 24-h urine samples, and the Kolmogorov–Smirnov test was used to examine the normality of the distribution. Subsequently, ROC curves were constructed to establish the cutoff values for the metabolites in the random urine samples with fasting that would be indicative of a high probability of observing hypercalciuria, hyperoxaluria, and hypocitraturia in the 24-h urine samples (gold standard). Differences were considered significant at $p \leq 0.05$. The SPSS 17.0 program was used for the data analyses (SPSS, Inc., Chicago, IL).

Ethics

The present study was approved by the Ethics Committee of San Cecilio University Hospital, and written informed consent was obtained from all the patients and controls according to the Helsinki Declaration.

Results

Of the 50 patients subjected to the analysis of random fasting and 24-h urine, 30 were men and 20 were women (M/F = 1.5). The average age of the subjects was 44.8 ± 12.7 years. Mean creatinine in 24-h urine was 92.48 ± 37.94 mg/dl.

After comparing the normality of the variables, Pearson's correlation analyses were performed with the calcium, oxalate, and citrate levels in the random fasting and 24-h urine. We found that three cases had a strong, positive, linear correlation that was statistically significant. The Pearson's coefficients (R) for the comparisons of calcium, oxalate, and citrate in the random fasting and 24-h urine were 0.717 ($p = 0.0001$), 0.838 ($p = 0.0001$), and 0.799
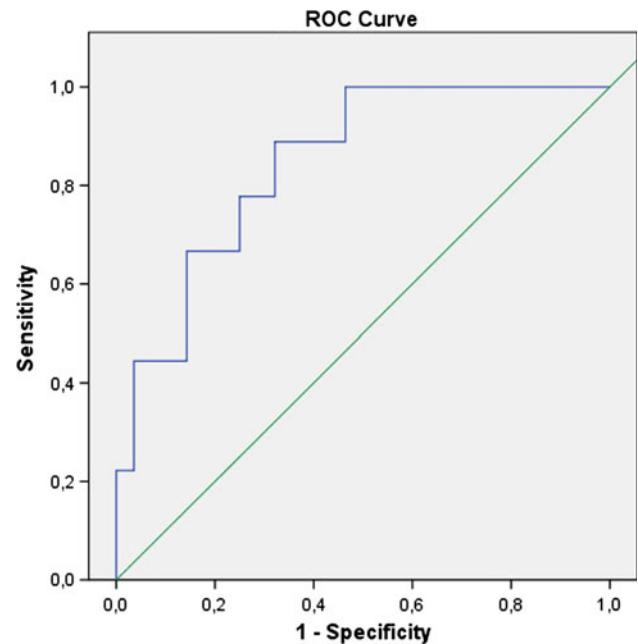
($p = 0.0001$), respectively. We also examined the correlations between the random fasting and 24-h urine for the ratios of calcium/creatinine ($R = 0.725$, $p = 0.0001$), oxalate/creatinine ($R = 0.564$, $p = 0.0001$), and citrate/creatinine ($R = 0.750$, $p = 0.0001$). An elevated and positive statistical significance was observed among all the variables analyzed.

A ROC curve was constructed to determine the optimal cutoff value for the level of calcium in the fasting random urine that would be indicative of the presence of hypercalciuria in the 24-h urine (area under the curve = 0.765, $p = 0.003$). We found that a calcium level greater than 10.15 mg/dl in the fasting random urine indicated that patients would have hypercalciuria (i.e., excretion greater than 200 mg/day) in the 24-h urine with sensitivity of 89 % and specificity of 59 % (Fig. 1). In men the cutoff value was 9.95 mg/dl (sensitivity of 88 % and specificity of 62 %) and in women the cutoff value was 10.8 mg/dl (sensitivity of 90 % and specificity of 56 %).

A second ROC curve was constructed to determine the optimal cutoff value for the level of oxalate in the fasting random urine that would be indicative of the presence of hyperoxaluria in the 24-h urine (area under the curve = 0.845, $p = 0.002$). We found that an oxalate level greater than 16.45 mg/l in the fasting random urine indicated that patients would have hyperoxaluria (i.e., excretion greater than 40 mg/day) in the 24-h urine with sensitivity of 89 % and specificity of 68 % (Fig. 2). In men
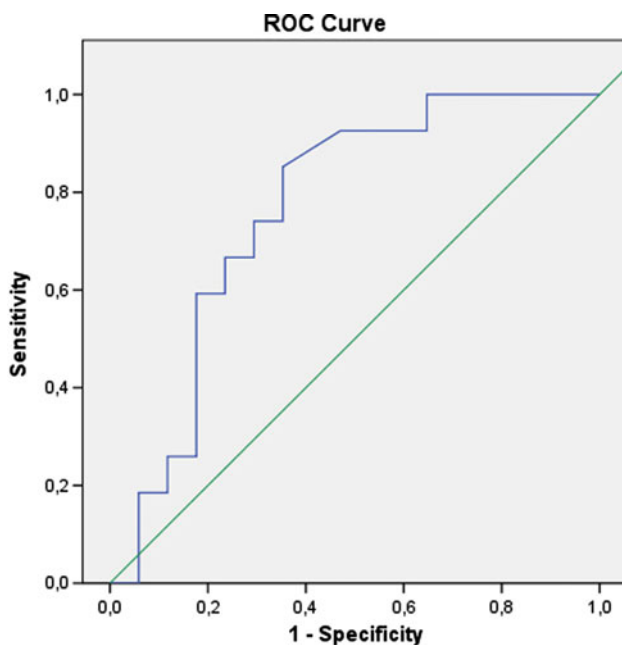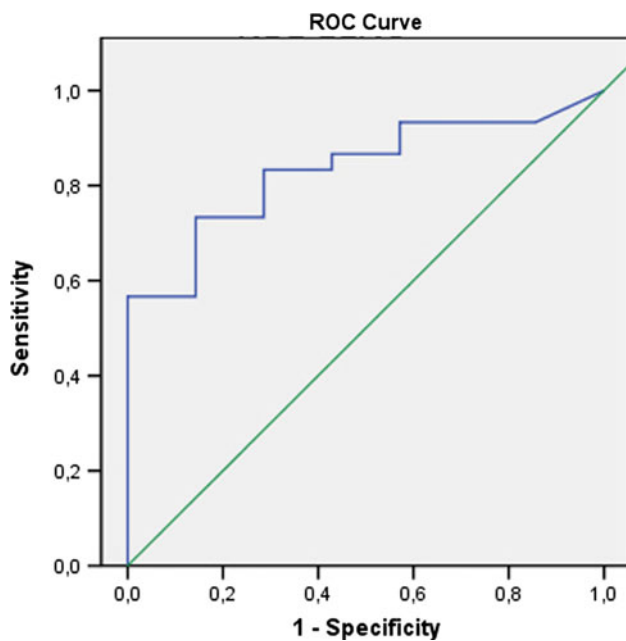

Fig. 2 A ROC curve was constructed to determine the optimal cutoff value for the diagnosis of hyperoxaluria in the fasting random urine that would be indicative of hyperoxaluria in the 24-h urine (area under the curve = 0.845, $p = 0.002$)

the cutoff value was 17.4 mg/l (sensitivity of 98 % and specificity of 75 %) and in women the cutoff value was 15.5 mg/l (sensitivity of 75 % and specificity of 58 %).

A third ROC curve was constructed to determine the optimal cutoff value for the level of citrate in the fasting random urine that would be indicative of the presence of hypocitraturia in the 24-h urine (area under the curve = 0.833, $p = 0.007$). We found that a citrate level less than 183 mg/l in the fasting random urine indicated that patients would have hypocitraturia (i.e., excretion less than 320 mg/day) in the 24-h urine with sensitivity of 86 % and specificity of 57 % (Fig. 3). In men the cutoff value was 183 mg/l (sensitivity of 83 % and specificity of 67 %) and in women the cutoff value was 164 mg/l (sensitivity of 91 % and specificity of 50 %).

Discussion

Urinary lithiasis is a prevalent disease that results in elevated medical costs due to the renal colic episodes treated in emergency rooms, instrumental treatment for lithiasis, and derivatives from hospital admissions [12–15]. Once the lithiasic episode is resolved, recurrences occur over time in a high percentage of patients; thus, many cases would benefit from a metabolic study to determine possible metabolite changes in the blood and urine [1, 2]. Metabolic studies involve the extraction of 24-h blood and urine samples in which different metabolic parameters, such as

Fig. 1 A ROC curve was constructed to determine the optimal cutoff value for the diagnosis of hypercalciuria in the fasting random urine that would be indicative of hypercalciuria in the 24-h urine (area under the curve = 0.765, $p = 0.003$)

Fig. 3 A ROC curve was constructed to determine the optimal cutoff value for the diagnosis of hypocitraturia in the fasting random urine that would be indicative of hypocitraturia in the 24-h urine (area under the curve = 0.833, $p = 0.007$)

calcium, phosphorous, citrate, and oxalate, are measured [16, 17]. Patients should carefully collect the 24-h urine sample because the instructions for urine collection are complex, which can create problems for less-educated populations. In many cases, the patients failed to collect the urine properly or to follow the instructions during the day of the collection. For example, patients had to follow a strict diet on the day of collection, which was different from their normal diet. In addition, the patients ingested more liquids than usual on the day of collection; thus, the results that were obtained were inconsistent with reality. Due to the elevated costs resulting from the treatment, the metabolic diagnosis, and the difficulties in the collection of the 24-h urine sample, we decided to establish a screening method that used the fasting random urine to assess the main alterations in urine (e.g., hypercalciuria, hyperoxaluria, and hypocitraturia) that are observed in lithiasis. Our objective was to establish a screening method to detect urine metabolic abnormalities, such as hypercalciuria, hyperoxaluria, and hypocitraturia. So, a good screening test must have high sensitivity (our method has around 85 % of sensitivity) and the specificity is important for a diagnostic test (although our specificity for screening is not low, around 60 %). We agree with Curhan that there is no clear evidence in the values of calciuria, but in some articles a cutoff for hypercalciuria, hyperoxaluria, and hypocitraturia has been established that give us the opportunity to classify the patients in different groups of metabolic abnormalities with the objective to prescribe the most appropriate

treatment. Post-prandial calciuria can be useful to diagnose absorptive hypercalciuria, but fasting calciuria is more sensitive to diagnose renal and resorptive hypercalciuria (fasting hypercalciuria) as we have indicated in the text [2, 11]. We have shown in the previous studies that both hypercalciuria and hypocitraturia are two of most important biochemical value determinants of recurrent calcium stones [18]. We exclusively applied the complete 24-h metabolic study to the patients who met certain criteria, with the objective of reducing costs and improving performance. Obviously, the more metabolic studies are performed, the more alterations can be found. In these times of global economic problems, we try to establish a cheap method to find possible metabolic alterations to be confirmed later in a complete metabolic evaluation. Strohmaier et al. [9] demonstrated that there was an excellent linear correlation between all the parameters measured in the random and 24-h urine. Therefore, the metabolite levels can be assessed in random urine to evaluate patients with lithiasis. Importantly, a third of the 24-h urinalyses are inaccurate. Regarding pH, the fasting random urine samples showed only a moderate correlation with that of the 24-h urine samples; thus, some studies have suggested that a 24-h urine sample is necessary in the evaluation of patients with changes in urinary pH [19]. Hong et al. examined 30 patients with lithiasis and assessed the correlation between the morning urine and 24-h urine. Hong et al. [20] observed a positive linear correlation for calcium, oxalate, and citrate with respect to the ratios of these metabolites to creatinine in the morning urine. In the present study, a strong, positive, linear correlation was observed between the levels of calcium, citrate, and oxalate in the fasting and 24-h urine (in all cases, $R > 0.7$). Moreover, a positive linear relationship was observed between the ratios of these metabolites to creatinine in the fasting urine. A ROC curve was constructed by defining hypercalciuria as the excretion of more than 200 mg of calcium per day [2] and taking into account the correlation between the fasting random and 24-h urine. Interestingly, we found that when patients presented with a value greater than 10.15 mg/dl in the fasting random urine, the sensitivity required for the 24-h urine sample to indicate hypercalciuria was 89 %. Patients with fasting hypercalciuria (renal or resorptive causes) [21] may benefit from this screening, as it measures the calciuria in a random sample urine after 8 h of fasting nocturnal of solids and liquids. The same operation was performed with oxalate, and hyperoxaluria was defined as the excretion of more than 40 mg of oxalate per day [2]. A ROC curve was constructed, and we observed that when patients presented with a value greater than 16.45 mg/l in the fasting random urine, the sensitivity required for the 24-h urine sample to indicate hyperoxaluria was 89 %. In regards to citrate,

hypocitraturia was defined as the excretion of less than 320 mg [2] of citrate, and the ROC curve showed that for patients with citrate excretion less than 183 mg/l in the fasting random urine, the sensitivity required for their 24-h urine sample to indicate hypocitraturia was 89 %.

The present study demonstrated that the fasting random urine samples serve as a reliable screening method for the detection of hypercalciuria, hyperoxaluria, and/or hypocitraturia in a 24-h urine sample. In addition, using a fasting random urine sample allows the doctors to screen patients and request a more complete metabolic study when necessary, which permits the diagnosis of the primary metabolic risk factors [22–26]. Using a fasting random urine sample as a screening method minimizes expenses because further metabolic studies will only be performed in patients with calcium lithiasis and with hypercalciuria, hyperoxaluria, or hypocitraturia. However, this metabolic screening does not replace the complete metabolic study that is indicated in different situations.

As a conclusion, screening for hypercalciuria, hyperoxaluria, and hypocitraturia can be performed with a fasting random urine sample with an overall sensitivity greater than 86 %. We recommend that patients with calcium lithiasis ask for the fasting random urine test because it is cheaper and simpler than the 24-h urine test and has demonstrated a comparable performance.

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