Intestinal *Oxalobacter formigenes* Colonization in Calcium Oxalate Stone Formers and Its Relation to Urinary Oxalate*

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**ABSTRACT**

**Background and Purpose:** *Oxalobacter formigenes* is an anaerobic commensal colonic bacterium capable of degrading oxalate through the enzyme oxalyl-CoA decarboxylase. It has been theorized that individuals who lack this bacterium have higher intestinal oxalate absorption, leading to a higher urinary oxalate concentration and an increased risk of calcium oxalate urolithiasis. We performed a prospective, controlled study to evaluate *O. formigenes* colonization in calcium oxalate stone formers and to correlate colonization with urinary oxalate and other standard urinary stone risk factors.

**Patients and Methods:** Thirty-five first-time calcium oxalate stone formers were compared with 10 control subjects having no history of urolithiasis and a normal renal ultrasound scan. All subjects underwent standard metabolic testing by submitting serum and 24-hour urine specimens. In addition, all subjects submitted stool samples for culture and detection of *O. formigenes* by Xentrix® *O. formigenes* Monitor.

**Results:** Intestinal *Oxalobacter* was detected in only 26% of the stone formers compared with 60% of the controls (*p* < 0.05). Overall, the average urinary oxalate excretion by the two groups was similar (38.6 mg/day vs 40.8 mg/day). Among stone formers, however, there were statistically higher urinary oxalate concentrations in *O. formigenes*-negative patients compared with those testing positive (41.7 mg/day vs 29.4 mg/day) (*p* = 0.03). Furthermore, all 10 stone formers with hyperoxaluria (>44 mg/day) tested negative for *O. formigenes* (*p* < 0.05).

**Conclusions:** Calcium oxalate stone formers have a low rate of colonization with *O. formigenes*. Among stone formers, absence of intestinal *Oxalobacter* correlates with higher urinary oxalate concentration and an increased risk of hyperoxaluria. Introduction of the *Oxalobacter* bacterium or an analog of its enzyme oxalyl-CoA decarboxylase into the intestinal tract may be a treatment for calcium oxalate stone disease.

**INTRODUCTION**

The discovery of nanobacteria as a potential nidus for renal calculi renewed interest in the role of bacteria in the pathophysiology of urolithiasis. *Oxalobacter formigenes* is a commensal colonic bacterium capable of naturally degrading intestinal oxalate through the enzyme oxalyl-CoA decarboxylase. Its potential importance in urinary stone formation was suggested by studies correlating the absence of *O. formigenes* colonization with calcium oxalate stone formation risk.1–4 In addition, colonization with *O. formigenes* has been shown to lower the urinary oxalate concentration in rats.1 There are few data regarding the relation between *O. formigenes* colonization and urinary oxalate in humans. The purpose of our study was to evaluate *O. formigenes* colonization in calcium oxalate stone formers and determine its relation to urinary oxalate and other urinary risk factors for calcium oxalate stone formation.

**PATIENTS AND METHODS**

A series of 35 patients treated for calcium oxalate stones (12 SWL, 12 ureteroscopy, 3 percutaneous nephrolithotomy, 8

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spontaneous passage) aged 30 to 79 years were asked to submit stool samples for detection of *O. formigenes* in addition to undergoing standard metabolic testing. The study was restricted to first-time stone formers with known stone compositions demonstrating >60% calcium oxalate. Patients with a history of significant bowel disease (jejunoileal bypass, inflammatory bowel disease, bowel resection) were excluded from the study. Patients submitted two stool samples for culture and detection of *O. formigenes* by polymerase chain reaction (PCR). All subjects additionally underwent standard metabolic testing to evaluate their risk for recurrent stone formation. Metabolic testing consisted of serum and 24-hour urine chemistries while on a random diet. Serum analysis consisted of SMA-7, calcium, and uric acid. The urine analysis determined the concentrations of calcium, oxalate, citrate, magnesium, uric acid, sodium, phosphorus, volume, and pH (Mission Pharmacoal Co., San Antonio, TX). At the time of testing, all patients were stone free and free of any urinary foreign bodies or infection.

Patients were compared with a control group of five women and five men aged 23 to 59 years with no prior or current urolithiasis or renal disease. One 40-year-old man had a family history of calcium oxalate stone disease (father). Control subjects underwent renal ultrasonography to document the absence of urinary stones, hydronephrosis, and renal parenchymal abnormalities. Similarly, controls submitted samples for detection of *O. formigenes* and serum and urine chemistry analysis.

Stool samples were coded and evaluated for the presence of *O. formigenes* using the Xentrix *O. formigenes* Monitor (Ixion Biotechnology, Inc., Alachua, FL). Subjects shipped fresh stool samples collected as fecal swabs using the BBL culture swab collection and transport system (Becton Dickinson, Sparks, MD). Genomic DNA was isolated from fecal swabs by the method of Phipps and associates and used as a template for PCR amplification with 5′ and 3′ primers specific for the *oxc* gene of *O. formigenes*. Each batch of samples was tested with a negative control consisting of a reaction mixture containing all of the PCR components except the template DNA and a positive control containing a DNA template with the *oxc* gene. The PCR-amplified products were confirmed by Southern blot analysis.

The relation between *O. formigenes* colonization and urinary oxalate was determined. Other urinary factors (calcium, sodium, uric acid, citrate, magnesium, volume, pH) known to affect calcium oxalate stone formation were similarly analyzed. Intestinal *O. formigenes* detection and urinary risk factors were compared in calcium stone formers and controls. A nonparametric Mann-Whitney U test was utilized for statistical analysis.

RESULTS

Stool samples demonstrating *O. formigenes* were significantly less common in stone formers than in control subjects. Only 26% (9/35) of the stone formers were colonized versus 60% of the controls. There was no correlation between *O. formigenes* colonization and age or sex.

No patient had received a course of oral antibiotic treatment within 6 months of stool sampling. However, patients requiring surgical intervention received a single dose of intravenous antibiotic on call to the operating suite. The negative colonization rate was similar for stone-forming patients requiring intervention and patients who spontaneously passed their stones (74% v 75%). For those patients who received one dose of intravenous antibiotics, the time to stool sampling averaged 19.3 weeks. There was no significant difference between the interval prior to stool sampling in those patients who were *O. formigenes* positive and those who were negative (23.1 v 18.5) (*p* = 0.488).

Overall, urinary oxalate excretion was similar in stone formers and controls. Stone patients excreted an average urinary oxalate of 38.6 ± 15.2 (SD) mg/day compared with 40.8 ± 14.8 mg/day for controls (Fig. 1). Among stone formers, however, there was statistically higher urinary oxalate excretion in *O. formigenes*-negative (41.7 ± 16.1 mg/day) than in *O. formigenes*-positive patients (29.4 ± 6 mg/day) (*p* < 0.05) (Fig. 2). In addition, all 10 stone patients with hyperoxaluria (>44 mg/day) demonstrated absence of *O. formigenes* colonization (Fig. 3).

Other urinary risk factors known to influence calcium oxalate stone formation were similar in *O. formigenes*-positive and -negative subjects. The urinary chemistries of *O. formigenes*-positive and -negative stone formers are compared in Table 1. The differences in urine, volume and pH reach statistical significance. An average of 2.5 urinary risk factors were found in *O. formigenes*-negative stone formers and 1.7 in *O. formigenes*-positive stone formers. Overall, stone formers demonstrated a higher number of risk factors, averaging 2.3 versus 1.5 for control subjects.

DISCUSSION

Urolithiasis is a significant health problem, affecting more than 10% of the US population. Greater than 65% of newly diagnosed stones are composed of calcium oxalate. While many factors influence calcium oxalate stone formation, urinary oxalate concentration is felt to be particularly important. It was initially believed that absorbed intestinal oxalate con-
tributed only 10% of excreted urinary oxalate. However, recent work by Holmes and colleagues\(^1\,\,\,12\) suggests that as much as 40% of urinary oxalate may come from intestinal oxalate absorption. These findings suggest that limiting intestinal oxalate absorption may be an important method of minimizing stone risk in some patients.

The interest in \textit{O. formigenes} stems from its ability to degrade intestinal oxalate and thus limit oxalate availability for intestinal absorption. Three prior studies have demonstrated a correlation between the lack of \textit{O. formigenes} colonization and an increased risk of calcium oxalate stone disease. Kleinschmidt and associates\(^2\) initially showed that lower colonization rates are found in stone formers with a higher number of stone episodes. In their study, all patients with four or more stone episodes lacked colonization with \textit{Oxalobacter}. Sidhu and coworkers\(^1\) similarly reported absence of \textit{Oxalobacter} colonization correlating with an increased number of stone episodes. In contrast to 75% of tested controls, only 38% of their patients reporting two to five stone episodes and 13% reporting more than five episodes were colonized with \textit{Oxalobacter}. A recent study by Kwak et al\(^3\) also found only a 37% colonization rate in calcium oxalate stone formers compared with 77% in the normal Korean population.

The findings of our study add to a growing body of evidence suggesting absence of \textit{O. formigenes} colonization adversely influences one’s risk for the formation of calcium oxalate stones. In our study, only 26% of first-time calcium oxalate stone formers demonstrated the presence of intestinal \textit{O. formigenes} compared with 60% of controls. Colonization with \textit{O. formigenes} typically occurs during the first years of life, with nearly 100% of children being colonized by age 6 to 8 years.\(^13\) This rate then falls to the 60% to 80% documented in the adult population.\(^14\) Loss of colonization has been associated with frequent antibiotic usage and a variety of gastrointestinal disorders. To control for these variables, we excluded any patient with a history of significant gastrointestinal disorder or frequent use of antibiotics. None of our patients received a course of oral antibiotics within 6 months of being tested, and there was no difference in colonization rates between those patients who spontaneously passed stones and those who received a single perioperative dose of antibiotics prior to surgical intervention. The low colonization rate found in our stone formers is far below what is expected in normal populations and that seen in our control subjects.

Unlike previous studies, in addition to evaluating colonization, we correlated \textit{Oxalobacter} colonization with urinary oxalate and other urinary risk factors known to influence calcium oxalate stone formation. Stone formers who tested negative for \textit{O. formigenes} had higher urinary oxalate excretion than those who tested positive (29.4 mg/day vs 38.6 mg/day, \(p = 0.03\)). Moreover, all 10 patients exhibiting hyperoxaluria (>44 mg/day) tested negative for the presence of \textit{Oxalobacter}. Although we did not control for oxalate intake, these findings strongly suggest a direct correlation between the absence of \textit{O. formigenes} colonization and higher urinary oxalate concentrations. There have been three prior studies that have evaluated urinary oxalate in relation to \textit{O. formigenes} colonization. Kleinschmidt and associates\(^4\) compared urinary oxalate in calcium oxalate stones formers and control subjects. However, they did not correlate their findings with \textit{O. formigenes} positivity versus negativity nor with other known urinary risk factors. Neuhaus and coworkers\(^5\) found an 8% rate (1/12) of \textit{O. formigenes} colonization in children with non-primary hyperoxaluria compared with 46% (6/13) among age- and sex-matched controls. They found no statistical difference in the urinary oxalate:creatinine ratio for control subjects colonized with \textit{O. formigenes} and those who were not colonized.\(^15\) Sidhu and colleagues\(^1\) evaluated urinary oxalate concentrations in rats prior to and after colonization with \textit{O. formigenes} and documented a significant decrease after colonization. To our knowledge, our study is the first in adult humans to directly link absence of \textit{O. formigenes} colonization with higher 24-hour urinary oxalate excretion in first-time stone formers.

Hyperoxaluria is only one of many factors contributing to calcium oxalate stone formation. Calcium oxalate supersaturation can also be influenced by urinary calcium, uric acid, sodium, citrate, and magnesium concentrations. \textit{Oxalobacter formigenes} colonization had little influence on urinary risk factors other than oxalate. Urinary volume and pH were statistically different between the two groups; however, the pH for both groups remained within the normal range. Hyperoxaluria was an isolated risk factor in only one stone patient, while

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**FIG. 2.** Urinary oxalate excretion by calcium oxalate stone formers testing positive and negative for \textit{O. formigenes}.

**FIG. 3.** Percentage of stone patients demonstrating hyperoxaluria.
five other patients demonstrated hyperoxaluria and low urine volume. These findings emphasize the complexity of the urinary milieu and that reduction of hyperoxaluria is only one of many factors in lowering one’s risk of recurrent stones.

The absence of \( O. \) *formigenes* colonization in calcium oxalate stone formers directly correlates with a higher urinary oxalate concentration. This implies that recolonization with the bacterium may be of therapeutic benefit by decreasing urinary oxalate. Sidhu and associates\(^1\) have recently demonstrated this to be possible in a rat model. They established \( O. \) *formigenes* using esophageal gavage and later oral administration of the bacterium with feedings.\(^2\) Both methods achieved colonization and subsequent lowering of urinary oxalate. However, they found that concomitant administration of an oxalate-rich diet and repeated inoculations were necessary to maintain colonization.

While recolonization has been shown to be possible, technical limitations may preclude it from being used clinically. The oxalate-degrading enzymes of \( O. \) *formigenes* have been cloned and expressed.\(^3,4\) Sidhu and associates\(^5\) have been successful in reducing urinary oxalate in a rat model through the administration of an enteric-coated capsule containing these oxalate-degrading enzymes. Clinically, this may be a more feasible form of treatment than introduction of the bacterium.

**CONCLUSION**

Intestinal \( O. \) *formigenes* colonization is less common among calcium oxalate stone formers than in control subjects. Among stone formers, absence of colonization correlates with hyperoxaluria. Introduction of \( O. \) *formigenes* or the enzyme oxalyl-CoA decarboxylase may lower urinary oxalate and reduce the risk of stone recurrence in some patients with calcium oxalate stone disease.

**REFERENCES**


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