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The Effect of Renal Cysts on the Fragmentation of Renal Stones During Shockwave Lithotripsy: A Comparative *In Vitro* Study

Husain Alenezi, MD,[1] Daniel Olvera-Posada, MD,[1] Peter A. Cadieux, PhD,[2,3]
John D. Denstedt, MD, FRCSC, FACS, FCAHS,[1] and Hassan Razvi, MD, FRCSC[1]

Abstract

Purpose: To assess the potential effect of simple renal cysts (SRC) on stone fragmentation during shockwave lithotripsy (SWL) in an *in vitro* model.

Materials and Methods: The *in vitro* model was constructed using 10% ordnance gelatin (OG). Models were created to mimic four scenarios: Model A—with an air-filled cavity (suboptimal for stone fragmentation); model B—without a cavity (normal anatomy); model C—with a 3-cm serum filled cavity (to represent a small SRC); model D—with a 4-cm serum filled cavity (to represent a larger SRC). SWL was applied to 24 standardized phantom stones (weight of 2 ± 0.1 g) in each model using a standardized protocol. Stone fragments were retrieved, then dried overnight at room air temperature. Fragmentation coefficient (FC) was calculated for each stone, for fragments <4 mm and <2 mm.

Results: The OG *in vitro* model was robust enough for the proposed research. There was no fragmentation evident in model A as expected. The mean FC was 29.7 (± 20.5) and 39.7 (± 23.7) for <4 mm fragments ($P = 0.069$) and 7.6 (± 4.1) and 10.6 (± 6.7) for <2 mm fragments ($P = 0.047$), for noncystic and cystic models, respectively. The mean FC was 29.7 (± 20.5), 38.8 (± 26.2) and 40.7 (± 21.3) for <4 mm fragments ($P = 0.213$) and 7.6 (± 4.1), 11.1 (± 8) and 10.2 (± 5.3) for <2 mm fragments ($P = 0.138$), for models B, C, and D, respectively.

Conclusion: Our *in vitro* experiment confirms better stone fragmentation associated with SWL in the presence of adjacent SRC.

Introduction

Although shockwave lithotripsy (SWL) has been established as a noninvasive treatment option for urolithiasis since its successful introduction by Chaussy and associates[1] early in the 1980s, basic research is still warranted to both discover factors affecting the efficiency of SWL and determine its limitations.[2] To date, little is known about the effect of simple renal cysts (SRC) on the fragmentation of adjacent renal stones during SWL, and only a limited number of small cohort retrospective studies have examined the success rate of SWL in treating patients with renal stones with adjacent SRC.[3,4]

The literature has reported a decreased success rate of SWL in treating patients with renal stones with adjacent SRC. Those reports, however, suffered from missing important data such as the location of the stones in relation to SRC and the inclusion of heterogeneous groups of patients including SRC, autosomal dominant polycystic kidney disease (ADPKD), and other cystic conditions.[5,6] Both renal stones and SRC are commonly prevalent worldwide,[7–9] and a higher rate of renal stones in patients with SRC has been reported.[10] Therefore, it is important to know if SRC truly affect the rate of renal stone fragmentation during SWL to determine whether SWL is suitable for management of stones in patients with concurrent SRC.

We present the data from a comparative study of stone fragmentation in a novel *in vitro* model mimicking SRC.

Materials and Methods

Construction of in vitro *models*

The *in vitro* models were constructed from 10% ordnance gelatin (OG), following the same modifications applied by

[1]Division of Urology, Department of Surgery, Schulich School of Medicine and Dentistry, and [2]Department of Microbiology and Immunology, The University of Western Ontario, London, Ontario, Canada.
[3]School of Health Sciences, Fanshawe College, London, Ontario, Canada.

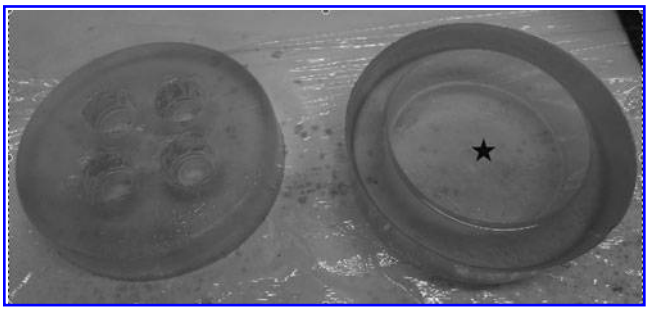

FIG. 1. The top (left) and the bottom (right) segments used to construct the cystic models (*cavity representing the cyst space).

Mendez-Probst and colleagues[11] to the original recommendations in the construction of ballistic studies models using OG by Fackler and Malinowski.[12] Four different models were created to mimic four different clinical scenarios:

1. Model A: Two-segment OG model with a 3-cm air-filled cavity within the bottom segment as a negative control with no expected stone fragmentation.
2. Model B: One segment OG model with no interior cavity, representing normal anatomy.
3. Model C: Two-segment OG model with a 3-cm cavity within the bottom segment filled with artificial serum to mimic a small SRC (Fig. 1).
4. Model D: Two-segment OG model with a 4-cm cavity within the bottom segment filled with artificial serum, representing a larger SRC (Fig. 2).

All of the models contained four stone wells on the top surface. The stone wells were contained within the boundaries of the underlying cavity in all of the cystic models to ensure the ''cysts'' were in the blast path of the shockwaves (SW). All stone wells were 2.5 cm in depth (Fig. 3A), and the total height of each model was 9.5 cm (Fig. 3B); thus, all stones were treated at the same distance from the SWL therapy head (7 cm).

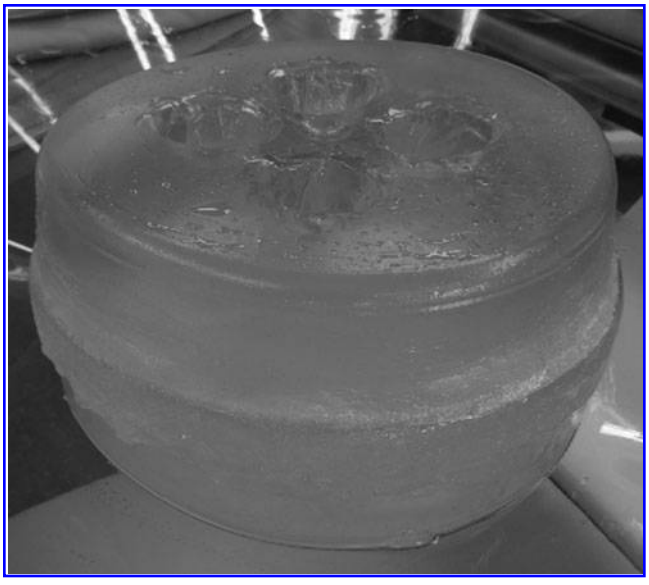

FIG. 2. The cystic model with the top and bottom segments glued together using 10% ordnance gelatin.

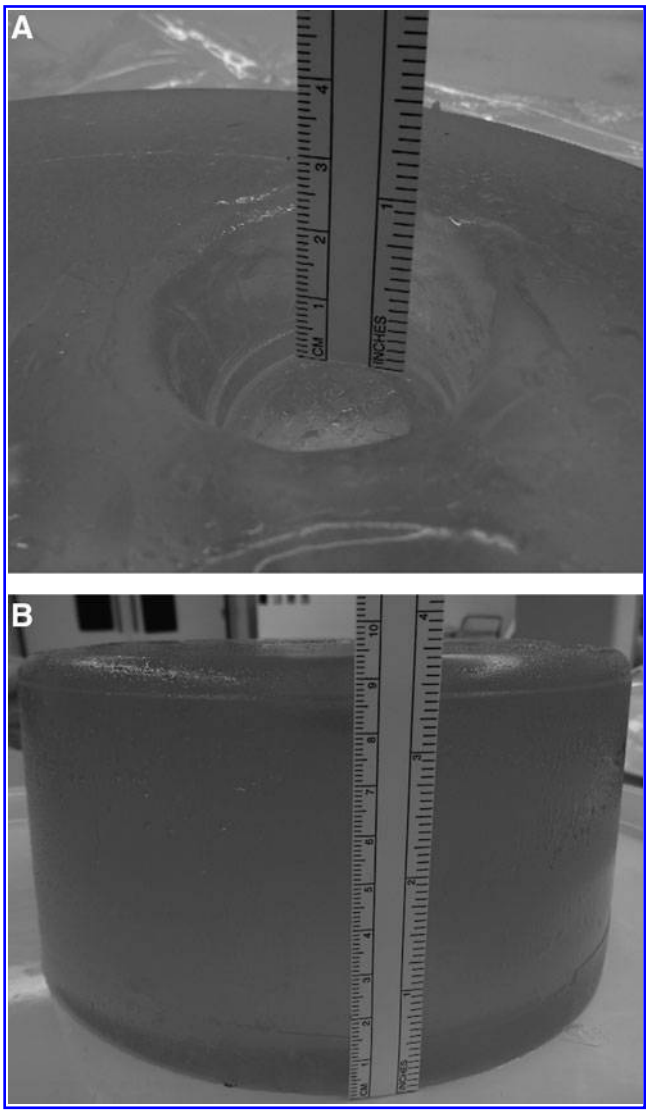

FIG. 3. (**A**) Stone well with the standardized depth of 2.5 cm. (**B**) Model B, one segment with no internal cavity and a height of 9.5 cm (standard height of all models).

Biochemical analysis of SRC fluid in previous reports revealed a composition similar to that of serum.[13,14] Therefore, the cystic cavities in models C and D were loaded with artificial serum to closely replicate SRC. Artificial serum was prepared by using 4.5 mM KCl, 5 mM CaCl2, 4.7 mM (D +)-glucose, 2.5 mM urea, 0.1% human serum albumin, and 145 mM NaCl, which is a commonly used formula in biochemical studies to represent serum.[15,16]

Cylindrical phantom stones were prepared using Begostone plus plaster (Bego Canada, QC, Canada) with a powder to water ratio of 5:1. All stones were sanded to a final weight of 2 ± 0.1 g with final dimensions of approximately 8×13 mm. The stones were soaked in sterile human urine (negative nitrites and leukocytes, pH = 6.5 and specific gravity = 1.01–1.02), obtained from healthy donors overnight before the lithotripsy procedure.

SWL procedure

SWL was conducted using the Modulith SLX-F2 lithotriptor (Storz Medical AG, Tägerwilen, Switzerland). Initially, an integrity test was performed to ascertain the durability of the OG model as follows: A 3-cm–cavity model was subjected to the standardized SWL protocol after filling the bottom segment cavity with methylene blue dye and filling the stone wells with contrast material. Each stone well was targeted at the F2 of the lithotripter. A thousand SW were delivered to each stone well (total of 4000 SW to the gel) at an energy level of 6. At the end of the procedure, the integrity of the stone wells was examined for any evidence of contrast leak using fluoroscopy, while the cystic cavity was manually examined for any breakage or gross leakage of the blue dye.

All OG models were kept in a cold area (at 4°C) after preparation until the planned SWL procedure. Each model was subjected to SW within 30 minutes after removing it from the cold area. Before the delivery of SWL, each stone well was loaded with 10 mL of sterile human urine before placing a phantom stone in each well. The stone wells were covered with a plastic wrap that was labeled above the corresponding stone to maintain orientation (Fig. 4). Coupling to the lithotripter was achieved using cold tap water, while the gels were held in position by a custom-made acrylic device to maintain efficient contact throughout the procedure (Fig. 5).

SW were delivered to each stone phantom using a standardized SWL protocol consisting of 1000 SW at an energy level of 6 and a frequency of 2 Hz. The precise (narrow) treatment focus (F2 diameter of 6 mm × 28 mm) was used to shock all the stone phantoms. All stones were shocked at the same distance from the point of contact to the lithotripter machine (7 cm) to standardize the ''skin to stone distance'' and eliminate its effect on stone fragmentation. Stone targeting was maintained by frequent fluoroscopy during the experiment (Fig. 6).

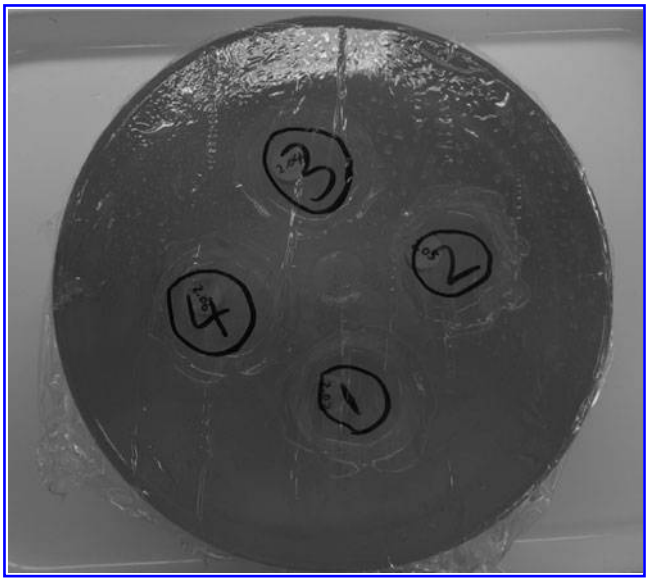

FIG. 4. Ordnance gelatin model with four stone wells loaded with 10 mL urine and containing a stone in each well; the model was covered with plastic wrap, and each well was labeled accordingly to maintain orientation and identification.

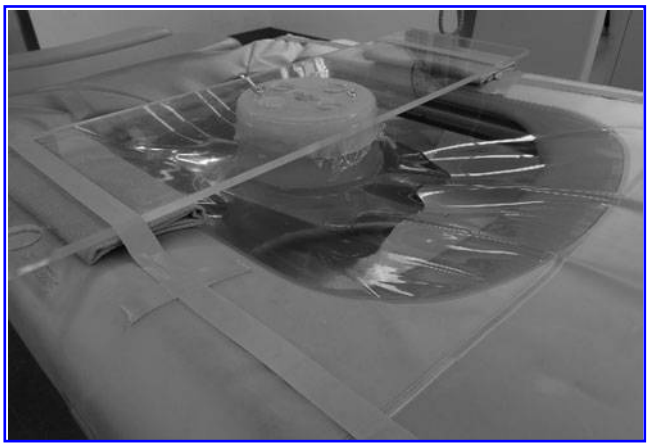

FIG. 5. Ordnance gelatin model positioned on the SLX-F2 lithotripter. Note the acrylic cover (taped to the bed) used to keep the gel in contact with the therapy head; also note the water surrounding the model for coupling purpose.

After the delivery of 4000 SW to fragment the stones in each gel, stone fragments were retrieved from each stone well separately. The fragments were then dried overnight at room air temperature. Fragments from each stone were sieved through a 4-mm filter, and the weight of the remaining fragments was recorded. The same process was repeated after sieving the fragments through a 2-mm filter. The Fragmentation Coefficient (FC) was calculated for each stone using the formula: FC = (pre-SWL weight – post-SWL weight) × 100/(pre-SWL weight).[17]

Statistical analysis

Data were analyzed using SPSS v22.0 software (IBM Corp. Released 2013. IBM SPSS Statistics for Macintosh, Version 22.0. Armonk, NY: IBM Corp.). Descriptive

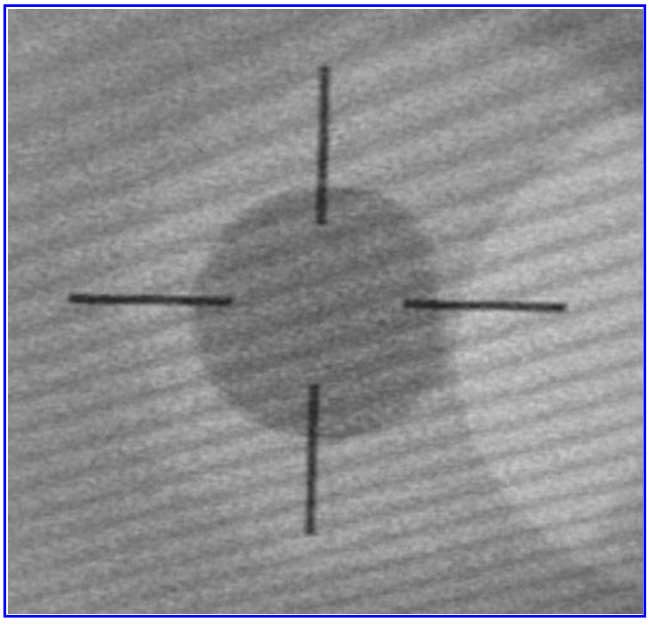

FIG. 6. Fluoroscopic image showing the targeted stone within the focus of the lithotripter.

statistics were presented as the means (± standard deviation). Data analysis was performed via unpaired, two-tailed Student *t* or Mann-Whitney *U* tests (model A *vs* models C and D) and one-way analysis of variance or Kruskal-Wallis test (model A *vs* model C *vs* model D), as appropriate. The Sidak *post hoc* test was performed for multiple group comparison. Significance was assessed at $P < 0.05$.

Results

The initial OG *in vitro* model was found to be robust enough after delivering 4000 SW to the gel during the integrity test. There was no evidence of contrast leakage from the stone wells (Fig. 7), and the methylene blue dye was retained completely within the cystic cavity. The test OG model was entirely intact without signs of melting or disruption.

SWL was applied to 24 phantom stones in each of the gel types, except model A that had only 12 phantom stones. No measurable fragmentation was evident in model A, as expected, because of the air interface within the cystic cavity, and thus model A was not included in the statistical analysis. To compare the effects of the cystic models on fragmentation to that of noncystic gels, the mean FC was measured for both model C and model D combined and compared with that of model B. The mean FC for fragments <4 mm was 39.7 (± 23.6) and 29.7 (± 20.5) for cystic gels (n = 48) and noncystic gels (n = 24), respectively.

There was a statistical trend toward better fragmentation with the cystic models ($P = 0.069$, Fig. 8). In addition, the cystic models had a statistically significant higher mean FC for fragments <2 mm when compared with that of noncystic models, with a mean FC of 10.6 (± 6.7) and 7.6 (± 4.1), respectively ($P = 0.047$, Fig. 8).

To assess the effect of the cystic cavity size on fragmentation, the mean FC of the three models (B, C, and D) was calculated and compared. For fragments <4 mm, the mean FC was 29.7 (± 20.5), 38.8 (± 26.2), and 40.7 (± 21.3) for models B, C, and D, respectively. There was no significant difference between the three groups ($P = 0.213$, Fig. 9). While the mean FC for fragments <2 mm was 7.6 (± 4.1), 11.1 (± 8.01), and

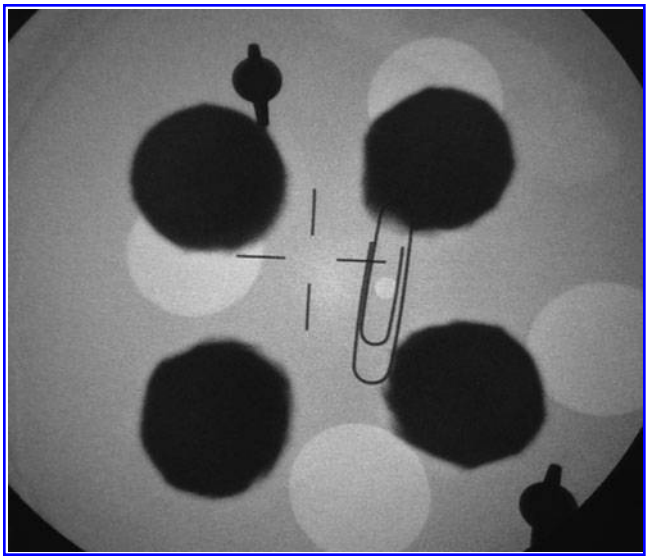

FIG. 7. Fluoroscopic image after the initial integrity test showing the four stone wells filled up with contrast material with no evidence of leakage. (Note: the paper clip and the metal bolts were above the model with the custom-made acrylic holding device and used to maintain orientation under fluoroscopic guidance.)

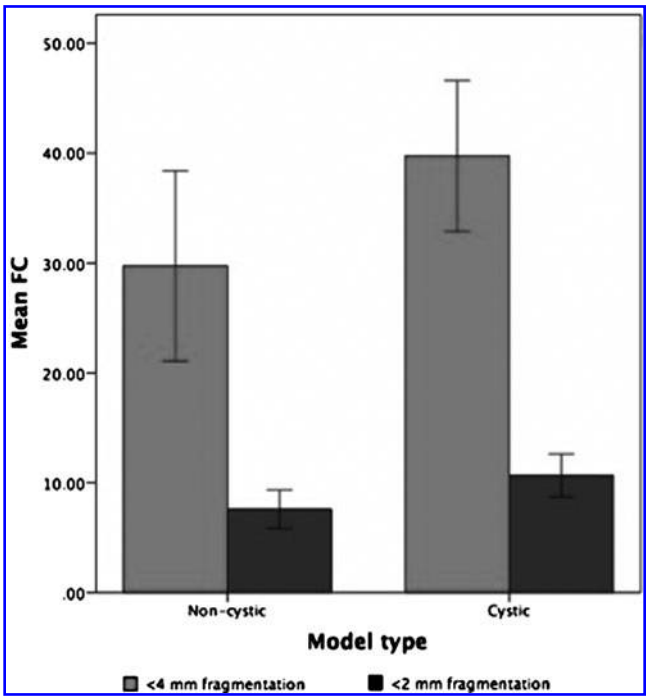

FIG. 8. The mean Fragmentation Coefficient (FC) of cystic and noncystic models for fragments <4 mm and <2 mm. (Error bars with 95% confidence interval.)

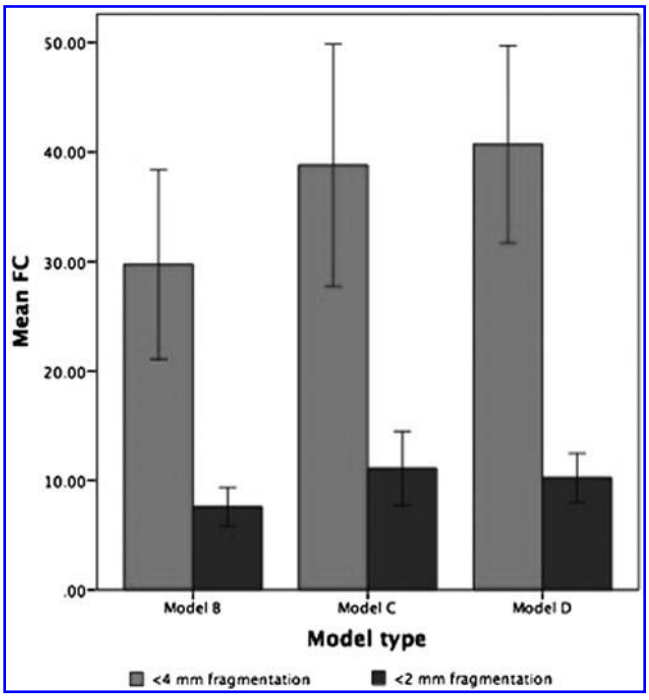

FIG. 9. The mean Fragmentation Coefficient (FC) for the three different models included in the analysis for fragments <4 mm and <2 mm. (Model B = no cavity, model C = 3-cm serum filed cavity, and model D = 4-cm serum filled cavity). (Error bars with 95% confidence interval.)

10.2 (± 5.3) for models B, C, and D, respectively, there was no significance between the three groups ($P = 0.138$, Fig. 9).

Discussion

SRC are common incidental findings during renal imaging by ultrasonography or CT in the general population, with an incidence of up to 50%.[9] Urolithiasis represents a very common disease worldwide,[7] and the high coincidence of both urolithiasis and SRC may explain the higher rate of stones in kidneys with SRC.[10] SWL is a widely used treatment option for renal stones, and currently SRC are not a contraindication to SWL.[18] The effect of SRC on the delivery of SW, however, and resultant stone fragmentation are not fully elucidated.

Although only a handful of publications have focused on SWL and SRC, a lower rate of SWL success has been reported. Cass[3] was the first to examine the effect of SRC on SWL efficiency.[3] He reported a 46% success rate after treating 13 patients with SWL, but this retrospective cohort was heterogeneous and included patients with SRC, multiple cysts, and ADPKD. Subsequent studies reported a success rate of 33% to 85% after the management of stones with SWL in patients with concurrent cystic kidneys. Unfortunately, those reports suffered from the same limitations of being retrospective small cohort studies including patients with different cystic disorders,[5,6] or patients with unrelated stones and SRC.[4]

None of the clinical studies have clearly delineated the exact reason why SRC negatively impact SWL efficiency. There are two possible theories for SRC to affect SWL results: By negatively interfering with the delivery of SW energy to adjacent stones and/or causing collecting system distortion or obstruction and preventing the spontaneous passage of the stone fragments after SWL. In this article, we wished to evaluate the effect of SRC on SW energy delivery and stone fragmentation.

The model used was based on the previous work by Mendez-Probst and coworkers[11,19] at our center, using OG for *in vitro* SWL studies. The modified model for the present experiments contained an internal cystic cavity that was filled with artificial serum to closely simulate the physiologic consistency of SRC.[13,14] Model durability was confirmed before performing the SWL experiments. Furthermore, to ascertain the effect of the model cavity on fragmentation of adjacent stones, model A was constructed to contain air within the cystic cavity as a negative control because of the known poor propagation of SW through even the smallest amount of air.[20,21] Twelve stones were shocked through model A with no resultant fragmentation, confirming that SWs had to travel through the cavity before reaching the stone wells.

The results of the present study confirmed better fragmentation after shocking stones adjacent to the cystic models, which was associated with the production of even smaller fragments than the noncystic model. Although the positive effect of cysts on the delivery of SW was not expected at the start of this study, it is a logical finding if we extrapolate data from the effect of SRC on ultrasound transmission.[22] Both SWL and ultrasound depend on the physical properties of the traversed tissues such as acoustic velocity and impedance. SRC are known to demonstrate acoustic enhancement with ultrasound because of the lower attenuation effect on the ultrasound waves compared with surrounding soft tissue.[22] SW are expected to behave in the same fashion through SRC resulting in better transmission of SW energy to the targeted stones, leading to higher energy density. Enhanced fragmentation would theoretically be expected.[23]

The current study is not without limitations. The *in vitro* nature of the experiments with standardized variables does not necessarily reflect the scenario within biologic tissues during SWL. It could be argued that the mean FC was low in the current study, although this can be explained by the known hardness of Begostone resembling the physical characteristics of calcium oxalate monohydrate stones.[24] Moreover, low FC has been observed in previous SWL experiments using Begostones.[24–26] Lastly, although the total number of shocked stones was high, each analyzed group had only 24 stones. Despite the relatively low number, there was a statistical significance observed (with fragmentation <2 mm). A larger sample might increase the significance of the observed difference.

Conclusion

The present study confirms an enhanced fragmentation rate for stones managed by SWL using an *in vitro* model simulating SRC, in comparison with fragmentation with a noncystic model. The presence of a cystic cavity was associated with the production of smaller fragments by SWL. This finding warrants further assessment with a large cohort, well-designed clinical study to determine the effect of SRC on SWL delivery and success rate.

Author Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Hassan Razvi, MD, FRCSC
Division of Urology
St. Joseph's Health Care London
268 Grosvenor Street
London, Ontario N6A 4V2
Canada

E-mail: hassan.razvi@sjhc.london.on.ca

Abbreviations Used

ADPKD = autosomal dominant polycystic kidney disease
CT = computed tomography
FC = fragmentation coefficient
OG = ordnance gelatin
SRC = simple renal cysts
SW = shockwaves
SWL = shockwave lithotripsy