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ORIGINAL PAPER

The concentration of Zn, Mg and Mn in calcium oxalate monohydrate stones appears to interfere with their fragility in ESWL therapy

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Abstract Extracorporeal shockwave lithotripsy (SWL) has remained the preferred method of treatment of urinary stones since its introduction in 1980. Although SWL is classified as a potential first-line treatment for renal stones smaller than 2 cm and its overall success rate is higher than 85% for stone clearance, not all renal calculi are successfully fragmented after SWL. Among the urinary stones, calcium oxalate monohydrate (COM) stone is one of the hardest stones to fragment. Several factors interfering with stone fragility are known to exist. In addition to technical properties for SWL to increase the quality and rate of stone disintegration, the composition of stones such as trace element levels may also interfere with the efficacy of SWL. Therefore, in the present study, we aimed to elucidate the correlation, if it exists, between fragmentation of renal stones and their trace element (Cu, Zn, Mg, Fe, Pb, Mn, Cr) concentrations. For this purpose, the patients admitted to our department who were identified with urinary stones (740 patients) and underwent SWL sessions were evaluated prospectively. Patients having 5–20 mm of solitary COM stone in the renal pelvis were included in this study. The trace element concentrations of renal stones that were successfully fragmented with SWL (SWL-S) were compared with those that were unsuccessfully fragmented after three SWL sessions (SWL-US) and removed surgically. Our measurements showed that the concentrations of Cu, Fe, Pb, and Cr were similar in both groups; by contrast, the concentration of Zn, Mg and Mn was significantly lower in SWL-US renal stones. The present results suggest that low concentrations of Zn, Mg and Mn in COM stones appear to make them resistant to SWL fragmentation and may offer a critical distinction for the choice of a treatment program.

Keywords Trace element · Stone fragility · ESWL · Zinc

Introduction

Kidney stone diseases are estimated to affect 1–15% of the population with a higher prevalence (10–15%) in industrialized countries [1]. Open surgery was the sole intervention option for all patients, except for those having spontaneous stone pass, prior to introduction of extracorporeal shockwave lithotripsy (SWL). After its introduction into clinical practice in 1982, SWL has remained the first-line treatment modality in the kidney and proximal ureter calculi [2, 3]. Nevertheless, SWL treatment is unsuccessful in 9.4–26.3% of urinary stones [4, 5]. The failure of SWL to clear stones in urolithiasis results from either a high stone burden due to difficulties in the clearance of fragments or from a primary failure of stone fragmentation. The degree of stone fragmentation by SWL is critical in the choice of a treatment program in patients with stones. Initially, Dretler [6] introduced the concept of stone fragility and its impact on fragmentation. Later, several investigators have shown the relative susceptibilities of different stone compositions to

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SWL [7, 8]. Struvite, uric acid and calcium oxalate dihydrate calculi tend to fracture into small particles that can pass relatively easily, whereas calcium oxalate monohydrate (COM) tends to break up into larger pieces that are more difficult to pass. The reason for the differences in the fragility of COM stones is not well established.

Many factors are involved in the formation of urolithiasis. The absence of crystallization inhibitors and the presence of crystallization promoters are critical to renal stone formation [9]. Various element concentrations are also found to be extremely variable in different categories of stones [10–12].

There are conflicting results regarding the role of Zn and Mg in urolithiasis. Zn is reported to act as a potent inhibitor of crystallization and is found to be abnormally low in the urine of stone formers [13, 14]. In contrast, several studies show increased excretion of Zn in the urine of stone formers [15] or no significant difference between healthy controls and stone formers [10, 16, 17]. Moreover, early studies showed that dietary Mg deficiency causes experimental urolithiasis. High excretion of Mg in urine reduces the concentration of oxalate available for calcium (Ca) oxalate precipitation [18]. By contrast, more recent studies indicate that Mg deficiency may have no role in the initiation of stone formation in recurrent calcium urolithiasis [19]. Environmental and dietary heavy metals and elements can also enhance or retard Ca-compound deposition, thereby interfering with the crystallization process [16, 20].

Although several studies have demonstrated that several elements are involved in the formation of urinary calculus, few studies have focused on the effect of elements on stone fragility. In contrast to intense studies on stone categories and well-known inhibitory function of citrate, phytate, pyrophosphate and magnesium in renal stone formation, little attention has been paid to the function and role of the elements in stone fragility. In the present study, we therefore aimed to determine element concentrations in COM stones with respect to SWL fragility.

Materials and methods

In this study, renal stone patients admitted to our clinic between March 1999 and August 2003 and treated with SWL (Stonelith V3 electro-hydraulic lithotriptor, Ankara, Turkey) were assessed prospectively. All the patients were evaluated with intravenous urography (IU) before SWL. During this period, 740 renal stone patients with stone size smaller than 20 mm underwent SWL performed as an outpatient procedure. During the delivery of the first 25 shock waves, the voltage was gradually increased to maximum levels and the patients received 80 shocks per minute (Tables 1, 2). In each session, 3,000 shock waves were applied to patients (Tables 1, 2). The patients were reviewed 10 days after the first SWL session using plain kidney–ureter-bladder (KUB) film and renal ultrasound to assess fragmentation and the presence of renal obstruction. Repeat treatment was carried out if there was inadequate fragmentation of the stone. SWL treatment was successful in 708 patients (95.68%). In contrast, it failed to fragment renal stones in 32 patients (4.32%) after 3 SWL sessions and these patients were referred for surgery. Of the 32 patients, 25 showed up for the surgery (percutaneous nephrolithotomy) and their stones were removed, while the rest were lost to follow-up.

Table 1 Patients and lithotripsy profiles in SWL-US group

Name	Patient No	Age	Gender	Stone localization	Stone diameter (mm)	SWL		
						Voltage (kV)	Sessions	Shock wave number (in each session)
KY	1	45	M	R	13	21	3	3,000
EO	2	40	M	L	12	20	3	3,000
SK	3	61	M	L	18	21	3	3,000
GK	4	57	F	L	20	18	3	3,000
CG	5	38	F	R	15	22	3	3,000
IG	6	64	M	R	6	21	3	3,000
DE	7	64	F	R	20	18	3	3,000
IE	8	25	M	R	10	20	3	3,000
HD	9	60	F	L	16	18	3	3,000
EC	10	24	F	L	14	19	3	3,000
IB	11	51	M	R	10	21	3	3,000
AA	12	44	M	R	20	21	3	3,000
SA	13	62	M	L	10	21	3	3,000
EA	14	28	F	R	15	17	3	3,000

Table 2 Patients and lithotripsy profiles in SWL-S group

Name	Patient No	Age	Gender	Stone localization	Stone diameter (mm)	SWL		
						Voltage (kV)	Sessions	Shock wave number (in each session)
AID	1	48	M	L	10	18	3	3,000
CT	2	33	M	L	9	17	2	3,000
HT	3	45	F	R	10	18	1	3,000
SO	4	54	F	L	17	21	1	3,000
NO	5	50	F	R	15	20	1	3,000
MN	6	43	M	L	11	22	3	3,000
FO	7	41	M	R	15	19	1	3,000
EK	8	42	F	R	10	22	1	3,000
FK	9	31	F	L	15	21	1	3,000
LK	10	37	M	R	18	19	1	3,000
RG	11	17	M	L	13	18	1	3,000
NG	12	49	F	R	20	20	1	3,000
KC	13	38	M	L	20	21	2	3,000
OA	14	47	M	L	15	17	1	3,000
CB	15	22	M	R	20	21	1	3,000
NY	16	57	F	R	17	20	7	3,000
NO	17	45	F	L	13	21	7	3,000
AEK	18	45	M	L	20	17	5	3,000
SG	19	42	F	R	12	18	5	3,000
DA	20	39	M	L	8	20	6	3,000
HO	21	76	M	R	12	20	5	3,000
MAK	22	40	M	L	20	19	5	3,000
RG	23	22	F	R	14	17	4	3,000
OC	24	38	M	L	20	19	7	3,000

Assessment of stone fragmentation

Three urologists (one was out of the study) judged the presence of fragmentation of the stones by examining KUB films taken before and after each SWL session. The absence of scattering and expansion of the stones on the KUB films were indicative of intactness. Accordingly, for this study we classified the renal stones into two groups as "SWL unsuccessful stones" (SWL-US) that were not fragmented, stayed intact after three SWL sessions (no scattering and expansion on KUB) and were removed surgically, and "SWL successful stones" (SWL-S) that were fragmented, at least partially, within the first three SWL session (there was indication of scattering and expansion in any of the first three sessions), and passed spontaneously.

Collection of stones

The patients were asked to collect their urine in a plastic container at the beginning of the treatment till they became stone-free, and the passed stones were recovered by sieving the urine and collecting in sterile plastic bags. All the stones collected during SWL treatment were included in the study. Before storing, the samples were cleaned using distilled water in order to remove any blood clots and urine.

Stone analysis and inclusion criteria

The urinary stones collected from each patient were randomly separated in two equal parts. One part was used to determine the composition of the stone using the X-ray diffraction (Phillips PW 1830-40) method and the other was used to measure the concentration of the elements. The patients having only one COM stone localized in the renal pelvis, showing normal urinary anatomy on IU and having stone size between 5 and 20 mm, were included in this study; patients with body mass index greater than 25 kg/m^2, with solitary kidney and multiple stones in their kidneys, and pediatric patients were excluded from the present study. Based on these criteria, 14 of 25 surgically and 24 of 708 SWL-treated patients had the inclusion criteria. The profiles of patients and renal stones grouped as SWL-US and SWL-S are illustrated in Tables 1 and 2, respectively. The elements analyzed were copper (Cu), zinc (Zn), magnesium (Mg), iron (Fe), lead (Pb), manganese (Mn) and chromium (Cr).

Determination of elements

The elements were determined using an atomic absorption spectrophotometer (Hitachi Polarized Zeeman Effect, 180/170 AAS). The samples were powdered with an agate mortar and pestle and dried; 200 mg of the powder was weighed accurately and digested in the mixture consisting of 6 unit of HNO_3 (Merck) and 1 unit $HClO_4$ (Merck). The digestion was carried out for 1 h in a 70°C water bath and the final digestion solution was adjusted to 50 ml. At the end of the 1 h period, most of the stones ($n = 32$) were completely digested, while six stones in SWL-S were partially digested. These were sieved and the digested solution was adjusted to 50 ml by adding distilled water. All measurements for element content were done using 50 ml digestion solutions. During measurements, all precautions were taken to prevent metal contamination and lab devices having direct contact with the stones were cleaned with 2% HNO_3, rinsed in distilled water and heated to 600°C. During the study, the instrument was calibrated at every ten readings to increase the reliability of the measurements. Burning unit was used to measure Cu, Zn, Mg and Fe content in renal stones and the concentration of these elements was expressed in mg/kg; a more sensitive graphite furnace unit was used to determine the concentration of Pb, Mn, Cr in the stones and element concentration was stated as μg/kg.

Statistical analysis

Statistical analyses were performed using the SPSS 15.0 for Windows Evaluation Version. Data were first checked for normality by Shapiro–Wilk test. The comparison of stone size and applied voltage between SWL-US and SWL-S was done using a *t* test. SWL sessions and element levels of stones were compared by using the Mann–Whitney *U* test.

Results

The SWL-US group contained eight men and six women with a mean age of 47.35 ± 3.92 (range 24–64) years; the SWL-S group consisted of 14 men and 10 women, and their mean age was 41.70 ± 2.49 (range 17–76) years. Mean stone size and SWL parameters (voltage and session) between groups are shown in Table 3. The concentrations of Cu, Zn, Mg, Fe, Pb, Mn and Cr were determined in 14 SWL-US and 24 SWL-S by atomic absorption spectrophotometer (Tables 4, 5). Overall, the concentration of Zn, Mg and Mn were significantly lower in SWL-US ($P < 0.01$, $P < 0.05$, and $P < 0.05$, respectively) than in SWL-S (Fig. 1a–c). By contrast, there was no statistically significant difference between the concentration of Cu, Fe, Pb and Cr in SWL-US and SWL-S (Fig. 2a–d). The quantities of the elements in SWL-US and SWL-S are illustrated in

Table 3 Median SWL sessions, mean voltage and mean stone size in SWL-US and SWL-S groups

	n	Mean stone diameter (mm)	Mean voltage (kV)	Median SWL sessions
SWL-US	14	14.21 ± 1.16 (6–20)	19.86 ± 0.42 (17–24)	3
SWL-S	24	14.75 ± 0.81 (8–20)	19.38 ± 0.33 (17–24)	2 (1–7)
P		0.702^{NS}	0.374^{NS}	0.372^{NS}

NS > 0.05, ± standard error

Table 4 Element concentrations in SWL-US group

	Cu (mg/kg)	Zn (mg/kg)	Mg (mg/kg)	Fe (mg/kg)	Pb (μg/kg)	Mn (μg/kg)	Cr (μg/kg)
1	83.47	131.21	4,775.75	181.81	4.18	3.81	3.60
2	32.23	85.24	3,785.35	92.16	3.16	0.10	0.10
3	29.22	53.07	2,363.68	96.08	3.11	2.96	0.55
4	112.29	126.52	8,856.82	394.56	4.99	0.94	2.38
5	21.94	70.65	7,476.44	51.26	3.17	0.96	0.82
6	58.37	102.53	3,634.92	83.80	9.14	1.19	1.15
7	14.56	43.47	1,211.82	26.57	1.0	0.20	0.35
8	33.74	91.21	4,021.13	55.93	3.40	0.45	1.82
9	47.14	98.54	2,929.90	34.41	7.80	0.76	3.88
10	20.53	38.31	1,731.95	38.08	1.05	0.27	2.22
11	54.97	458.26	6,962.13	143.28	5.59	1.93	8.95
12	16.34	24.95	2,319.69	26.62	1.50	0.34	0.96
13	24.98	62.95	2,582.62	52.56	4.72	0.84	4.10
14	11.04	12.06	1,037.76	42.61	1.19	0.26	1.88

Table 5 Element concentrations in SWL-S group

	Cu (mg/kg)	Zn (mg/kg)	Mg (mg/kg)	Fe (mg/kg)	Pb (μg/kg)	Mn (μg/kg)	Cr (μg/kg)
1	61.77	21.40	3,463.03	21.40	2.40	2.52	4.08
2	62.71	230.60	6,310.39	143.48	15.08	2.19	2.91
3	83.69	352.64	6,245.47	38.01	8.79	1.23	2.38
4	33.75	1,120.50	47,357.59	46.45	9.81	1.50	0.50
5	31.61	84.04	3,962.89	47.25	2.72	0.20	2.28
6	37.42	330.40	6,223.10	115.51	8.32	4.07	5.69
7	32.38	126.91	2,996.16	61.12	3.50	0.65	1.50
8	46.41	155.01	21,632.15	751.38	3.26	13.70	4.56
9	42.83	225.98	4,063.44	84.59	7.85	1.75	0.36
10	56.47	241.17	2,358.82	125.88	2.76	1.82	15.52
11	2.78	556.60	12,301.88	205.66	5.09	0.75	7.30
12	65.14	211.98	4,398.91	78.05	2.01	0.62	4.59
13	197.13	389.05	17,834.02	319.01	4.59	11.68	47.44
14	13.30	91.74	1,935.06	28.99	1.79	0.26	0.37
15	40.90	669.34	14,946.88	89.88	5.28	1.48	0.63
16	50.10	77.99	2,256.43	112.31	3.78	2.58	0.65
17	136.72	69.20	15,346.02	1,366.78	4.84	11.07	8.30
18	71.62	281.51	6,799.71	258.05	5.60	0.98	1.61
19	290.77	171.90	7,088.72	270.79	4.70	2.58	0.45
20	28.81	56.61	7,841.06	333.33	7.17	3.83	16.31
21	23.92	21.52	2,077.75	23.94	4.49	5.49	1.55
22	71.60	406.48	3,946.98	92.04	3.11	0.58	8.37
23	24.97	281.92	6,024.73	44.92	2.77	0.58	2.19
24	24.81	111.78	3,809.59	47.23	2.59	0.84	0.70

Tables 4 and 5, respectively. There was no noteworthy difference in the median Cu concentration between the groups, which was 30.73 mg/kg in SWL-US and 46.41 mg/kg in SWL-S (Fig. 2a). In contrast to Cu, Zn concentration in SWL-US was significantly lower ($P < 0.01$) than in SWL-S and the values were 70.65 and 218.98 mg/kg, respectively (Fig. 1a). There was a large variability in the concentration of Zn among individual stones, both in SWL-US and SWL-S, ranging from 12.16 to 458.26 mg/kg in SWL-US and 21.40–1,120.50 mg/kg in SWL-S. However, the exclusion of the lowest and the highest values in both groups from the analyses did not change the P values (not included). The same observation was also true for the rest of the elements studied here. Mg concentration, comparable to Zn, was significantly lower ($P < 0.05$) in SWL-US than in SWL-S, the values being 3,282.41 and 6,123.91 mg/kg, respectively (Fig. 1b). Iron was another element we looked at in our measurements. Median Fe content was similar ($P > 0.05$) in SWL-US (52.56 mg/kg) and SWL-S (89.88 mg/kg) (Fig. 2b). In addition to Cu and Fe, the median concentration of Pb (3.28 μg/kg) and Cr (1.82 μg/kg) was not considerably different between SWL-US and SWL-S (Fig. 2c, d). Moreover, similar to Zn and Mg, the concentration of Mn was significantly ($P < 0.05$) lower in SWL-US (0.80 μg/kg) than in SWL-S (1.62 μg/kg) (Fig. 1c). Finally, some of the stones, having nearly the same size, did require different numbers of shock waves as well as treatment sessions in order to be disintegrated in the SWL-S group. When these stones were evaluated, although not very consistent, it was apparent that as the concentration of Zn, Mg and Mn increased, the number of SWL sessions required for disintegration appeared to decrease.

Discussion

Although SWL is widely used in clinics for the treatment of patients with urinary stone, who have no complications of acute urinary tract infection, sepsis, pregnancy, bleeding dyscrasia or obstruction distal to the stone, it is ineffective in around 15% of cases [21]. The patient's health status, stone size and composition, and renal anatomy are all critical factors that may interfere with SWL efficiency [7]. For example, patient weight is an important factor for response to SWL treatment. Patients whose body mass index (BMI) is less than 26.9 kg/m^2 are shown to have a better success rate for SWL [22]. We, therefore, excluded patients having BMI greater than 25 kg/m^2 for the current study.

Stone composition appears to affect the success rate of SWL by interfering with stone fragility. Stones composed

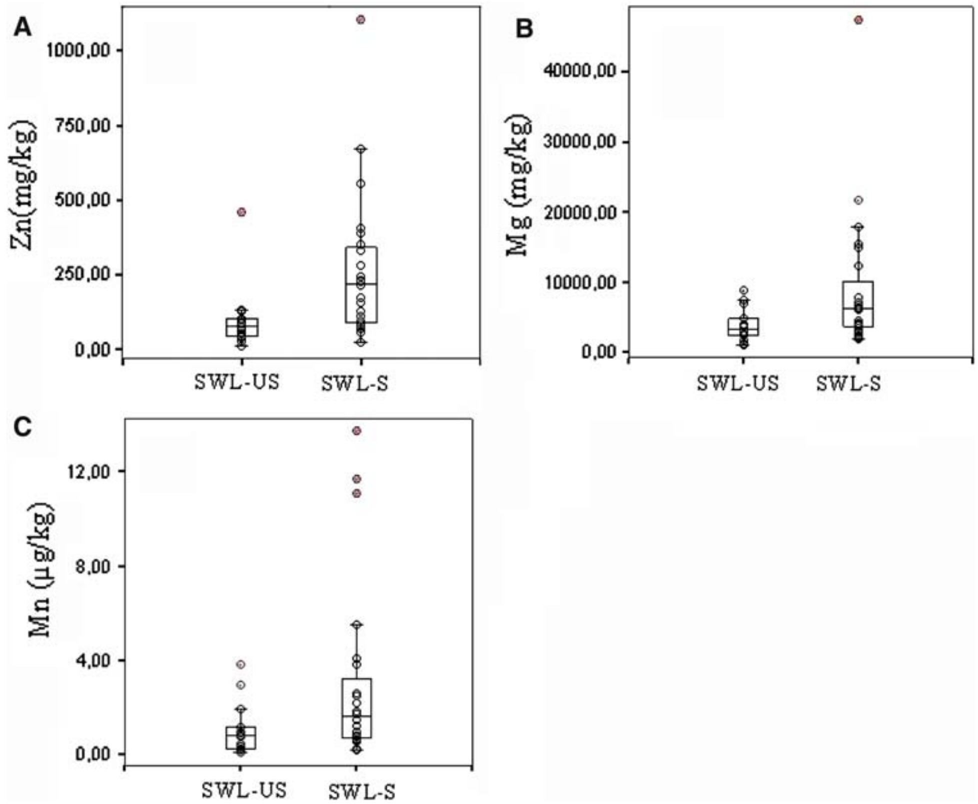

Fig. 1 Zn, Mg, Mn concentrations in SWL-US and SWL-S. **a** Zn concentration, **b** Mg concentration, **c** Mn concentration. Note that Zn, Mg and Mn concentrations in SWL-US are significantly lower ($P = 0.002$, $P = 0.034$, $P = 0.033$, respectively)

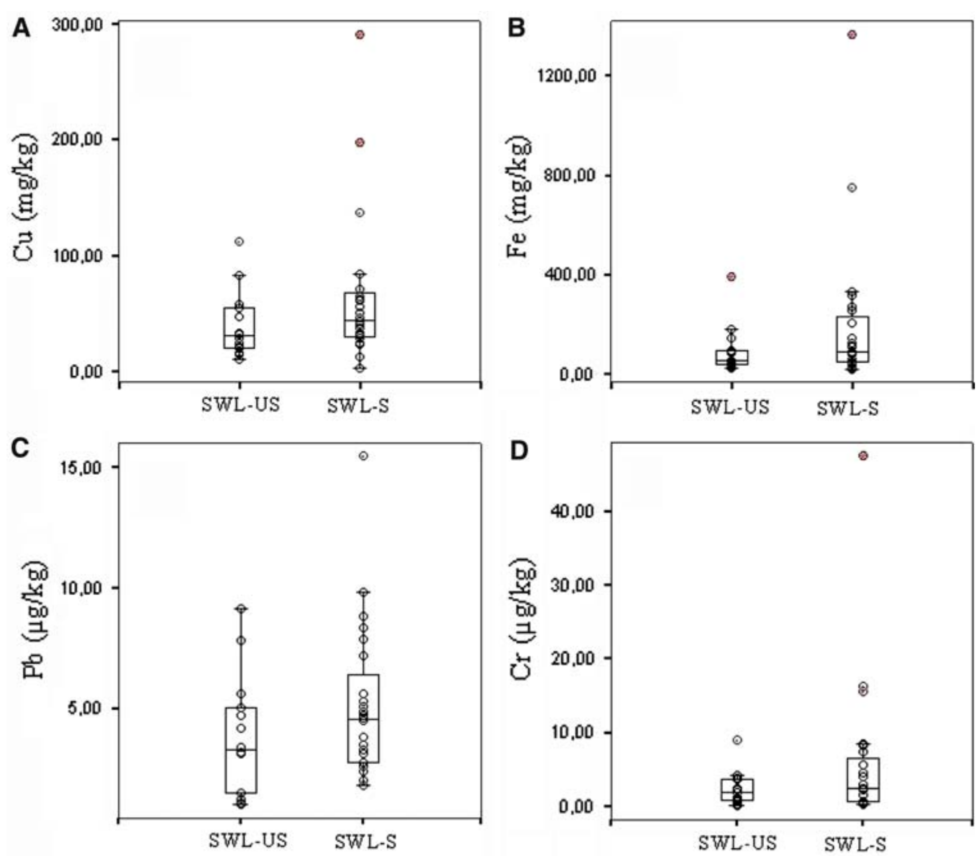

Fig. 2 Cu, Fe, Pb, Cr concentrations in SWL-US and SWL-S. **a** Cu, concentration, **b** Fe concentration, **c** Pb concentration, **d** Cr concentration. Note that Cu, Fe, Pb, and Cr concentrations in SWL-US and SWL-S are similar ($P = 0.074$, $P = 0.172$, $P = 0.256$, $P = 0.157$, respectively)

of COM and cystines have a lower coefficient of fragmentation than those composed of uric acid and calcium oxalate dihydrate [6, 8]. Moreover, COM is one of the hardest inorganic stones to fragment [6]. Why some of the COM stones cannot be fragmented by SWL has not been well understood. Sarica et al. [23] showed that the content of matrix glycosaminoglycan in COM stones is important for the disintegrative effect of high-energy shock waves.

Variations in internal structure of COM stones might be a potential factor affecting the fragility. Some elements appear to interfere with COM stone fragility [24]. In this study, therefore, we planned to determine the contribution, if any, of Mg and various elements such as Cu, Zn, Fe, Pb, Mn and Cr to urinary stone fragility in SWL treatment. When sensitive methods of analysis are used, almost all trace elements are found in urinary stones [17]. Trace element concentrations in urine and urinary stones vary considerably and no regularity exists among the various categories of stones [10]. Nonetheless, a correlation between trace element content in serum and urinary stones occurs in patients with stone [17].

Zn and Cu have received much attention in that they are found in urine and urinary stones at the trace levels. Several studies have reported conflicting results regarding the concentration of Zn and Cu excretion in urine and stones [25, 26]. Some studies indicate that Zn and Cu excreted in the urine are present at high levels, while others show no difference in urinary Zn and Cu excretion between stone formers and healthy controls [10, 16, 17]. Increases in urinary Zn excretion in stone formers are not well established; however, dietary animal protein intake and increased levels of cysteine and histidine amino acids in serum are shown to greatly increase Zn excretion in urine [27, 28]. Moreover, the presence of zinc is identified to flaw Ca-oxalate precipitation and the crystallization process that precedes stone formation [29]. Thus a reduction in Zn concentration of a renal stone might make the stone harder to fragment.

Mg, needed for more than 300 biochemical reactions and normal muscle and nerve function [30, 31], was another element that was significantly reduced in the SWL-US group. Magnesium-deficient animals are shown to be more likely than normal to develop calcium oxalate crystals in their kidneys, making stones more likely. Most trials have shown that supplementing with magnesium and/or vitamin B6 significantly lowers the risk of forming kidney stones [25, 26, 32]. In addition, calcium oxalate stone formers as a group excrete less Mg than normal controls and have a lower urinary Mg to Ca ratio [33]. The present results suggest that a decrease in Mg concentration may also interfere with stone fragility.

Mn, an essential nutrient in all forms of life, was the third trace element that was present at significantly lower levels in the SWL-US group in the present study. Mn concentration in the serum and urine of active stone patients is shown to be lower than that of stone-free patients and healthy people [17]. Contradictory observations yet subsist in the literature regarding the quantity of Mn in calcium oxalate stones. Joost et al. [10] showed the presence of Mn at below 0.5 ppm in all 24 renal stones they had studied. In our study, we were also able to measure Mn in all the COM stones we examined and the presence of Mn in SWL-US patients was significantly lower than in the SWL-S group, indicating that a low level of Mn may interfere with the fragility of urinary stones in SWL therapy. In addition, urinary stone patients are found to have significantly lower Mn, Ni and Li in urine than healthy patients. When patients with a history of urolithiasis were free of stones for 1 year after treatment, no differences in the Mn, Ni and Li between the urine of the patients and the healthy controls were found, indicating the significance of diverse elements in the formation of urinary stones [17].

In vitro studies have shown that Fe has a weak effect on prevention of calcium oxalate stone formation, yet addition of citric acid enhances its inhibitory effect [34]. In our studies, even though Fe quantity was lower in SWL-US than in SWL-S, it was not statistically significant. Cr was the last trace element we measured. Similar to Fe, Cr concentration was lower in SWL-US, but was not statistically consequential. Cr is shown to be effective in the inhibition of renal stone formation at pH 7.4 whether or not citric acid is present; when pH is lowered to 6.0, however, Cr loses its effectiveness under experimental conditions.

Shockwave lithotripsy fragments the renal stones at the lines of the circumferential or radial laminations and at the interfaces of crystals of differing composition [35]. It might be that Zn, Mn and Mg deposit between the interfaces of crystals of differing composition and thereby may create laminations and relatively more brittle lines in stones. It is also possible that in the presence of insufficient concentrations of these elements, crystallization may become more compact and homogeneous, thereby stone breakage gets harder. The role and localization of Zn, Mg and Mn in crystallization and growth of stones remain to be uncovered. Whether SWL-US form in the presence of low Zn, Mg and Mn or these elements are lost during urolithiasis remains a challenge. Would an increase in dietary Zn, Mg and Mn make renal stones more brittle to SWL? All of these fall short of explaining the mechanism and reason for the decrease in concentration of Zn, Mg and Mn in SWL-US. Additional work remains to be done to answer these questions.

Furthermore, evaluation of the stones having nearly the same size but requiring different numbers of shock waves, as well as treatment sessions, in order to be disintegrated in the SWL-S group indicated that as the concentration of Zn, Mg and Mn increased, the number of SWL sessions required for disintegration appeared to decrease. This observation suggests that the concentration of Zn, Mg and Mn is involved in the determination of the number of shock waves as well as treatment sessions required for disintegration.

In conclusion, the present results suggest that the concentration of Zn, Mn, and Mg in stones is likely to impinge on the response to SWL and on the stone fragility.

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