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ORIGINAL PAPER

Treatment of renal calculi by lithotripsy: minimizing short-term shock wave induced renal damage by using antioxidants

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Abstract Treatment with extracorporeal shock wave lithotripsy (ESWL), the preferred method of treating kidney stones <3 cm in size, has been shown to induce silent and often self-limiting acute and chronic lesions in the kidneys and adjacent organs. We conducted a randomized clinical trial to determine whether ESWL produces ischaemia and reperfusion injury in the kidneys and whether oral administration of antioxidants reduces the degree of short-term renal injury in patients treated with ESWL. The study included 120 patients with renal stones (1–3 cm in size) treated with ESWL. The patients were divided into three groups—patients in group A ($n = 39$) served as a control group and were not given any antioxidants; patients in group B ($n = 41$) were given two capsules of antioxidants "Nature Made κ" 2 h before ESWL, and 2 and 8 h after ESWL; and patients in group C ($n = 40$) were given two capsules of the antioxidants 2 and 8 h after ESWL. Double 'J' stents were inserted in patients before treatment with ESWL. Blood and urine samples were obtained from all patients just before the start of treatment with ESWL, and at 2 and 24 h and on 7th and 28th day after ESWL. Serum levels of malondialdehyde (MDA), α-tocopherol, cholesterol, albumin and ascorbic acid, and α-tocopherol/cholesterol ratio were determined. Urinary levels of albumin and β_2 microglobulin were also determined as measures of renal tubular injury. At 24 h after ESWL, patients given antioxidants (groups B + C) had significantly reduced mean serum concentration of MDA ($P < 0.001$); higher levels of serum ascorbic acid ($P < 0.001$) and serum albumin ($P < 0.001$); lower α-tocopherol/cholesterol ratio, lower urinary albumin and β_2 microglobulin levels compared with patients who did not receive antioxidants (group A). These findings suggest that treatment with ESWL generates free radicals through ischaemic/reperfusion injury mechanism, and that oral administration of antioxidant may protect these patients from short term renal injury caused by ESWL.

Keywords Renal calculi · Shock waves · Renal injury · Antioxidants · Humans

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Introduction

Extracorporeal shock wave lithotripsy (ESWL) is the preferred method for treating kidney stones <3 cm in size [1]. Although the procedure is non-invasive, concerns have been recently expressed about its safety [2]. The shock waves have been shown to induce acute and chronic lesions in the kidney and in other organs. Hypertension and loss of renal function have been reported occasionally [3]. ESWL has also

been associated with enlargement of the kidney, subcapsular or perinephric fluid collection or hematoma and decrease in renal function as assessed by radionuclide studies [4]. However, these morphological abnormalities tend to regress and renal function is partially restored over time. In the majority of cases these complications follow a silent clinical course.

While ESWL has been shown to cause acute but reversible decreases in function of the treated kidney, moderate, partially reversible decreases in function of the untreated kidney have also been reported [2]. In contrast, following pyelolithotomy the function of the treated kidney is rapidly improved and there is no effect on the function of the opposite kidney. The mechanism by which ESWL given to one kidney affects the function of the untreated or contralateral kidney is not well known. Two hypotheses have been put forward: (1) that the shock waves induce ischaemia and reperfusion injury with subsequent release of free radicals, (2) that the scatter of the shockwaves administered affect the function of the other kidney and possibly other organs as well [5, 6].

The first hypothesis is supported in part because free radical scavengers like verapamil, allopurinol and nifedipine have been shown to have a protective effect on shock wave induced lesions [7, 8]. However, these drugs have other properties like calcium channel blocking, improvement of renal plasma flow, which may explain why they ameliorate shock wave induced lesions in the kidney. If the correct mechanism for renal damage following treatment of renal calculi by ESWL is via the production of free radicals, it should be possible to reduce or even completely eliminate this complication by the use of antioxidants like acetylcysteine, vitamin A, C and E, that have been shown to prevent oxidative damage to the kidneys, heart and lungs in human and laboratory animals [9, 10]. It is recognized that treatment with ESWL can produce damaging effects in immature kidneys in the paediatric age group [11, 12] and in elderly patients with limited kidney nephron reserve [13]. Furthermore, renal stone disease has been recently associated with high oxidative stress and damage to renal tubular cells [14]. Also, mannitol has been shown to protect renal function in patients receiving ESWL when this is given before ESWL presumably through decreasing the amount of renal injury [15]. However, the underlying mechanism(s) responsible for these observations have not been fully established. The objective of this study was to determine whether ESWL produces ischaemia and reperfusion injury in the kidneys; and can oral administration of antioxidants reduce the degree of renal injury in the short term.

Patients and methods

Patients presenting with 1–3 cm calculi in the renal pelvis were recruited into the study. Double 'J' stent was inserted prior to randomization. Standard "blocked randomization" method [16] was used to allocate subjects into the three treatment groups as illustrated in Fig. 1. All investigators were blinded to the treatment groups of the patients. The purpose of the study was explained to the patients and an informed consent was obtained. Approval was obtained from the local ethics committee prior to the commencement of the clinical trial.

Baseline assessment on day 0

The patients had baseline assessment of renal function (serum creatinine, BUN, K^+, Na^+ and uric acid estimation), liver function test, urine for culture and intravenous urography (IVU) before randomization. All the patients had Tc 99 m MAG3 renogram before commencement of treatment and 3 months after, by which time they had all completed their treatments. All renograms were performed by one nuclear medicine consultant (I. L). Creatinine clearance was measured before commencement and at the end of treatment, by 3 months.

Inclusion criteria

All the patients had normal renal function (serum creatinine <120 μmol/l) and normal blood pressure (BP < 130/90 mmHg) on day 0. They were not on any of the following drugs: steroids, calcium channel blockers—verapamil, nifedipine or allopurinol. Before the final renogram at 3 months, the patients had completed their treatment and the "J" stents removed.

Exclusion criteria

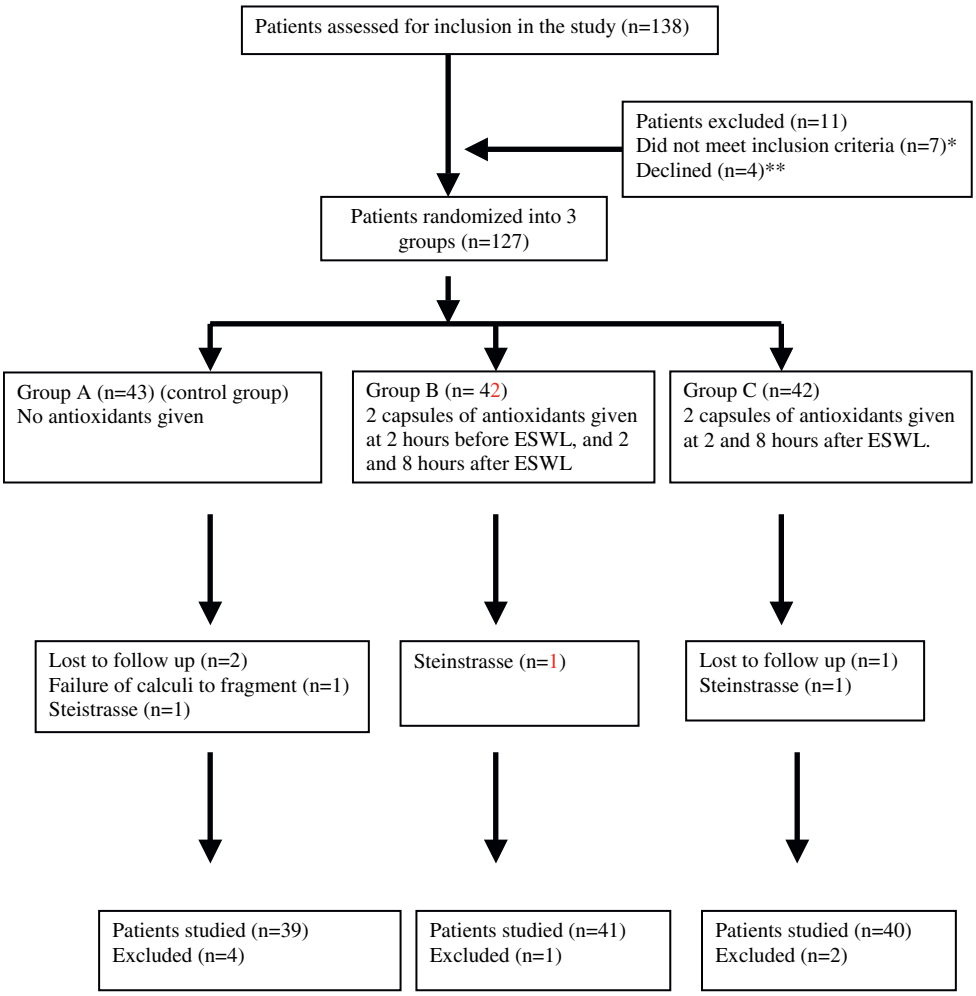
Patients weighing more than 120 kg, and those with coagulopathy, cardiac or spinal anomalies were excluded from the study. Patients with calculi in a kidney with a congenital anomaly were also excluded. Patients who developed post ESWL complications like steinstrasse, sepsis or ureteric obstruction who required further intervention were also excluded from the study.

Treatment groups

Figure 1 shows the different phases of the clinical trial. Before receiving ESWL, the patients were divided into three treatment groups as follows:

1. Group A ($n = 39$): Patients in this group served as a control group and were not given any antioxidant.
2. Group B ($n = 41$): Patients received 2 capsules of "Nature Made ᴿ" antioxidant 2 h before ESWL on the day of treatment, and then 2 and 8 h post ESWL.

Fig. 1 Study flow diagram



*Patients with myocardial infarction on anticoagulants (n=3), weight >120kg (n=2), calculi in kidneys with congenital anomaly (n=2).
**2 patients planned to leave the country before the end of treatment period; 1 patient gave no reason for non participation; 1 patient declined because of work commitments.

3. Group C ($n = 40$): Patients received 2 capsules of "Nature Made ʀ" antioxidant, 2 and 8 h post ESWL. To ensure compliance with drug intake, patients in groups B and C were admitted to the ward for about 24 h.

Blood and urine samples were collected from all patients according to the following schedule: 2 h before ESWL was started (Sample 1), immediately before ESWL (Sample 2), 2 h after completing ESWL (Sample 3), 24 h after completing ESWL (Sample 4), 7 days after completing ESWL (Sample 5) and 28 days after completing ESWL (Sample 6). Blood and urine samples 5 and 6 were taken before ESWL was administered on days 7 and 28, respectively. Ten milliliter of whole blood was taken on each occasion. The serum was separated and stored at −85°C. Analysis of serum samples took place, in batches within 3–6 months of collection.

Using the HPLC method, the serum samples were analyzed for levels of serum Vitamin E (α-tocopherol). Using test kits (Randox Laboratories Ltd, Co., Antrim, UK) serum samples were analysed for malondialdehyde (MDA), ascorbic acid and cholesterol, and α-tocopherol/cholesterol ratio was determined. Serum albumin was determined on the Beckman LX 20 automated analyzer.

The urine samples were refrigerated at −20°C immediately after collection. The following assays were carried out within 7 days of urine collection. Using Randox kits, the urine was analyzed for levels of urinary albumin (evaluates glomerular leakage of proteins). Urine β_2-microglobulin level was also determined with Immulite auto analyzer (DPC, Webster, USA).

ESWL

The authors (HAH and AA) administered ESWL to all the patients. The number of shock waves given was 2,500 SW per session and the energy setting was 16 KV for all

patients using Siemen's LithoStar C machine. The patients had ESWL on a weekly basis until they were completely stone free. No more than 7 ESWL sessions were given to any patient.

Antioxidants used

Each "Nature Made ʀ" antioxidant capsule (Pharmavite Corporation, Mission Hills, CA, USA) contains high levels of the following antioxidants, Vitamin A (as β- carotene) 10,000 i.u., Vitamin C 250 mg, and Vitamin E 200 i.u., and mineral supplements like zinc 7.5 mcg and selenium 15 mcg. The capsule was chosen because it contains more than the daily recommended allowances of most of the antioxidants.

Statistical analysis

All data management and analyses were conducted using the SPSS program. The chi square (χ^2) test was used to assess the significance of association between categorical variables. The *t* test was used to compare the means of two independent groups. Variables with skewed distribution were logarithmically transformed before analysis. Because of the multiplicity of tests, the Bonferroni correction was applied to each set of three tests; hence $P < 0.016$ was considered statistically significant.

Results

Table 1 shows the clinical characteristics of the patients studied. There was no material difference between the groups with respect to the number of males, females randomized to each group. The numbers of patients without any systemic diseases or with mild and controlled DM were approximately similar in the three groups. There were more patients with hypertension (all had normal BP with drug treatment before randomization) in group C compared to

Table 1 Clinical characteristics of patients in the study

Parameter	Group A	Group B	Group C
Total no. of patients initially recruited	43	42	42
Sex			
Male	36	36	37
Female	7	6	5
Medical conditions[a]			
None	33	29	28
Diabetes	7	7	6
Hypertension	3	6	8
Urine culture (Day 0)			
+ve	4	1	2
−ve	39	41	40
Urine culture (Day 28)			
+ve	7	4	5
−ve	36	38	37
	Mean ± SD		
Age (years)	43.9 ± 13.2	41.6 ± 12.1	43.2 ± 10.3
Weight (kg)	77.8 ± 9.1	79.7 ± 9.4	78.6 ± 9.9
Height (cm)	170.2 ± 3.8	170.1 ± 3.4	170.3 ± 3.0
Creatinine (μmol/l) (Day 0)	93.5 ± 21.5	93.7 ± 18.2	93.6 ± 18.0
Na^+ (mmol/l)	140.2 ± 2.0	139 ± 2.4	138.8 ± 2.4
K^+ (mmol/l)	4.3 ± 0.4	4.4 ± 0.5	4.5 ± 0.5
Uric acid (μmol/l)	346.9 ± 81.1	344.7 ± 62.1	348.8 ± 71.7
Stone size (mm)	13.6 ± 3.6	14.0 ± 2.6	14.1 ± 3.3
Stent duration (days)	40.9 ± 13.7	40.1 ± 11.4	44.8 ± 12.4
ESWL sessions (*N*)	4.7 ± 1.5	4.6 ± 1.4	5.5 ± 1.6
No. of patients excluded from analysis on day 28[b]	4	1	2
Adverse events (nausea only)	0	2	1
No. of patients in the final analysis	39	41	40

DM diabetes mellitus, *HTN* hypertension

[a] Patients with DM or HTN were mild cases and well controlled before randomization. None was on any drugs known to affect ischaemia or reperfusion

[b] Due to: loss to follow up × 3, steinstrasse × 3, failure of calculus to fragment × 1

groups A and B. The groups were almost similar for other parameters (i.e., number of patients with infected urine, age, weight, baseline blood biochemistry profile, number of shockwave given). There was no significant difference between the three groups in terms of adverse events, serious adverse events or any specific symptoms. Three patients in group B + C complained of minor gastrointestinal tract symptoms (mostly mild nausea). Overall the antioxidants tablets were well tolerated in groups B and C patients.

Serum MDA

The results of serum MDA levels are shown Table 2 and Fig. 2. Two hours after ESWL was given to the patients, group B patients had lower MDA compared to group A ($P < 0.0001$) and group C ($P < 0.0001$), whereas there was no significant difference between serum MDA levels in groups A and C who did not receive antioxidants tablets (group C patients received the antioxidants after blood sample 3 was taken at 2 h). By 24 h, group B patients had the lowest MDA levels followed by group C patients, while the control group A had the highest MDA levels. By day 7 the MDA levels in the three groups were about the same.

Table 2 Mean ± SD of serum concentrations of MDA in patients receiving ESWL for renal calculi

Time	Group A ($n = 39$)	Group B ($n = 41$)	Group C ($n = 40$)
MDA (nmol/ml)			
−2 h	2.36 ± 1.54	2.36 ± 1.54	2.36 ± 1.54
0	2.36 ± 1.54	2.30 ± 0.61	2.36 ± 1.14
2 h	2.36 ± 1.56[1,2]	2.21 ± 0.45[1,3]	2.41 ± 1.58[2,3]
24 h	2.31 ± 0.94[4,5]	2.06 ± 0.40[4,6]	2.23 ± 1.18[5,6]
Day 7	2.28 ± 0.87	2.28 ± 0.98	2.48 ± 1.15

[1]$P < 0.0001$, [2]$P = 0.47$, [3]$P < 0.0001$, [4]$P < 0.0001$, [5]$P = 0.10$, [6]$P < 0.0001$

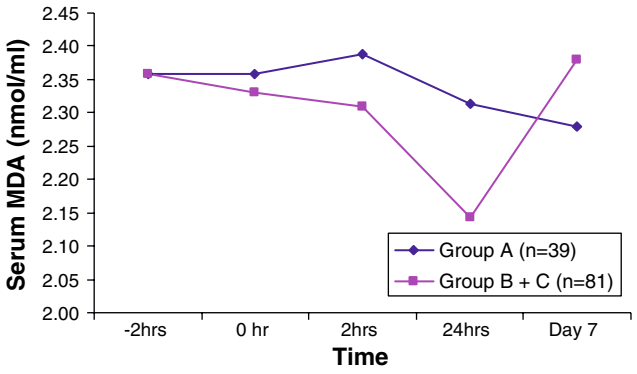


Fig. 2 Mean serum MDA in Group A versus Group B + C patients

Serum ascorbic acid

Table 3 and Fig. 3 show the serum ascorbic acid levels in the three groups of patients. Two hours after oral administration of antioxidants to group B patients the level of serum ascorbic acid rose to 5.69 mg/ml compared to group A 3.20 ng/ml ($P = 0.14$) and group C 2.76 mg/ml ($P = 0.09$). There was no difference in the serum levels of ascorbic acid between groups B versus A and C 2 h after group B received the antioxidants. This may indicate that the amount of antioxidants given was not adequate to affect serum levels of ascorbic acid. At 24 h, the 2 groups given ascorbic acid, i.e., group B and C had higher but statistically insignificant serum ascorbic acid levels (Fig. 3). However, the two groups who received ascorbic acid (i.e., groups B + C) had significantly higher levels of ascorbic acid compared to controls by day 28 ($P < 0.001$).

Serum albumin

The groups given antioxidants had persistently higher serum albumin compared to the control group for the entire duration of the study (Table 4; Fig. 4). The differences were statistically significant on day 7. Whereas, the control group had lower serum albumin level even at day 28. As albumin is a known negative acute phase reactant, the higher levels of albumin in the treated group indicates prolonged protection of body pool by the antioxidants.

α-Tocopherol/cholesterol ratio

Ischaemia/reperfusion injury is associated with decrease in the α-tocopherol/cholesterol ratio. As shown in Table 5 and Fig. 5, group A patients had higher α-tocopherol/cholesterol ratio compared with groups B and C individually or combined. This indicates that the injury caused by ESWL was at least, in part, secondary to ischaemia and reperfusion injury.

Table 3 Mean ± SD of serum concentration of ascorbic acid in patients receiving ESWL for renal calculi

Time	Group A ($n = 39$)	Group B ($n = 41$)	Group C ($n = 40$)
Ascorbic acid (mg/ml)			
−2 h	2.98 ± 1.77	2.98 ± 1.77	2.98 ± 1.77
0	3.20 ± 1.84[1,2]	5.69 ± 2.62[1]	2.76 ± 1.27[2]
2 h	3.03 ± 1.97	5.67 ± 1.52	3.19 ± 1.27
24 h	4.45 ± 2.86[3,4]	5.32 ± 1.42[3]	4.69 ± 2.71[4]
7 days	3.58 ± 1.20	4.52 ± 1.84	3.53 ± 1.81
28 days	2.66 ± 1.21[5,6]	4.90 ± 1.90[5]	4.90 ± 5.00[6]

[1]$P = 0.14$, [2]$P = 0.09$, [3]$P = 0.02$, [4]$P = 0.41$, [5]$P = 0.08$, [6]$P < 0.0001$

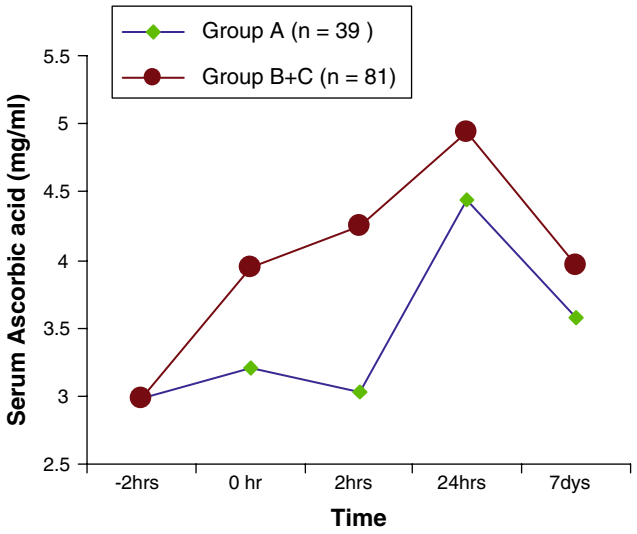


Fig. 3 Mean serum ascorbic acid in Group A versus Group B + C patients

Table 4 Mean ± SD of serum concentrations of albumin in patients receiving ESWL for renal calculi

Time	Group A ($n = 39$)	Group B ($n = 41$)	Group C ($n = 40$)
Albumin (g/l)			
−2 h	40.75 ± 5.66	40.75 ± 5.66	40.75 ± 5.66
0	38.18 ± 10.63	39.32 ± 6.86	43.29 ± 6.66
2 h	37.28 ± 11.51	41.70 ± 6.77	42.43 ± 6.80
24 h	36.61 ± 11.43[1,2]	42.59 ± 8.05[1]	41.57 ± 6.27[2]
7 days	39.09 ± 10.98[3,4]	42.39 ± 5.32[3]	44.86 ± 6.64[4]

[1]$P = 0.04$, [2]$P < 0.003$, [3]$P < 0.0002$, [4]$P = 0.01$

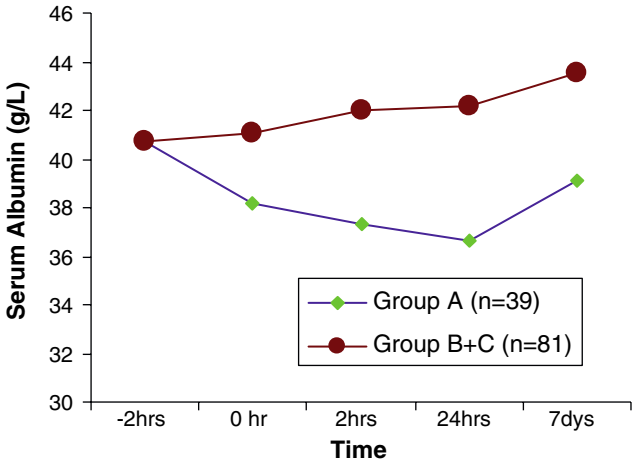


Fig. 4 Mean serum albumin in Group A versus Group B + C patients

Urine albumin

There was increased urinary excretion of albumin in the groups that received antioxidants as opposed to control group as shown in Table 6 and Fig. 6. This was because the group that received the antioxidants had higher serum levels of albumin compared to control group. Hence, albumin being a natural antioxidant was able to protect the pool of antioxidants in serum thereby minimizing the damaging effects of ESWL. The protective effect of the antioxidants was best seen at 24 h when urinary albumin level in group

Table 5 Mean ± SD of serum α-tocopherol/cholesterol ratio in patients receiving ESWL for renal calculi

Time	Group A ($n = 39$)	Group B ($n = 41$)	Group C ($n = 40$)
α-tocopherol/cholesterol ratio			
−2 h	0.0230 ± 0.016	0.0230 ± 0.016	0.0230 ± 0.016
0	0.023 ± 0.016[1,2]	0.016 ± 0.011[1]	0.023 ± 0.036[2]
2 h	0.021 ± 0.015[3,4]	0.015 ± 0.012[3]	0.012 ± 0.030[4]
24 h	0.017 ± 0.011	0.015 ± 0.014	0.013 ± 0.019
7 days	0.024 ± 0.014[5,6]	0.022 ± 0.019[5]	0.013 ± 0.007[6]

[1]$P = 0.08$, [2]$P < 0.0003$, [3]$P = 0.27$, [4]$P < 0.002$, [5]$P = 0.1247$, [6]$P < 0.002$

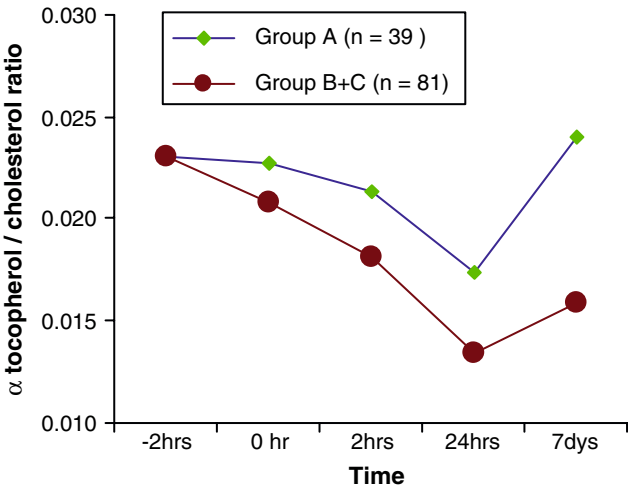


Fig. 5 Mean serum α-tocopherol/cholesterol ratio in Group A versus Group B + C patients

Table 6 Mean ± SD of urine concentrations of albumin in patients receiving ESWL for renal calculi

Time	Group A ($n = 39$)	Group B ($n = 41$)	Group C ($n = 40$)
Albumin (g/l)			
−2 h	1.27 ± 0.9	1.27 ± 0.9	1.27 ± 0.9
0	0.90 ± 0.73	1.20 ± 1.26	1.64 ± 1.40
Immediate[a]	1.59 ± 1.69[1,2]	3.01 ± 4.52[1]	2.28 ± 2.29[2]
2 h	1.47 ± 1.59[3,4]	1.82 ± 3.27[3]	2.28 ± 2.50[4]
24 h	0.77 ± 0.84[5,6]	1.23 ± 1.77[5]	1.64 ± 2.46[6]
7 days	0.72 ± 0.68[7,8]	0.68 ± 1.14[7]	0.96 ± 0.89[8]

[a] Urine sample taken immediately after completion of ESWL

[1]$P < 0.0001$, [2]$P < 0.06$, [3]$P < 0.0001$, [4]$P = 0.01$, [5]$P < 0.0001$, [6]$P < 0.0001$, [7]$P < 0.003$, [8]$P = 0.087$

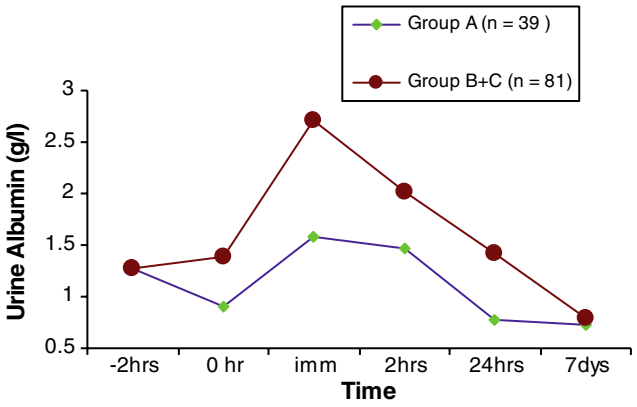


Fig. 6 Mean urine albumin in Group A versus Group B + C patients

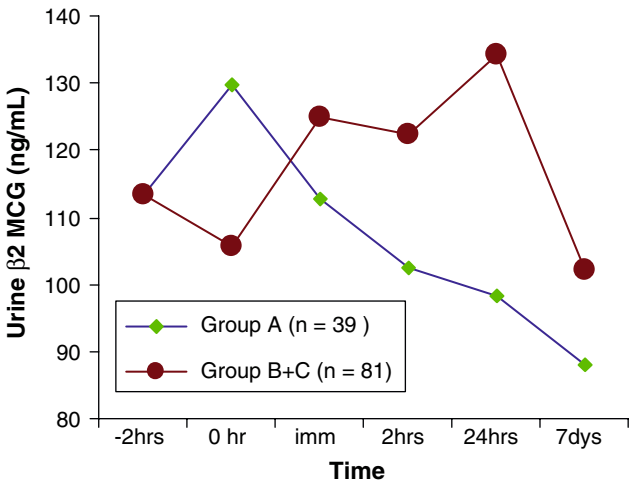


Fig. 7 Mean urine β-microglobulin levels (βMCG) in Group A versus Group B + C patients

A patients was 0.77 g/l, compared to group B 1.23 gm/l ($P < 0.001$) and group C 1.64 gm/l ($P < 0.001$).

Urine β_2 microglobulin

There was increased urinary excretion of β_2 microglobulin after ESWL in all three groups of patients (Table 7, Fig. 7). The excretion rate was higher in the treated groups (B + C) compared to the control group. By day 7 and 28, the urinary excretion rate of β_2 microglobulin had returned to about baseline values for all groups of patients (i.e., treated versus control).

Discussion

Shock-wave lithotripsy is considered safe and effective for fragmenting urinary tract calculi. However, recently an increasing number of investigators have questioned the adverse effect of this technique. Clinical studies have shown that hematuria and postoperative pain or discomfort frequently occurs in almost all patients undergoing shock-wave treatment. The procedure is also demonstrated to cause microtrauma or hemorrhage in normal kidney. Varying degrees of perirenal and intrarenal injuries have been reported [17]. The renal parenchymal injury secondary to shock-wave treatment has been well characterized by the appearance of urinary marker proteins and enzymes, such as β-microglobulin and *n*-acetyl-beta-*o*-glucosaminidase (NAG) [15, 18]. Initially the adverse effects of ESWL were attributed to renal damage resulting only from the direct action of shock-wave energy, which has been known to produce gross areas of hematomas with subsequent cortical fibrosis. The mechanism of tissue injury was believed to be mechanical trauma to renal vasculature and tubules through action of cavitation bubbles or shear stress [19, 20]. More recently free radical formation was considered to be an integral element in shock-wave induced renal damage through an indirect mechanism. In in vivo experiments, vascular injuries caused by the direct action of shock waves could induce areas of tissue ischemia and hypoxia, which becomes more susceptible to free radical production as reperfusion occurs. Metabolic alterations caused by ischemia with reperfusion can result in abnormally high levels of free radicals [21, 22]. Thus free radical formation and subsequent damage to the kidney during the procedure seem to be in the same manner as in the ischemia reperfusion models [21–23]. The toxicity of free radicals is attributed to their ability to initiate lipid peroxidation of cellular membranes. Following alteration of cellular membrane integrity, the cellular equilibrium is lost and cell death typically ensues [24]. It is difficult to detect free radical species because of their volatile and transient nature. However the presence of the free radicals can be measured from the elevated lipid perxidation products or antioxidant consumption under such conditions. MDA is believed to be a reliable free radical marker of free radical-mediated lipid peroxidation. As the breakdown products of cellular lipids, MDA could

Table 7 Mean ± SD of urinary β-microglobulin levels in patients receiving ESWL for renal calculi

Time	Group A ($n = 39$)	Group B ($n = 40$)	Group C ($n = 41$)
β-microglobulin (urine) (ng/ml)			
−2 h	113.39 ± 87.21	113.39 ± 87.21	113.39 ± 87.21
0	129.83 ± 167.72	113.85 ± 143.38	96.95 ± 107.21
Immediate[a]	112.70 ± 118.00[1,2]	144.29 ± 137.93[1]	101.36 ± 83.33[2]
2 h	102.49 ± 87.96[3,4]	132.97 ± 133.48[3]	110.49 ± 105.38[4]
24 h	98.36 ± 137.72[5,6]	141.33 ± 185.92[5]	126.94 ± 157.76[6]
7 days	88.16 ± 92.63[7,8]	99.09 ± 113.05[7]	105.27 ± 129.50 [8]

[a] Urine sample taken immediately after completing of ESWL

[1] $P = 0.16$, [2] $P = 0.02$, [3] $P < 0.005$, [4] $P = 0.13$, [5] $P = 0.03$, [6] $P = 0.20$, [7] $P = 0.11$, [8] $P = 0.02$

reflect the degree of tissue oxidative injuries [25]. Enzymes such as SOD, catalase and glutathione peroxidase can functionally protect against the toxic effects of free radicals. Substrates such as Vitamin C, tocopherol, albumin, haptoglobulin can also protect against the toxic effects of free radicals [10]. These substances are collectively called natural antioxidants [26–29]. At present, there is no ideal assay to assess accurately the effect of free radical damage [27, 28]. However, Young et al. [29] have shown that more than one independent assay system be used as different assay systems complement each other. Moreover, doing this will most likely produce scientifically correct results and valid conclusions. Most workers in this field agree that the combination of assays that truly reflect the extent of free radical damage in an ischaemic/reperfusion scenario should include: (a) measurements that reflect free radical damage to macromolecules—being a more direct measurement of activity such as lipid perioxidation or its product malondialdehyde (MDA), (b) measurement of total antioxidant status and (c) measurement of a few individual antioxidants like α-tocopherol, retinol, ascorbic acid, albumin etc [27–29]. Classical I/R injury produces a rise in plasma lipid peroxides, a fall in total antioxidant capacity, a decrease in α-tocopherol/cholesterol ratio and a decrease in antioxidant levels [28, 29]. Recently, Krambeck et al. [19], reported that diabetes melitus and hypertension were associated with ESWL treatment of renal and proximal ureteral stones after 19 years follow up. It would therefore appear that renal calculi treated by ESWL may not only produce short-term reversible renal injuries, but may be associated with long-term sequelae, thus the need to continue the search to make the use of ESWL as safe as possible.

In this study, serum MDA, ascorbic acid, albumin and changes in α-tocopherol/cholesterol ratio as well as urinary excretion of markers of tubular dysfunction like urinary albumin and β2 microglobulin were used to evaluate ESWL induced oxidative stress in the subjects. The results demonstrated that ESWL could increase the level of serum MDA and exhaust serum ascorbic acid. Treatment with "Nature Made R" antioxidants resulted in significant reduction in serum MDA level and elevation of ascorbic acid level. These observations confirm previous in vivo and ex vivo data suggesting that apart from morphological damage, ESWL treated kidneys also suffered from ischaemic/reperfusion injury [7, 30–32]. Similarly, the changes produced by the antioxidants in the levels of urinary albumin and β microglobulin excretion can be attributable to the protecting effects of the antioxidants on the treated groups (B + C) compared to the untreated group (A). These findings in our study are consistent with previous reports, reporting the ameliorating effects of various antioxidants on renal damage induced by ESWL. Some of these antioxidants are vitamins [30], selenium [31], astragalosides [32] and others. Other agents like melatonin [33] mannitol [15] and verapamil [7] have also been shown to have protective effect against shock wave induced renal oxidative stress. These latter agents work by improving the blood flow (verapamil) or encouraging excretion of toxic agents (mannitol) after ESWL. From a practical point some of these agents that have been shown to protect against shockwave induced renal damage by whatever mechanism are difficult to incorporate into clinical practice. For example, mannitol usage will require an i.v. line infusion. Verapamil is an antihypertensive agent and may therefore cause hypotension when used in normotensive patients. The main advantage of the "Nature Made R" antioxidant capsule is that it contains vitamins A, B, and C, zinc, copper, manganese and selenium in levels that are not toxic, but effective when used to prevent renal injury post ESWL (as shown in our study). The patients can be instructed to take the tablets before reporting for ESWL. From our data, it would appear that "Nature Made R" capsule antioxidants will have maximal beneficial effect when taken about 2 h prior to the start of ESWL. Even then taking it after ESWL would appear to still confer some protective effect as the renal injury that occurs after ESWL still produces measurable side effects up to 4 weeks after initiating the treatment, as our data showed. The correct dose of the drug to be used is yet to be determined. Further studies are warranted to resolve whether more than 2 capsules taken before ESWL will provide more effective protection than the 2 capsules that we used in this study.

Ascorbic acid is a potent free radical scavenger that protects unsaturated fatty acids in cell membranes from attack by peroxides. Since patients given the antioxidants have higher serum ascorbic acid levels compared to control ($P < 0.001$), this indicates that the capsule is an effective antioxidant as it helps to maintain a high ascorbic acid level in those taking the capsules. Similarly, patients given antioxidants had lower serum MDA levels, indicating protection against ischaemic/reperfusion injury by the antioxidants. These findings suggest that ESWL generates free radicals through ischaemic/reperfusion injury mechanism and the use of antioxidants is associated with significant reduction in the severity of the injury. Renoprotection must be considered in groups where further deterioration of renal function may be a sequel of treatment of renal calculi by ESWL. The high risk groups include children (immature kidneys), patients with solitary kidney, those with pre-ESWL significant renal dysfunction and those whose renal calculi have recurred in a previously treated kidney requiring further ESWL for recurrent calculi in the same kidney [7, 33, 34]. More research on whether antioxidants will reduce the incidence of recurrence of urolithiasis in known stone formers are warranted. A phase II study is also warranted to determine whether the oral administration of

"Nature Made R" before the weekly ESWL sessions will provide better protection for the kidneys compared to the schedule of antioxidant intake used in this study.

Conclusion

In the light of our findings and the results reported in the literature, we believe that production of free radicals and the subsequent tissue damage is one of the contributing factors to shock wave induced renal parenchymal injury. In our study, we have demonstrated the potential protective effect of oral "Nature Made R" antioxidants on the shock wave induced oxidative stress in human kidneys. Further investigations are needed to determine the dose–response relationship between the damaging effects of ESWL and prior administration of antioxidants.

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