

URINARY AND SERUM CYTOKINE LEVELS IN PATIENTS UNDERGOING SWL

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ABSTRACT

Cytokines may have a role as non-invasive markers of renal damage and inflammation. In this study, we aimed to evaluate urinary and serum cytokine levels in patients undergoing shock wave lithotripsy (SWL). Twenty-one patients (5 females, 16 males), with the mean age 42 (range: 32-63), were enrolled in this study. None of them had any additional systemic diseases. Routine urine examination and urine culture were obtained to exclude urinary infection. Two hours after the SWL application, urine and serum samples were obtained. Urine and serum cytokine levels of Interleukin-1 β (IL-1 β), Interleukin-6 (IL-6) and Tumor Necrosis Factor- α (TNF- α) were measured by IMMULATE hormone analyzer via chemiluminescent immunometric assay using BIODPC (Los Angeles, USA) before and 2 hours after SWL. Mean values \pm SEM (pg/ml) for IL-1 β , IL-6 and TNF- α in urine were 21.14 ± 9.10 , 20.18 ± 4.10 and 9.43 ± 1.02 , respectively before SWL, while 24.00 ± 7.22 , 26.01 ± 4.74 and 10.07 ± 1.52 , respectively after SWL. Mean serum values \pm SEM (pg/ml) for IL-1 β , IL-6 and TNF- α were 5.30 ± 0.30 , 15.66 ± 7.02 and 10.02 ± 3.84 , respectively before SWL and 4.94 ± 0.05 , 8.54 ± 1.13 and 6.54 ± 1.74 after SWL. No statistically significant difference was observed in serum and urine cytokine levels before and after SWL. SWL does not seem to cause an inflammatory response detectable with IL-1 β , IL-6 and TNF- α . However, further studies are needed to get more accurate results.

Key words: kidney; lithiasis; lithotripsy; urolithiasis; cytokine
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INTRODUCTION

Urolithiasis is one of the most common urological diseases with various modalities for its treatment. One of these modalities is shock-wave lithotripsy (SWL), which is preferred by the urologist and patients due to its low morbidity and high treatment success (1). Although it is mostly non-invasive in nature, shock waves are reported to cause acute and rarely chronic damage in the kidneys and other organs (2).

Some markers including proinflammatory cytokines are gaining importance in urological practice (3-5). Cytokines are a group of peptides that regulates the humoral and cellular components of immune system and in vivo inflammatory responses. Interleukin-6 (IL-6) is an inducer of activation and

differentiation of B and T cells during inflammatory responses. It also activates the vascular endothelium in the process of inflammation (6).

Tumor Necrosis Factor- α (TNF- α) is produced by many cells in vivo. Increased and prolonged release of TNF- α is harmful and causes inflammation and tissue damage (7). IL-6 and TNF- α in urine can be used as a marker to predict renal paranchymal damage (8).

Another proinflammatory cytokine, interleukin-1 (IL-1) is a prototype of proinflammatory cytokines. Similar to TNF- α , IL-1 can affect any kind of cell (9).

In our study, we investigated the acute injurious effects of SWL on the renal parenchyma by using proinflammatory cytokines as markers.

Table 1 - Cytokine levels in the urine before and after SWL (\pm SEM)

SWL	IL-1 β (p = 0.460)	IL-6 (p = 0.136)	TNF- α (p = 0.763)
Before	21.14 \pm 9.10	20.18 \pm 4.10	9.43 \pm 1.02
After	24.00 \pm 7.22	26.01 \pm 4.74	10.07 \pm 1.52

SEM: standard error of mean

MATERIAL AND METHODS

Twenty-one patients (5 females, 16 males), with the mean age 42 (range: 32-63), were enrolled in this study. None of the patients received any previous treatment for stones, nor did they have any additional systemic diseases. Patients on immunosuppressive agents were excluded from study. Routine urine examination and urine culture, before and after SWL, were obtained to exclude urinary infection. The patients did not have an indwelling stent. The SWL procedure was undertaken at the lithotripsy unit of Aydın State Hospital via electrohydraulic bathless lithotripter of Elmed (1001, Turkey). Approximately 3000-4000 shock waves at the range of 8 - 20 kilovolt were applied to every individual. Fentanyl (1.5 μ g/kg) was used for the anesthesia and cephalosporine (1 g) for prophylaxis. Before and two hours after SWL, urine and serum samples were obtained to evaluate the acute effects of the procedure.

The blood samples were centrifuged as soon as possible at 4000 rpm for 10 minutes at 4°C. The serum samples were divided into aliquots and stored at -85°C for the assessment performed in weekly intervals. The same process was applied to urine samples.

Cytokine concentrations were measured using the commercial BIODPC (Products Corporation, Los Angeles, CA, USA) kit (cat. No: LKL11 for IL-1 β ; cat no: LK6P1 for IL-6; cat no: LKNF1 for TNF- α)

by IMMULATE hormone autoanalyzer via chemiluminescent immunometric assay.

The reference value for IL-1 β was < 5.0 pg/ml for healthy controls. The reference range for IL-6 was nondetectable to 5.4 pg/ml for healthy controls. The reference range for TNF- α was nondetectable to 8.1 pg/ml for healthy controls. To note date, 99% of samples yielded results that were nondetectable in given procedure. 500 μ l sample was put into sample cuvettes for IL-1 β , IL-6 and TNF- α determination. Approximately, 100 μ l sample was taken by probe automatically for cytokine determination.

Statistical Analysis

Wilcoxon test was used for the statistical analyses, via the software of SPSS.

RESULTS

We found no significant differences between the cytokine levels in the urine samples taken before and two hours after SWL (p > 0.05) (Table-1). Some changes in serum cytokine levels were observed, but they were not significant (Table-2) (p > 0.05).

No evidence of infection before and after SWL in the urine examination and cultures were found. Macroscopic hematuria was observed in 6 patients while in the remaining 15 patients microscopic hematuria was detected.

Table 2 - Cytokine levels in the serum before and after SWL (\pm SEM).

SWL	IL-1 β (p = 0.180)	IL-6 (p = 0.535)	TNF- α (p = 0.310)
Before	5.30 \pm 0.30	15.66 \pm 7.02	10.02 \pm 3.84
After	4.94 \pm 0.05	8.54 \pm 1.13	6.54 \pm 1.74

SEM: standard error of mean

DISCUSSION

Numerous studies have examined the effects of SWL on renal tubular and glomerular cells. Gilbert and associates demonstrated reversible, nephrotic-range proteinuria in patients after electrohydraulic shock wave lithotripsy (10). Reversible changes in urinary levels of substances such as N- β -acetylglucosaminidase, beta galactosidase, gamma-glutamyl/transferase, creatinine phosphokinase, lactate dehydrogenase and alpha₂ macroglobulin have also been demonstrated in both animal and human studies (1,2,11). Such substances may eventually serve as markers that allow the determination of optimal treatment parameters to minimize the possibility of renal damage after SWL.

Urine and serum levels of proinflammatory cytokines became important markers in the evaluation of urological disorders recently (3). Epithelial cells form a barrier between submucosa and environment and release the proinflammatory cytokines and other response molecules to activate the protective mechanisms against the harmful agents like bacteria and toxins. Activated epithelial cells interact also with other cellular elements in the submucosa and at distant locations (12). The lack of correlation between urine and serum levels of cytokines indicates that these cytokines are produced locally (13).

Various localizations of human urinary system were stained in favor of evidence for cytokines. There are differences in the cytokine staining between conditions with and without pathology of the urinary tract epithelium (14). Epithelial cells of bladder and kidney can produce IL-6 as a response to external stimuli (15). It is also a product of inflammatory cells like mononuclear phagocytes, mast cells and lymphocytes. On the other hand, IL-6 is a cytokine that is expressed in epithelial and smooth muscle cells, endothelium and fibroblasts of the normal bladder, as well. Due to its widespread cellular sources, IL-6 expression increases in various conditions and thus it can serve as a useful marker (16).

Elevation of IL-6 in urine has been detected in interstitial cystitis, mesangial glomerulonephritis and urinary infection (13,16). A close correlation has been observed between pain severity and IL-6 level in

interstitial cystitis. Although IL-6 levels in urine samples obtained from ureters were similar, IL-6 levels in bladder samples were different depending on the severity of the disease. These results strongly suggest the bladder to be the production-site of IL-6 in interstitial cystitis (16). In another study, Rhee has showed that urinary IL-6 level increases in urolithiasis independently, which can be explained by stone irritation (18). Also serum IL-6 level increases in acute bacterial infections and probably accompanied by fever (19). Urinary IL-6 elevation in urolithiasis may help to differentiate from bacterial cystitis. While IL-1 α , IL-1 β and IL-6 are increased in bacterial cystitis, only IL-6 among them displays an increase in urolithiasis. IL-6 and TNF- α elevations were also detected in patients with microhematuria (20).

IL-1 β , like TNF, affects almost all types of cells (9). Significant elevations in IL-1 β and α levels were observed in patients with bacterial cystitis and microscopic hematuria (17). Correlation between pyuria and IL-1 β was more prominent (17,21). It was thought that peripheral monocytes infiltrating the kidney were the main source of TNF. However, recent evidence indicates that glomerular mesangial cells are important sources of TNF (22). So, elevated TNF levels can be expected due to urolithiasis and shock waves of SWL.

We found elevated cytokine levels in urine samples after SWL, but this elevation was not statistically significant.

Igarashi et al. evaluated the serum IL-6 level one day after different urological operations. They found the increase of serum IL-6 level in minimally invasive surgery like endourology, laparoscopy and SWL to be lower than that in the open surgery (4). Other studies have shown that the maximum levels of urinary cytokines were observed 2 to 8 hours after bacillus Calmette-Guerin therapy for bladder cancer. The cytokine levels returned to baseline values within 24 hours (23,24). Plasma TNF level increased within 1 hour and then returned to baseline within 3 hours after endotoxin administration (25). Also the plasma concentration of IL-6 showed an increase in 2 to 4 hours following intravenous endotoxin (26). IL-1 was detected in 60 min and high levels occurred in 3 hours following lipopolysaccharide stimulation of monocytes (27). Peak levels of IL-1 β were also observed at 3 hours

during experimental endotoxemia (28). Based on this data, in our study the cytokine levels were measured only at the second hour after SWL.

Changes in urinary cytokines had not been investigated previously in patients undergoing SWL. In our study, we performed a single application of SWL for each patient. The total number of shock waves (4000 shock waves) and total energy for each patient were the same except two patients who had pain and thus the number of shock waves was reduced to 3000. After every 500 shock waves, energy was increased by 2 kilovolts (8-20 kilovolts).

We found no significant differences between the cytokine levels in urine samples taken before and two hours after SWL. In urine samples obtained after SWL, cytokine levels were slightly elevated, but this was not statistically significant. This slight increase after SWL may be the result of shock waves or the irritation caused by the stone itself. This finding is consistent with the Rhee's study. Some changes in serum cytokine levels were observed, but they were not remarkable, either. There was no concordance between serum and urine cytokine levels. This finding supports that cytokines are produced locally.

The lack of the significance in the increase of plasma IL-1, TNF- α and IL-6 levels may be due to the sampling method, which collect urine and serum once at a specific time. Further studies that measure the cytokine levels at various intervals after the SWL treatment may help to investigate the association between cytokine levels and SWL treatment.

CONCLUSIONS

These results did not support a definitive role of proinflammatory cytokines in the evaluation of effects of SWL on kidneys. We conclude that more elaborated studies should be designed to get more accurate results.

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