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ORIGINAL PAPER

Evaluating ESWL-induced renal injury based on urinary TNF-α, IL-1α, and IL-6 levels

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Abstract Extracorporeal shockwave lithotripsy (ESWL) has dramatically changed the treatment of urinary lithiasis and has been the first treatment option for the majority of patients for more than two decades. Despite its significant benefits, it induces acute renal injury that extends from the papilla to the outer cortex. We evaluated the severity of the inflammatory response to ESWL by measuring the urinary excretion of the cytokines TNF-α, IL-1α, and IL-6. The study included 21 selected patients and 14 control subjects. All patients underwent the same ESWL procedure (2,500 shockwaves at 100 shockwaves/min and 0.039 J from the lithotripter). Urine TNF-α, IL-1α, and IL-6 levels were measured using standard ELISA kits. In the study population (patients and controls), we did not detect TNF-α in the urine samples. The levels of both IL-1α (2.5 pg/ml) and IL-6 (3.8 pg/ml) measured before ESWL were not significantly different from the control group (2.5 and 5.2 pg/ml, respectively; $p > 0.05$). Twenty-four hours after ESWL, in contrast to IL-1α (4 pg/ml), urine IL-6 (19.7 pg/ml) increased significantly ($p < 0.05$). Fourteen days after ESWL, IL-1α increased to 5 pg/ml, while IL-6 (7 pg/ml) decreased to the control level. Urine cytokine levels may be used to evaluate the inflammatory response to ESWL. After ESWL, IL-6 levels increased in the early phase, while IL-1α levels increased later. These two markers may be used to measure the severity of inflammation. In contrast to IL-1α and IL-6, urine TNF-α excretion was not increased by ESWL. We believe that the inflammatory response to ESWL can be detected by the urinary excretion of IL-1α for up to 14 days.

Keywords ESWL · Renal injury · TNF-α · Interleukin-1α · Interleukin-6

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Introduction

Although the main target of the shockwaves applied during an extracorporeal shockwave lithotripsy (ESWL) procedure is stones located in the kidney, the surrounding tissues and other organs are traumatised during the intervention. Despite being a non-invasive treatment method, the shockwaves induce tissue injury that impairs renal function in most patients. Consequently, the effects of shockwaves on renal morphology and function must be elucidated [1, 2]. There are two main traumatic effects of shockwaves on renal tissue: traumatic vascular injury induced by the physical force of the waves and ischaemic injury caused by renal vasoconstriction, and intraparenchymal bleeding [3, 4]. In addition, an inflammatory response known as lithotripsy nephritis may develop at the sites of vascular injury [5]. The severity and duration of inflammation may be accepted as a definitive factor of ESWL-induced renal injury.

Cytokines are multifunctional peptides used by cells for intercellular communication and controlling environment which they operate [6]. They are produced by many cell types that have important roles in the inflammation, healing

and systemic response to injury. Some cytokines such as IL-1, TNF-α and IL-6 which promote inflammation, make disease worse are called proinflammatory cytokines [7]. Elevated levels of proinflammatory cytokines can be accepted as a signal of inflammation process. IL-1 plays a primary and central role in the inflammatory response and tissue repair. At low concentration, it acts as a mediator of the local immunoinflammatory reaction, whereas at high concentration it diffuses into the blood, inducing fever and increases the synthesis of acute-phase proteins. IL-6 is the principal inducer of acute phase proteins in the liver and stimulates the mesangial proliferation in kidney. In endothelial cells, it also increases the secretion of TNF-α and IL-1. TNF-α is a major inducer of acute phase proteins and important link between immune and inflammatory reaction. It mediates the induction of adhesion molecules and other cytokines such as IL-1 and IL-6. In common IL-1, TNF-α and IL-6 are endogenous pyrogens which induce fever, stimulate acute-phase proteins and promote inflammation [6, 8]. These three cytokines can be used to evaluate the inflammatory response for a long period.

In this study, we evaluated the inflammatory response to ESWL by monitoring the urinary excretion level of the cytokines TNF-α, IL-1-α, and IL-6 for up to 14 days.

Materials and methods

Patient population

The study protocol was approved by Kartal Training and Research Hospital Ethical Committee and informed consent was obtained from each subjects. The study population consisted 21 patients and 14 control subjects with similar age. The study population characteristics are summarised in Table 1. The enrolled subjects had no history of hypertension, diabetes, hypercholesterolaemia, or any other chronic disease. By performing intravenous pyelography (IVP) or contrast enhanced spiral computed tomography at the baseline of the study, we confirmed that the patient had a functional and non-obstructed urinary tract before the SWL treatment. Patients with infections and/or obstructions, as well as the ones demonstrating minor or major complication during treatment (which may in turn affect urinary cytokine levels) were excluded from the study (Fig. 1).

Table 1 Characteristics of study population

Characteristics	Patients ($n = 21$)	Control subjects ($n = 14$)
Age, years (mean, range)	41.1 (24–47)	39.3 (28–48)
Male/female (n)	17/4	6/8
Stone size (mean, range; mm)	11.4 (10–14)	–
Location		
Left kidney	12	–
Mid calyx	8	–
Inferior calyx	4	–
Right kidney	9	–
Mid calyx	7	–
Inferior calyx	2	–
Thickness of the parenchymal tissue around stone (mean, range; mm)	1.3 (1–2.5)	–

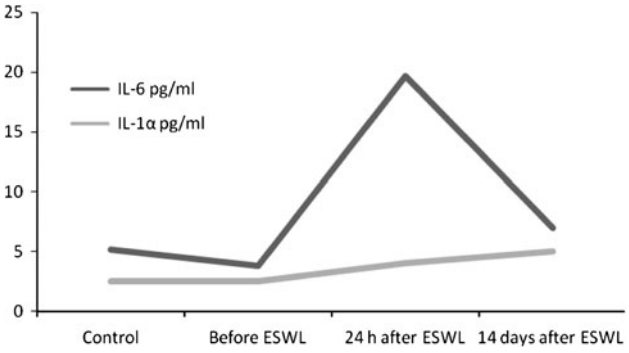

Fig. 1 Urinary IL-6 and IL-1α levels before and after ESWL

All patients underwent the same ESWL and medication procedures during the study period. Briefly, all patients were given 2,500 shockwaves at 100 shockwaves/min and 0.039 J from the lithotripter (Dornier Compact Sigma, Med Tech, Kennesaw, GA, USA). Before ESWL, all patients were given a single dose (75 mg) of diclofenac sodium and after ESWL they received 500 mg of paracetamol three times daily for 5 days.

Methods

Urine samples were obtained from the patients and control subjects. Morning mid-urine samples were collected from the patients before ESWL and at 24 h and 14 days after ESWL. The samples were stored at −80°C until analyzed. All samples were processed within 1 month.

Biochemical assays

The urine IL-1α, IL-6, and TNF-α levels were determined using standard enzyme-linked immunosorbent assays (ELISA) (RayBiotech, Norcross, GA, USA). The limits of detection and within-assay coefficients of variation of the tests are given in Table 2.

Statistical analysis

All data are expressed as the median and range. The Kolmogorov–Smirnov test was used to evaluate the normality

Table 2 Limit of detection (LOD) and coefficient of variation (CV) of IL-1α, IL-6 and TNF-α

Tests	LOD (pg/ml)	CV (%)
IL-1α	<2	<10
IL-6	<3	<10
TNF-α	<30	<10

of the data. Our data were not normally distributed and therefore we used medians and ranges instead of means and standard deviations. The Wilcoxon signed-rank test was used to evaluate the significance of differences between the control and patient groups. The differences among patient groups were assessed using the Friedman nonparametric test. A paired sample *t* test was used to evaluate the difference between patient groups. Values of $p < 0.05$ were deemed statistically significant.

Results

The urine IL-1α, IL-6, and TNF-α levels are summarised in Table 3.

IL-1-α

The median IL-1α levels measured before (2.5 pg/ml) and 24 h after (4 pg/ml) ESWL were not significantly different from the control group (2.5 pg/ml) ($p > 0.05$). However, 14 days after ESWL, IL-1α increased to 5 pg/ml and this difference was significant ($p = 0.04$).

IL-6

Similar to IL-1α, the median IL-6 level (3.8 pg/ml) measured before ESWL was not significantly different from the level in the control group (5.2 pg/ml) ($p > 0.05$). However, 24 h after ESWL, the urine IL-6 level (19.7 pg/ml) increased significantly ($p = 0.01$). In contrast to IL-1α, 14 days after ESWL, IL-6 (7 pg/ml) decreased to the control level (5.2 pg/ml) ($p < 0.05$).

TNF-α

In both the patient and control groups, we did not detect TNF-α in the urine samples, except in one patient (at 24 h and 14 days after ESWL the values were 53 and 66 pg/ml, respectively).

Discussion

Despite its widespread use in patients, ESWL has many effects on the renal and surrounding tissues, and these effects have been the subject of many studies [1, 2, 9, 10]. In this study, we showed that the inflammatory response to ESWL continues for at least 14 days after intervention and that the severity of inflammation can be assessed by measuring urine proinflammatory cytokine levels. We did not find an inflammatory response in patients with stone disease.

Stones located in soft tissues are destroyed by the physical effects of the shockwaves, which are thought to have harmful effects on the surrounding tissues. Consequently, an inflammatory response to ESWL is expected. In addition to shockwaves, the size, composition, and location of the stone, anatomy of the urinary tract, pre-treatment evaluation of the patient, and availability of modern equipment can affect the treatment outcome [11].

In addition to ESWL, the inflammatory responses in patients with stone disease need to be evaluated. Rhee et al. [12] measured the urine IL-1α, IL-1β, and IL-6 levels and found a significant elevation in IL-6 level in patients with stone disease. By contrast, Rieder et al. [13] did not find a significant elevation in IL-6 level in stone-forming patients. We also did not find any inflammatory response in patients with stone disease. In our study, we selected subjects who could be treated by ESWL, and consequently the patient

Table 3 Urine IL-1α, IL-6 and TNF-α levels of the patient and control groups

Tests	Control group (*n* 14)	Patients (*n* 21)		
		Before ESWL	24 h after ESWL	14 days after ESWL
IL-1α (pg/ml) Median (Min–Max)	2.5 (1.9–6.5)	2.5 (1.2–16.6)	4.0 (1.5–36.4)	5.0 (1.9–39.0)[a,b]
IL-6 (pg/ml) Median (Min–Max)	5.2 (2.9–9.8)	3.8 (2.9–47.7)	19.7 (2.9–174.2)[a,b,d]	7.0 (2.9–129.5)
TNF-α (pg/ml) Median (Min–Max)	ND	ND	ND	ND

ND not detected

Significantly different from [a]control group, [b]before ESWL, [c]24 h after ESWL and [d]14 days after ESWL

characteristics might have a critical role in the inflammatory response to stone disease.

The inflammatory response can be evaluated using individual biochemical markers. However, the use of a single marker is not sufficient to evaluate the entire response over a long period. The sensitivity and specificity of each marker may be quite different and might not reflect the entire response adequately. Therefore, we evaluated the inflammatory response to ESWL using three urine proinflammatory cytokines (TNF-α, IL-1α, and IL-6) for up to 14 days.

Many agents increase the TNF-α level, including bacteria, viruses, some cytokines, immune complexes, hepatic regeneration, ischaemia/reperfusion, and reactive oxygen intermediates [6, 14–16]. In this study, we detected TNF-α in urine samples from one patient only. However, this does not mean that TNF-α is not present in the urine of these subjects; rather, TNF-α may be present in the urine, but we cannot quantify it by our method. As shown in Table 2, the detection limit of our method for TNF-α was 30 mg/ml. This means that in clinical practice, we may need more sensitive methods with lower detection limits to measure urine TNF-α accurately. Some authors have measured the urine TNF-α level. In comparison to a sham group, Clark et al. [4] showed that the urinary excretion of TNF-α level increased significantly in ESWL-treated pigs and remained elevated thereafter, albeit at lower levels. However, they collected urine from each kidney directly, rather than from the urinary bladder. In our study, we collected bladder-pooled urine excreted in the morning. The collection of urine from the kidney is not practical clinically and cannot be applied to patients. The number of shockwaves is critical to the TNF-α response [17]. In another study, Clark et al. [17] did not detect a significant change in the urinary excretion of TNF-α in pigs receiving either 500 or 1,000 shockwaves. However, when they increased the number of shockwaves, they found that urinary TNF-α excretion level was increased significantly in pigs receiving 2,000 shockwaves at 1 h after ESWL, and declined thereafter. Dundar et al. [18] measured urine TNF-α level before and 2 h after ESWL and found no significant changes. In light of the literature and our findings, we speculate that TNF-α is not a useful clinical marker of ESWL-induced renal injury.

In our study, the urinary excretion of IL-6 increased to a high level 24 h after ESWL, demonstrating that there is a strong inflammatory response to ESWL in this period. Dundar et al. [18] suggested that there is no inflammatory response to ESWL because they did not detect IL-6 in urine samples after intervention. However, there is a methodological difference between the two studies: they measured IL-6 before and only 2 h after ESWL, while we measured it for up to 14 days. We also did not find a significant difference between control group and patients before ESWL, while 24 h later, the urinary excretion of IL-6 was five times higher than before ESWL. Subsequently, it declined and 14 days after the intervention its level was not significantly different from that in the control group. Clark et al. [17] measured the IL-6 level in pig kidney tissue and found an elevated level 4 h after ESWL. They also found a significant correlation between the IL-6 level and number of shockwaves. These results show that IL-6 may be used to assess the inflammatory response to ESWL in the early phase, but not in the late phase.

IL-1 is produced mainly by activated mononuclear phagocytes, but after stimulation almost all nucleated cells are capable of creating IL-1α and IL-1β, the two main forms of IL-1. Their biological activities are essentially equivalent and they act both locally and systemically [6]. Dundar et al. [18] measured IL-1β level in urine samples before and 2 h after ESWL and found no significant changes. In our study, we did not find a significant change in urine IL-1α level before and 24 h after ESWL. However, 14 days later its level was significantly higher than in the control group. These results show that IL-1α may be a useful marker in the late phase, but not in the early phase after ESWL treatment.

Conclusions

The inflammatory response is a complicated metabolic process that may take a long time. Consequently, a single biochemical marker and measurements over short periods may not be sufficient to evaluate the entire response adequately. In contrast to previous studies, we examined three proinflammatory cytokines and measured their urinary excretion over a long time period. We found that before ESWL, there was no significant elevation. By contrast, 24 h after ESWL, there was a strong response that could be detected by measuring IL-6. The response continued at a slower rate, and 14 days after ESWL the inflammatory response could be assessed based on urinary IL-1α.

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