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## ■ Original Article

# Clinical use of specific markers TAS, TOS, PON and IL-6 by the evaluation of kidney damage in patients receiving SWL treatment

## *SWL tedavisi alan hastalarda böbrek hasarının değerlendirilmesinde özel markırlar: TAS, TOS, PON ve IL-6*

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### ABSTRACT

**Aim:** Beside efficacy of the shock wave lithotripsy (SWL) procedure, also its negative effects on the kidneys, its relation with the oxidant/antioxidant balance and the search after biomarkers for the detection of this negative effect gained interest in the recent years. The aim of the study is to investigate the possible usage of total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), Paraoxonase-1 (PON-1) and Interleukin 6 (IL-6) parameters as biomarkers for renal injury/trauma in the early period by patients undergoing SWL due to kidney stones.

**Material and Methods:** Forty patients receiving SWL therapy due to kidney stones were included to study by collecting their blood samples before and 2 hours after the procedure.

**Results:** It was observed that SWL therapy has deteriorated the oxidant/antioxidant balance in terms of the oxidants by analyzing the increase of IL-6 (P <0,01) and decrease in PON-1 (P = 0.049). There was no change observed in TAS (P = 0.178) and TOS (P = 0.175) and OSI (P = 0.551) parameters.

**Conclusion:** This has shown that IL-6 and PON-1 may be more sensitive markers of renal injury after SWL in early period.

**Key Words:** Shock Wave Lithotripsy (SWL), Total Antioxidant Status (TAS), Total Oxidant Status (TOS), Oxidative Stress Index (OSI), Paraoxonase-1 (PON-1), Interleukin-6 (IL-6).

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## ÖZ

**Amaç:** Şok dalga litotripsi (SWL) prosedürünün böbrek taşları üzerine açık etkinliğinin yanı sıra böbrekler üzerindeki olumsuz etkileri, oksidan/antioksidan denge ile ilişkisi ve bu olumsuz etkinin saptanması için biyomarkır arayışları son yıllarda ilgi çekmektedir. Bu çalışmanın amacı böbrek taşı nedeniyle SWL tedavisi uygulanan hastalarda erken dönemde böbrek hasarı/travma belirlenmesinde biyomarkır olarak toplam antioksidan status (TAS), toplam oksidan status (TOS), oksidatif stres indeksi (OSI), Paraoksonaz-1 (PON-1) ve İnterlökin-6 (IL-6) parametrelerinin olası kullanımını araştırmaktır.

**Gereç ve Yöntemler:** Böbrek taşları nedeniyle SWL tedavisi alan 40 hasta işlemden önce ve 2 saat sonra kan örnekleri toplanarak çalışmaya dahil edildiler.

**Bulgular:** SWL tedavisinin oksidan/antioksidan dengesini, IL-6 artışı (P <0,01) ve PON-1 azalması (P = 0.049) ile oksidan parametreler yönünde bozduğu belirlenmiştir. TAS (P = 0.178), TOS (P = 0.175) ve OSI (P = 0.551) parametrelerinde herhangi bir değişiklik gözlenmemiştir.

**Sonuç:** IL-6 ve PON-1'in SWL sonrası erken dönemde renal hasarın belirlenmesinde daha hassas belirteçler olabileceği gösterilmiştir.

**Anahtar Kelimeler:** Şok Dalga Litotripsi (SWL), Toplam Antioksidan Status (TAS), Toplam Oksidan Status (TOS), Oksidatif Stres İndeksi (OSI), Paraoksonaz-1 (PON-1), İnterlökin-6 (IL-6).

## Introduction

Urolithiasis is one of the most common disorders of the urinary system and is considered as a serious health problem which may lead to renal function disorder and permanent renal failure [1].

SWL has substituted surgical procedures by the treatment of renal stones in selected patients due to its advantages like easy application, no workforce loss and no need for hospitalization after the procedure [2].

The effect of SWL on renal function has started to be questioned due to its wide use. Many complications like subcapsular-perirenal haematoma, rupture in the urothel, haematuria, ureteral obstruction, formation or aggravation of hypertension, perirenal fibrosis, ureteral fibrosis, decrease in renal blood flow, loss of renal function have been reported after the SWL procedure [3].

Renal injury after SWL is considered to be developed due to oxidative stress caused by transient renal ischemia and reperfusion [4]. In recent years, practical methods are developed to measure the total level of oxidants and antioxidants in serum and plasma. The measurement of "Total Oxidant Status (TOS)" and "Total Antioxidant Status (TAS)" are much easy to perform and cost effective when comparing with individual measurement of the all oxidants and antioxidants [5].

Inflammatory response may be evaluated by using one biochemical marker but using one marker may not be

sufficient for evaluation in the time period required. Therefore we have evaluated the inflammatory response to SWL by using different parameters individually [6].

Interleukin-6 (IL-6) is a significant mediator of the acute phase response activated by inflammation and an efficient proinflammatory cytokine with anti-inflammatory and protective features [7]. Paraoxonase-1 (PON-1) is a calcium dependent esterase and an enzyme which prevents atherosclerosis by decreasing the oxidation of the low density lipoprotein (LDL). Decrease in PON-1 is an indicator for reduction of the antioxidant activity and increase of oxidative stress [8].

In this study we aimed to compare the TAS, TOS, OSI, PON-1 and IL-6 parameters as biomarkers for renal injury in the early period by patients undergoing SWL.

## Material and Methods

Forty voluntary patients over the age of 18 which applied to our outpatient clinic and received SWL treatment indication due to kidney stones according to international guidelines are included to our study.

Patients having renal pathologies other than kidney stones, systemic diseases affecting the kidneys, like diabetes mellitus and hypertension, infection and tumors of urinary system or other systems, alcohol and tobacco consumers, antioxidant drug users and organ failure were excluded from study.

TAS, TOS, PON-1 and IL-6 levels were measured in blood

samples obtained before and 120 minutes after the SWL procedure.

**Preparation of the samples**

Five cc. antecubital vein blood samples were taken from patients right before and 120 minutes after the SWL procedure. Serum samples were preserved at -80 °C for TAS, TOS, PON-1 and IL-6 analysis.

**Reactives for TAS Measurement**

An automatic method developed by Erel which measures total antioxidant capacity of the body against free radicals [9].

**Reactive 1:** Prepared by solving of 10 mM o-Dianisidine and 45 mM Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O in 75 mM Clark buffer ( pH=1.80).

**Reactive 2:** Prepared by solving of 7,5 mM hydrogen peroxide in 75 mM Clark buffer ( pH=1.80).

**Measurement of TOS**

A calorimetric method developed by Erel [9].

**Reactives:**

**Reactive 1:** Main solution is prepared by solving of 25 mm H<sub>2</sub>SO<sub>4</sub> in 140 mm NaCl solution. Then (10%) Glycerol is resolved in the main solution and 250 µm Xlenol orange is added to the total volume.

**Reactive 2:** Prepared by solving 10 mm o-Dianisidine dihydrochloride in the main solution with 5 mm ammonium ferrum sulfate.

**Measurement of PON-1**

PON-1 is measured by commercial kits. Paraoxon hydrolysis ratio (diethyl p nitrogenyl p-phosphate) is measured at 37o C by observing the decrease in absorption at 412 nm. The forming p-nitrophenol is calculated with 18.290 M-1 molar absorption ratio at pH: 8.50 and paraoxonase activity is expressed as u/l. Phenyl acetate is used as a substrate in order to measure the arylesterase activity. Enzyme activity is measured according to molar absorption ratio of 1310 M-1 cm. One unit of arylesterase activity is expressed as 1mmol phenol formed under the above mentioned conditions.

**IL-6 Measurement**

Serum of the obtained blood samples are centrifuged 10 minutes long by the speed of 3000/min and preserved at -80°C in two Eppendorf tubes. Interleukin 6 level is measured with Human ELISA (DIAsource ImmunoAssays S.A. B-1400, Nivelles, Belgium) kit. This kit is preserved at +2 - +8°C. The measurement range of the kit is between 0- 2560 pg/ml.

**Statistical Analysis**

IBM SPSS 20 software program is performed for statistical analysis. Shapiro Wilk Test is used for evaluating the proximity of the distribution platform of the continuous and non-continuous variables to normality. Descriptive statistic values are expressed as mean ± standard deviation or as median value (minimum - maximum) and categorical variables were shown as the number of cases and in percent (%).

Statistical analysis of the difference in TAS levels before and after SWL are determined with dependent t-test and the difference in TAS and PON levels with Wilcoxon signed rank test. And the differences of serum IL-6 and OSI levels before and after SWL are statistically analyzed with Mann – Whitney U test. P value < 0,05 is considered as statistically significant.

**Ethical Aspect**

The study is approved by the ethic committee for clinical studies of our institution on November.29.2016 with the reference number 23/15.

**Results**

The sociodemographic features, size, number and localization of the kidney stones of the evaluated 40 patients are described in the tables. (Table 1-2-3-4).

**Table 1.** Sociodemographic features of the patients

Sex	Male	31	77.5
	Female	9	22.5
Age, Mean ±SD (years)	36.53±11.37		
Min. – Max.	19-60		
BMI Mean ±SD (kg/m <sup>2</sup> )	26.18±2.15		
Min. – Max.	22.8-31.2		
Total		40	100.0

**Table 2.** Distribution of the stone localizations

Stone Localization	Number (n)	Percent (%)
Renal Pelvis	24	60.0
Upper Calyx	3	7.5
Mid Calyx	5	12.5
Lower Calyx	8	20.0
Total	40	100.0

**Table 3.** Distribution of the number of stones

Number of Stones	Number (n)	Percent (%)
1	28	70.0
2	8	20.0
3	3	7.5
4	1	2.5
Total	40	100.0



**Table 4.** Distribution of the size of the stones

Size of the Largest Stone	Size of the Smallest Stone	Mean Stone Size
17mm	6mm	8.75mm

Mean TAS level in the serum of the patients before SWL was determined as  $2.23 \pm 0.20$  mmol trolox eqv/l and 120 minutes after SWL as  $2.18 \pm 0.16$  mmol trolox eqv/l. There was no statistically significant difference detected between TAS levels before and after SWL ( $P = 0.178$ ).

Mean TOS level before SWL was determined as  $22.72 \pm 12.29$   $\mu\text{mol H}_2\text{O}_2$  eqv/l and 120 minutes after SWL as  $20.05 \pm 10.58$   $\mu\text{mol H}_2\text{O}_2$  eqv/l. There was also no statistically significant difference detected between TAS levels before and after SWL ( $P = 0.175$ ).

The mean PON-1 level in the serum of the patients before SWL was detected as  $230.10 \pm 129.95$  u/l and 120 minutes after the SWL procedure as  $187.03 \pm 106.23$  u/l. A statistically significant difference was detected between PON-1 levels before and after SWL ( $P = 0.049$ ).

Mean IL-6 level in the serum of the patients was detected as  $17.14 \pm 49.03$  pg/ml before SWL and as  $20.43 \pm 51.93$  pg/ml 120 minutes after SWL. There was a statistically significant difference detected between IL-6 levels before and after SWL ( $P < 0.01$ ).

It was observed that mean OSI level before SWL was  $10.538 \pm 6.52$  arbitrary units and  $9.44 \pm 5.48$  arbitrary units 120 minutes after the SWL procedure. There was no statistically significant difference detected between OSI levels before and after SWL ( $P = 0.551$ ).

TAS, TOS, PON-1 and IL-6 levels and the statistical differences between before and after SWL procedure are shown in Table 5.

**Table 5.** Evaluation of serum TAS, TOS, OSI, PON-1 and IL-6 levels (Mean  $\pm$  SD) before and after (120. min) SWL.

Serum TAS, TOS, PON-1 and IL-6 Results	Before SWL	After SWL (120. Min)	p Value
TAS (mmol Trolox Eqv./L)	$2.23 \pm 0.20$	$2.18 \pm 0.16$	0.178
TOS ( $\mu\text{mol H}_2\text{O}_2$ Eqv./L)	$22.72 \pm 12.29$	$20.05 \pm 10.58$	0.175
OSI (Arbutrary Units)	$10.538 \pm 6.52$	$9.44 \pm 5.48$	0.551
PON-1 (U/l)	$230.10 \pm 129.95$	$187.03 \pm 106.23$	0.049*
IL-6 (pg/ml)	$17.14 \pm 49.03$	$20.43 \pm 51.93$	<0.01*

\*: Statistically significant difference before and after SWL.

### Discussion

SWL is widely accepted as a low risk procedure. However its reliability started to be questioned because of the increasing incidence of diabetes mellitus and hypertension after SWL [10].

Many clinical studies evaluating the possible harming effect of SWL on the kidney and surrounding tissues may be found in the recent years [11,12]. Results of these studies show that SWL is not a harmless procedure as predicated before, may cause damage on kidney tissue and may have negative effects on renal functions [11]. Traumatic vascular damage, renal vasoconstriction, ischemic damage due to intraparenchymal bleeding and additionally the ischemia/reperfusion injury due to the inflammatory response which is called lithotripsy induced nephritis may occur and cause acute renal injury [13]. Renal function mostly recovers in hours but also it may be observed that functional renal tissue loss occurs due to scar formation around the traumatic area.

We are focused on markers of oxidative stress and antioxidant status in this study. We aimed to congregated independent markers like TAS, TOS, OSI, PON-1 and IL-6 whose signal paths are not clearly enlightened in the same study and evaluate their action by SWL depending renal injury in the early period in order to determine their possible contribution to the antioxidant stress agents secreted from renal epithelial cells and their regulation. Inflammatory response is a complicated metabolic process and generated in extended period of time. A single marker may be misleading on evaluating the process. Many authors have reported that SWL is increasing the oxidative stress after evaluating the renal injury through numerous oxidant and antioxidant parameters in blood and urine [14-16]. We had the opportunity to evaluate and compare different parameters in the same study group. Optimization is provided by using the before and after procedure blood samples of the same study group. Yilmaz et al. have studied 24 hour urine samples of patients with renal stones before and after the SWL procedure and detected an increase of TOS and OSI and no change in TAS in before SWL and 24 hours after urine samples. This study has shown the renal damage of SWL and detected a high level of TOS at the evaluation 24 hours after SWL and indicated that these parameters may be used as markers in the late period [17]. In our study we have not detected any change in TOS, TAS and OSI parameters after 2 hours of the procedure (Table 5). This may indicate that TOS, TAS and OSI may not be used as a marker of renal injury in the early period.

Proinflammatory cytokines like IL-6 are increasing the inflammation and contribute to the mechanism of renal injury [18]. To our knowledge increased level of the proinflammatory cytokines is considered as a sign of a potent inflammation in the tissue and proximal tubule damage and dysfunction are a clear initiator of acute renal failure. The decreased IL-6 metabolism due to proximal tubule dysfunction may be the reason for increased IL-6 level by acute renal failure. Paradoxically increased proximal tubule metabolism may cause an increase in the level of IL-6 in urine, because IL-6 will be intact in the urine due to its failed metabolism in the proximal tubule.

Some studies have found that oxidative stress markers decrease and anti-oxidative markers increase by the presence of IL-6 and the opposite occurs by the absence of IL-6 [19].

Dennen et al have detected higher urine IL-6 levels at the sixth hour of the acute renal injury and considered IL-6 as a diagnostic marker by acute renal injury. They especially draw attention on the fact that IL-6 is an early biomarker for detecting acute renal injury due to acute tubular necrosis [20]. While Rieder et al have not detected high levels of IL-6 by urolithiasis patients, Rhee et al have oppositely observed higher IL-6 levels [21,22]. We have found an increased level of IL-6 after two hours which is showing a strong inflammatory response (Table 5). Dundar et al have not detected an increase in IL-6 after 24 hours of SWL [6] but Clark et al have reported an IL-6 increase after 4 hours in their animal study and interpreted this increase as correlated with the number of the shock waves [23]. Greenberg et al have also shown that IL-6 may be used as a biomarker by acute renal injury [24]. Vriesa et al have reported a statistically significant increase in IL-6 on the 30th. minute of the reperfusion in their study [25]. We have detected presence of renal injury by showing IL-6 increase in blood in the early period after SWL and have also shown that IL-6 may be an antiinflammatory, protective and antioxidative stress marker.

Studies have shown that PON-1 may also be used for evaluation of the antioxidant defense system [26]. PON-1 decrease indicates a lower antioxidant activity and a rise in the oxidative stress [8]. Studies have shown that PON-1 has a protective effect against oxidative stress and plays an antioxidant role [27]. Premila et al have found that the PON-1 activity has shown an increase of 85% in the first 6 hours and 160% after 16 hours by cyclophosphamide induced renal injury. They have also reported that the blood level of PON-1 has turned back to the control values after 24 hours. In the same study they have reported an unchanged PON-1 activity after 6 hours

but a decrease of 35% in 16 hours which indicated that PON-1 may be used as an early biomarker by the evaluation oxidant/antioxidant response. Like we have achieved the same result differently in a clinically structured study [28]. In our study we have not detected a statistically significant difference between the samples taken before and 120 minutes after SWL in TOS, TAS and OSI parameters. This result may have been interpreted that SWL is not deteriorating the oxidant/antioxidant balance which may lead to renal injury. Whereas the other two markers we have evaluated in this study, IL-6 and PON-1 levels revised this interpretation. With the evaluation of former studies, we would expect SWL to increase the oxidant status, to have no effect on antioxidant status and finally to increase the OSI level. However, we observed the difference during post-SWL test period. We observed that in most of other studies that were investigating oxidant/antioxidant response against any kind of stress or trauma that samples were being taken after 6-12 hours or more waiting period. However we started our investigations 2 hours after ESWL with the opportunity of early stage evaluation on renal injury. We obtained oxidant/antioxidant response on IL-6 and PON-1 values before there was any alteration on TOS and TAS parameters. Detection of the acute renal injury following the SWL procedure could be done through early biomarkers which may play a crucial role to protect the renal functions and to reduce systemic complications.

### Conclusion

As a conclusion we can say that IL-6 and PON-1 may give reliable information in the early period of renal injury following SWL. Furthermore studies are needed in order to determine the mechanisms, possible damage and the prevention of renal ischemia/reperfusion injury and oxidative and antioxidative stress.

### Declaration of conflict of interest

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