

Variations in Nitric Oxide and Endothelin Serum Levels in Extracorporeal Shock Wave Lithotripsy-Treated Patients

Abstract

Introduction: Renal vasculature is extremely sensitive to vasoconstrictor effects of endothelin (ET), while nitric oxide (NO) has special role in several pathological renal conditions. **Aim:** The aim of this study to examine the presence, character, and degree of changes in NO and ET levels in the serum of extracorporeal shock wave lithotripsy (ESWL)-treated patients with nephrolithiasis. **Patients and Methods:** This study included a total of 60 patients that were divided in two groups: Group I ($n = 24$), in which a total of 2000SWs were administered; 0–2 units; (0.5 units per each 500SWs), and Group II ($n = 36$), in which a total of 4000SWs were administered; 0–4 units; (0.5 units per each 500SWs). **Results:** In the Group I median NO serum concentration increased in relation to pretreatment levels ($39.04 \pm 8.29 \mu\text{mol/L}$) specifically 30 min, 60 min, and 24 h following the treatment (39.11 ± 12.60), (41.80 ± 6.89), and (46.33 ± 9.03), where concentration growth after 24 h was statistically significant $P < 0.01$. The NO serum concentration in the Group II increased in relation to pretreatment levels ($38.90 \pm 10.33 \mu\text{mol/L}$) after 30 min (48.71 ± 30.09), 60 min (54.57 ± 39.76), and 24 h (97.95 ± 72.07). The NO concentration increase after 60 min and 24 h is statistically significant, respectively, $P < 0.03$ and $P < 0.0001$. **Conclusion:** NO and ET serum levels are changing under the influence of ESWL, and that the NO and ET changes are directly correlated with the number of administered shock waves and administered energy.

Keywords: Endothelin, nephrolithiasis, nitric oxide

Introduction

Extracorporeal shock wave lithotripsy (ESWL) is a treatment of urinary calculosis, which also carries pathophysiological consequences, which can be of great importance to the patient. Endothelin (ET) is a potent vasoconstrictor peptide isolated from cultivated porcine aortic endothelial cells.^[1] ET biosynthesis begins with creation of pre-pro-ETs, which, under the effect of specific endopeptidases and through pre-pro-ET hydrolysis, transform into pro-ET or big ET in the endoplasmic reticulum. During the further process, part of pro-ET generated in the cell is already processed on an intra-cellular level into mature ET, while the part is excreted in an unchanged form, and is transformed into a mature form in peripheral tissues.^[1,2] Immediately after this discovery, ET isoforms, ET-2 and ET-3, were soon thereafter isolated from endothelial cells. Besides porcine aortic endothelial cells, ET synthesis is present in numerous tissues.

Hence, for example, concerning ET-1, mRNA was found in the heart, lungs, liver, spleen, stomach, small and large intestines, muscles, and testicles. Concerning ET-2, mRNA was found in the stomach, small and large intestines, pancreas, and in muscles, while concerning ET-3, in the central nervous system, especially in supraoptical neurons. ET-2 shows close structural similarity to ET-1, with the only difference being the two amino-acids, while ET-3 differs in terms of distribution of six amino-acids. Renal vasculature is extremely sensitive to vasoconstrictor effects of ET-1, more than any other vascular bed.^[2] This unprecedented sensitivity is partly related to the high density of ETa receptors found in vascular smooth musculature in all renal resistant vessels.^[3] ETb receptors are generally more distributed, with a high concentration in renal collecting ducts. ET-1 is produced in endothelium, and also in glomerular mesangial and tubular epithelial cells. Renal effects of ET-1 include an increase in renal vascular resistance and a decrease in the rate of glomerular filtration and

**Emir Milisic,
Mustafa Hiros¹,
Edin Begic^{2,3}**

Department for Pediatric Surgery, Clinic of Pediatric Surgery, University Clinical Center Sarajevo, ¹Department for Urology, Clinic of Urology, University Clinical Center Sarajevo, ²Department of Cardiology, General Hospital "Prim. Dr. Abdulah Nakas", ³Department of Pharmacology, School of Medicine, Sarajevo School of Science and Technology, Sarajevo, Bosnia and Herzegovina

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Address for correspondence:

*Dr. Emir Milisic,
Clinic of Pediatric Surgery,
University Clinical Center
Sarajevo, Sarajevo,
Bosnia and Herzegovina.
E-mail: emirilejla@gmail.com*

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renal tubular sodium handling alteration, leading to natriuresis and diuresis. Nitric oxide (NO) is an important signaling molecule which is active in numerous tissues by regulating different volumes of physiological and cellular processes. Its role was first discovered by several groups of investigators, who tried to identify the agent responsible for vascular relaxation promotion and vascular tone regulation.^[4,5] The agent was named endothelium-derived relaxing factor, and it was initially assumed to be a protein, as are the majority of other signaling molecules. Intracellular NO synthesis may be regulated by various methods, such as cellular NO distribution, change in NO gene expression, enzyme activity and the presence of cellular NO activity inhibitors.^[4-6] In numerous pathological conditions, the role of NO is dependent on the stadium of disease, involvement of NOS and presence or absence of other modifying intrarenal factors. NO's role is special in several pathological renal conditions, such as acute renal insufficiency, inflammatory nephritis, diabetic nephropathy, and transplant rejection.^[6] Although NO was initially described as a chemokine vasodilator, it plays an important role in vascular biology, with its anti-thrombotic, anti-inflammatory, anti-proliferative, and anti-oxidative effects.^[6,7] All three NO isoforms are represented in individual segments of the kidney. eNOS is abundantly represented in renal microvasculature, glomerular endothelial cells, proximal tubular cells, the thin ascending limb of the loop of Henle and collecting tubules. Numerous studies have confirmed that NO regulates macrovascular and microvascular action, including glomerular hemodynamics. NOS inhibition reduces basal renal blood flow, with the preservation of auto-regulatory mechanisms. Intrarenal NO regulates the tone and afferent and efferent arterioles.^[7,8] The NO effect on renal flow and electrolyte transport is a result of net effects on renal hemodynamics, renal nerves, and direct tubular transport properties.^[8-10]

The present study was planned with aims to examine the presence, character, and degree of changes in NO and ET levels in the serum of ESWL-treated patients with nephrolithiasis and to determine whether there is a correlation between the changes in NO and ET concentrations in the serum of pre-ESWL treatment patients and the occurrence, character, degree, and localization of changes in vascular renal elements. The study's objective is also to investigate whether NO and ET stand in direct correlation with the number of applied shock waves and administered energy.

Patients and Methods

The study included a total of 60 patients at the Urology Clinic, Clinical Center University of Sarajevo, over a 3-year period. Inclusion criteria were as follows: patients older than 18 years with nephrolithiasis (kidney stone maximum 20 mm in diameter, stone must be visible on X-ray), first ESWL treatment (*de novo* cases), unilateral

lithotripsy treatment, without previous kidney surgery, without infravesical obstruction, without proven urinary infection, repeated use of one and the same analgesic, without anticoagulant therapy, without antihypertensive therapy, without use of nephrotoxic drugs prior to and during the treatment, normal blood pressure, general criteria— non-pregnant patients, patients with normal renal function. Exclusion criteria were as follows: kidney stone larger than 20 mm in diameter, acute urinary infection and systemic inflammation, urinary tract obstructions causing hydronephrosis, patients with congenital kidney or ureter anomalies, bilateral ESWL treatment, recurrent cases of urolithiasis, ureteral stones, patients with serum creatinine >350 $\mu\text{mol/L}$ over the past 3 months, hypertension, taking antihypertensive medicines, diabetes mellitus, previous surgeries on kidney, general criteria— pregnant patients, patients with impaired renal function, failure to sign informed consent.

The treated kidney stones were located exclusively in the renal duct system, pyelon (14), upper (11), middle (16), and lower calyces (19). The subjects were divided in two groups: Group I ($n = 24$), in which a total of 2000SWs were administered; 0–2 units; (0.5 units per each 500SWs), and Group II ($n = 36$), in which a total of 4000SWs were administered; 0–4 units; (0.5 units per each 500SWs). Samples for determining NO serum concentration were taken before, as well as 30 and 60 min, and 24 h after ESWL treatment, and immediately refrigerated at the temperature of -20°C pending analysis. Pre-refrigeration serum samples were deproteinized so that 0.05 ml of 30% ZnSO_4 solution was added to 1 ml of sample. After a few minutes, the sample was centrifuged over a period of 10 min at 700 g, after which the isolated supernatant was refrigerated at -20°C pending the determination of NO_3^{2-} concentration. The NO concentration was determined by measuring NO_3^{2-} and NO_2^{2-} concentration in the whole blood. The concentration was determined by NO_3^{2-} conversion into NO_2^{2-} using elementary zinc, and then by measuring NO_2^{2-} concentration by colorimetrically prepared Griess reagent, consisting of sulfanilamide and N-1-naphthyl ethylenediamine. After 10 min of vibrator stirring at room temperature, light absorption (optical density) was measured by 546 nm filter spectrophotometer. NO_2^{2-} concentration was read from the standard curve with known NaNO_3 concentration (of 1.56–100 nM). Distilled water with added Griess reagent was used as a blind trial run. The same procedure was applied for the direct determination of NO_2^{2-} , the only difference being that there was no addition of elementary zinc. Serum samples for determining NO levels were taken at the Urology Clinic, Department of Lithotripsy, while the analysis itself was made at the Laboratory of the Clinical Center University of Sarajevo and at the Institute for Physiology and Biochemistry of the Medical Faculty Sarajevo. Serum samples for determining ET concentration were taken at

the Urology Clinic, Department of Lithotripsy, before, immediately after, on the third and 7th day after ESWL treatment. Samples were refrigerated at -20°C, pending measurement. The ET 1 KIT, IBL Hamburg, standard ELISA method was used to determine ET concentration. The results were statistically processed so for each group of subjects, the average value (Mean), standard deviation, standard error of the mean, median value (MED) and percentage differences (P10, 25, 75 and 90%) were determined. Testing the significance of the differences in the average value of the groups of subjects was carried out using the Student's *t*-test (*p*), and where it could not be used, the statistical significance of the difference in the MED was tested using the Mann Whitney–Wilcoxon test. The values of *P* < 0.05 were considered statistically significant. For certain comparative groups of data of the subjects, Pearson's correlation coefficient (*r*) was determined, the level of significance of the same (*r* – *p*) and the correlation level for the significance level *P* < 0.05 (*r*1–*r*2). Ethical approval was obtained from the Ethical Committee of the Clinical Centre University of Sarajevo.

Results

In the subjects (*n* = 24) treated with 2000 SWs, the ET serum concentration increased on days 1, 3 and 5 after the ESWL treatment (3.48 ± 1.17), (3.38 ± 0.68), (3.40 ± 0.74) in relation to pre-treatment levels (3.27 ± 0.36 pg/ml), the increase being nonsignificant [Table 1]. In the group of subjects (*n* = 36) treated with 4000 SWs, the ET serum concentration shows variable movement. In relation to pre-treatment levels (3.20 ± 0.47 pg/ml), on day one after ESWL treatment the ET serum concentration shows a nonsignificant decrease (3.16 ± 0.48), while on days 3 and 5 following ESWL, the concentration increased in relation to pre-treatment levels, (3.24 ± 0.63)

and (3.22 ± 0.28), respectively, the increase being nonsignificant [Table 2]. In the subjects treated with 2000 SWs (*n* = 24), median NO serum concentration increased in relation to pretreatment levels (39.04 ± 8.29 µmol/L) specifically 30 min, 60 min, and 24 h following the treatment (39.11 ± 12.60), (41.80 ± 6.89), and (46.33 ± 9.03), where concentration growth after 24 h was statistically significant *P* < 0.01 [Table 1]. The NO serum concentration in the group of subjects (*n* = 36) treated with 4000 SWs increased in relation to pre-treatment levels (38.90 ± 10.33 µmol/L) after 30 min (48.71 ± 30.09), 60 min (54.57 ± 39.76), and 24 h (97.95 ± 72.07) [Table 2]. The NO concentration increase after 60 min and 24 h is statistically significant, *P* < 0.03 and *P* < 0.0001. The NO serum concentrations ratio between the two groups is shown in Figure 1.

Discussion

Observing ET variations, there are only two reports in the literature about the relationship between extracorporeal lithotripsy and changes in serum and urine concentration. A post-ESWL treatment increase in ET serum levels is present in this study too but is nonsignificant and there is no direct correlation in the function of time and the number of administered shock waves. These data do not confirm the part of hypothesis 1 pertaining to ET-1. Strohmaier *et al.* concluded that ESWL does not have a significant effect on ET serum concentration movement, and thus causes no significant renal trauma.^[11] That same year, Kirkali *et al.* claimed that post-ESWL treatment ET-1 plasma concentration grew significantly, while in urine that growth was nonsignificant, which means that ET released after this treatment induces hemodynamic changes in the kidney.^[12] The same study showed that medicamentous prevention with calcium channel blockers may prevent ET

Table 1: Statistic indicators of endothelin and nitric oxide changes following 2000 SWs, as measured in patients following extracorporeal shock wave lithotripsy treatment

Number of administered shock waves		Endothelin				Nitric oxide			
		0	1 day	3 days	5 days	0	30 min	60 min	24 h
2000 SWs	<i>n</i>	24	24	24	24	24	24	24	24
	Xsr	3.277	3.489	3.388	3.402	39.048	39.114	41.894	45.336
	SD	0.369	1.170	0.687	0.744	8.299	12.602	6.897	9.034
	SEM	0.075	0.239	0.140	0.152	1.694	2.572	1.408	1.844
	MED	3.210	3.217	3.146	3.211	39.710	38.122	42.093	45.269
	P10%	3.039	3.006	3.019	3.026	31.768	24.303	33.833	34.786
	P25%	3.094	3.105	3.094	3.097	34.945	32.959	37.725	41.298
	P75%	3.291	3.328	3.334	3.309	44.872	46.461	47.652	49.638
	P90%	3.497	3.803	3.756	3.550	48.287	55.753	49.241	56.706
	<i>P</i>		0.388 (NS)	0.420 (NS)	0.460 (NS)		0.975 (NS)	0.260 (NS)	0.012
	MWW		0.617	0.721	0.920		0.676	0.154	
	<i>r</i>		0.137	0.332	0.058		0.568	-0.257	0.148
	<i>P-r</i>		0.523	0.114	0.786		0.004	0.225	0.489
<i>r</i> 1		-0.282	-0.084	-0.353		0.213	-0.599	-0.272	
<i>r</i> 2		0.512	0.648	0.451		0.790	0.163	0.521	

SD: Standard deviation; SEM: Standard error of the mean; MED: Median; MWW: Mann–Whitney–Wilcoxon test; NS: Not significant

Table 2: Statistic indicators of endothelin and nitric oxide changes following 4000 SWs, as measured in subjects following extracorporeal shock wave lithotripsy treatment

Number of administered shock waves		Endothelin				Nitric oxide			
		0	1 day	3 days	5 days	0	30 min	60 min	24 h
4000 SWs	<i>n</i>	36	36	36	36	36	36	36	36
	Xsr	3.205	3.168	3.242	3.225	38.907	48.710	54.575	97.946
	SD	0.479	0.485	0.631	0.283	10.334	30.089	39.762	72.073
	SEM	0.080	0.081	0.105	0.047	1.722	5.015	6.627	12.012
	MED	3.092	3.055	3.102	3.105	39.388	38.601	42.539	51.205
	P10%	2.964	2.942	2.945	3.007	29.935	22.845	35.450	31.511
	P25%	3.043	3.000	3.009	3.054	33.086	31.511	37.813	37.813
	P75%	3.172	3.134	3.229	3.328	44.115	49.629	48.842	148.49
	P90%	3.352	3.244	3.513	3.575	51.993	99.259	74.050	200.88
	<i>P</i>		0.737 (NS)	0.763 (NS)	0.820 (NS)		0.069 (NS)	0.033 (NS)	0.00
	MWW		0.009	0.654	0.456		0.402		
	<i>r</i>		0.075	0.166	0.132		0.052	-0.125	0.329
	<i>P-r</i>		0.653	0.327	0.427		0.763	0.469	0.050
<i>r1</i>		-0.258	-0.170	-0.201		-0.281	-0.435	0.000	
<i>r2</i>		0.396	0.471	0.445		0.374	0.213	0.593	

SD: Standard deviation; SEM: Standard error of the mean; MED: Median; MWW: Mann-Whitney-Wilcoxon test; NS: Not significant

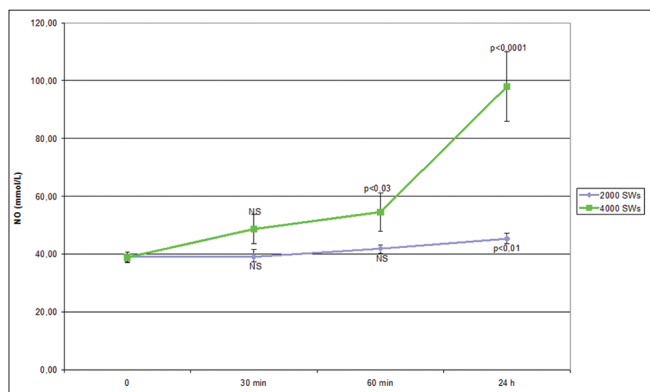


Figure 1: Comparative nitric oxide serum concentrations in subjects treated with 2000 SWs and 4000 SWs (after 30 min [30 min], after 60 min [60 min], after 24 h [24h])

concentration growth.^[12] NO, highly effective vasodilator peptide is enzymatically generated through endothelial NOS, to regulate vasodilation through vascular cells of smooth musculature, and antagonizes vasoconstrictor effect of angiotensin II, in the afferent arteriole, which helps regulate renal blood flow, glomerular filtration, and Na homeostasis. NO is also involved in auto-regulation of blood flow, renin secretion, activities of mesangial glomerular and epithelial cells and tubular function. While some authors have shown interest in NO's detrimental effect, it has been clearly proven that NO production increases during renal ischemia. In this study, NO serum concentration movement was monitored 30 and 60 min and 24 h after treatment. In the ESWL-treated group of patients, with the administration of 2000 SWs, the concentration level increased, where only the increase reported after 24 h was statistically significant ($P < 0.012$). In the patients treated with 4000 SWs, the NO serum concentration increased in all three set temporal categories, but significantly after 60 min ($P < 0.033$) and after

24 ($P < 0.001$). Given the NO serum concentration results obtained, which show a certain pattern, it was expected that the concentration after 24 h would show a tendency of stability and return to pretreatment levels.

In their 2003 study, Sarica *et al.* monitored NO and adrenomedullin serum and urine level movement, and concluded that levels of both tested parameters increased 24 h after treatment, and that the increase was proportionate to the number of administered shock waves. Seven days after treatment, the values were back to pretreatment levels.^[13] Park *et al.* concluded that ESWL raises the NO serum level immediately after, 30 and 60 min after treatment, and the increase is significant. The NO urine increase within the set temporal parameters was not significant.^[13] Cyclic 3',5' guanosine monophosphate, as an NO metabolite, increases after treatment within the set temporal categories, and the increase is significant. Aksoy *et al.* monitored the NO and malondialdehyde plasma and urine movement, with the aim to determine post-ESWL treatment changes in these categories, and what exactly is responsible for the adverse effect of ESWL, tension force, thermal effects of cavitation or formation of free radicals.^[14] During all set temporal parameters the NO plasma and urine levels grew significantly in relation to pretreatment values. The increase in malondialdehyde plasma levels was also significant, whereas the increase in the urine level was nonsignificant. In their paper, the authors speculate that the ESWL treatment triggers oxidative stress caused by renal ischemia and the reperfusion process.^[15-17] The NO production increase is interpreted as prevention from vasoconstriction-caused renal impairment.^[16-19] ET and NO values can be an indicator of an early parenchymal damage and damage of renal arterial circulation, and allows the clinician timely therapeutic and diagnostic approach.

Conclusion

Shock waves may affect renal hemodynamics by interfering with endothelial NO production or by stimulating the release of vasoactive substances such as NO and ET, very potent vasodilators, or vasoconstrictors, whose serum levels are monitored over the set periods of time, being very sensitive indicators of ESWL treatment-caused impairment at the cellular level. Based on the study results, it has been clearly proven that NO and ET serum levels are changing under the influence of ESWL, and that the NO and ET changes are directly correlated with the number of administered shock waves and administered energy.

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Conflicts of interest

There are no conflicts of interest.

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