SELECTIVE EVALUATION OF TWO URINARY ENZYMES (NAG AND AAP) BEFORE AND AFTER UNILATERAL SHOCK WAVE LITHOTRIPSY

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Abstract- Biological effects extracorporeal shock wave lithotripsy (ESWL) is not precisely known. We have evaluated two urinary enzymes activity N-acetyl-B-D-glucosamine (NAG) and alanine amino peptidase (AAP) before and after unilateral ESWL as markers for renal parenchymal damage. Forty eight patients with kidney stones (mean age 39) who had presented for the first time or at least one year after their previous lithotripsy underwent ESWL. Urinary specimens were collected before and after first, third and seventh days of lithotripsy and NAG, AAP were evaluated. These enzymes displayed the greatest activity 24 hours after ESWL with significant difference compared to the control group, ($P < 0.05$ versus 0.02). Elevation of urinary enzymes activity correlated with stone size particularly stones larger than 2 cm. These data suggest that there is some tubular and parenchymal damage induced by ESWL that needs time to get improved. The higher urinary enzyme activity in patients with larger stones (> 2 cm) is probably related to injury resulting from passage of smaller stones, produced after lithotripsy of a large stone, and it is suggested that these patients are treated with a safer procedure.

INTRODUCTION

Although, extracorporeal shock wave lithotripsy (ESWL) is presently the preferred procedure in treatment of renal calculus, its biological effects on kidney parenchyma is not precisely known (1).

Urinary enzymes levels have been assessed throughout the spectrum of kidney injuries due to antibiotics, heavy metals, analgesics, chemotherapy and graft rejection, but as soon as kidney function improves, the excretory enzymes return to their normal level (2).

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Hydrolytic lysosomal enzymes levels, like N-acetyl-glucose aminidase and galactosidase are precise indicators of these enzymes and have the greatest concentration in proximal tubules of mammals (3).

Another enzyme used in evaluating kidney parenchymal damage is alanine amino peptidase (AAP) (3).

There are three primary types of shock wave generation: electrohydraulic (spark gaps) electromagnetic and piezoelectric.

ESWL is now known to induce acute structural changes in the treated kidney in most treated patients. The two most common renal side effects seen immediately after ESWL are hemorrhage and edema within or around the kidney.

Electromagnetic machine produced complete cellular destruction at F2, which may explain the

higher rate of subcapsular hemorrhage for electro magnetic lithotriptors.

The decrease in renal function has been linked to the number of shocks administered.

After electromagnetic lithotripsy, level of NAG increases and it takes about one week to return to its previous level. These findings show that extracorporeal electromagnetic shock wave causes significant damage to the proximal tubular epithelium of the kidney, however, these changes return to normal in the first week (4).

In this study, levels of urinary enzymes (NAG and AAP) in patients who were treated with ESWL (with electromagnetic machine) before and after one week of this procedure were measured.

MATERIALS AND METHODS

Forty eight patients with kidney stones were included in this study. Mean age was 39 years (SD = ± 5 years). All of the patients had negative urinary culture and they had presented for the first time or at least one year after their previous lithotripsy. None of them had single kidney. All the stones were radio opaque. Twenty four (50%) patients had stones in pelvis, 2 (4%) in the inferior calix, 5 (10%) in middle calix, 2 (4%) at the uretero pelvic junction and 4 (8%) patients had staghorn calculi.

Seven (13%) patients had 0-1 cm stones, 35 (69%) had 1-2 cm stones and 6 (12%) had stones larger than 2 cm.

Extracorporeal shock wave lithotripsy was performed unilaterally with the Siemens Lithostar device which produced 3000 waves at 15-18 kV over 45 minutes. Urine samples were collected before and at first, second and seventh days after treatment. The collected samples were immediately transported to the laboratory. Our control group included 72 persons of approximately the same ages (38 ± 5).

Samples were gathered during one year beginning 1996. For measuring NAG activity, 5-10 m1 of urine sample was centrifuged and then 100 m1 of it was incubated with 8 mmol paranitrophenyl-N-acety-b-d glucosamine in (37°C) for 15 minutes. More than 200 ml glycerine buffer was utilized to stop the reaction and then absorption level was measured against a blank sample at 405 nm wavelength.

Finally enzyme activity level was calculated (1-3). Adynamic method was used to measure AAP activity level. After centrifugation of 5-10 ml of urine, 100 ml of it was mixed by 2700 ml of tris buffer and incubated in 30°C for 10 minutes, and then absorption changes were studied at 450 nm wave length for 2 minutes after adding 200 ml of l-alanine-p-Nitroanilide substrate. Data were analyzed by SPSS.

RESULTS

Considering high consumption of fluids after lithotripsy, the activity of the above mentioned enzymes were measured in relation to creatinine concentration so that the diuresis effect was omitted (u/gr of Cr). Results showed that NAG activity individuals with renal stone is higher than the normal control population even before lithotripsy (9.75 ± 1.65 u/gr of Cr against 3/84 ± 0/26 u/gr of Cr) (Table 1).

Overall the greatest activity of this enzyme is on the first day after lithotripsy but in patients with lower calix stones and those with stones larger than 2 cm, the urinary excretion of this enzyme is greatest on the 7th day post lithotripsy (Table 2, 3).

Table 1. Urinary NAG and AAP excretion before and after ESWL*

Enzyme	Control	Before Lithotripsy	After Lithotripsy		
			1 Day	3 Days	5 Days
NAG activity	3.84 ± 0.26	9.58 ± 1.68	17.01 ± 7.68	9.99 ± 1.63	11.79 ± 1.75
AAP activity	10.84 ± 0.50	12.88 ± 1.30	16.41 ± 2.90	14.07 ± 2.37	15.87 ± 2.03

*Data are given as mean ± S.E.M.
Abbreviations: ESWL, extracorporeal shock wave lithotripsy.

Table 2. Correlation between urinary AAP and NAG activities and stone size in patients who underwent lithotripsy*

Stone Size	Enzyme	Control	Before Lithotripsy	After Lithotripsy		
				1 Days	3 Days	7 Days
0-1 cm	NAG	3.84 ± 0.26	7.31 ± 1.49	7.42 ± 1.93	6.76 ± 1.8	7.19 ± 2.45
	AAP	10.84 ± 0.5	10.35 ± 1.95	15.46 ± 5.5	11.01 ± 2.5	10.66 ± 0.9
1-2 cm	NAG	3.84 ± 0.26	9.07 ± 1.11	9.88 ± 1.73	9.70 ± 2.25	9.99 ± 1.45
	AAP	10.84 ± 0.5	15.62 ± 2.18	16.77 ± 4.67	14.72 ± 2.33	15.01 ± 1.95
> 2 cm	NAG	3.84 ± 0.26	7.77 ± 3.13	11.99 ± 15.01	13.75 ± 4.65	19.46 ± 6.45
	AAP	10.84 ± 0.5	10.78 ± 2.56	21.41 ± 23.21	26.28 ± 13.69	19.76 ± 8.69

*Data are given as mean ± S.E.M.

The difference is statistically significant ($P < 0.001$, $P < 0.01$) compared to control groups but not in comparison with prelithotripsy. Urinary excretion of NAG in 23 patients with pelvic stones did not change considerably on different days of sampling (days 1, 3, 7) compared to prelithotripsy but the increments were statistically significant in comparison with our control group ($P < 0.001$) (Table 3).

We reached the same results in patients with 0-1, 1-2 and > 2 cm stones ($P < 0.01$ $P < 0.001$, $P < 0.01$) (Fig. 2). Our investigation on patients with different stone sizes exhibited that NAG urinary excretion level correlates closely with stones sizes (Fig. 2 and Table 2).

Regarding the urinary excretory level of AAP, it was demonstrated that the activity of this enzyme increased 24 hours post lithotripsy (16/42 ± 2/90 U/gr of Cr) which revealed significant difference with control group ($P < 0.05$) (Fig. 1, Table 1).

Excretory levels of AAP diminish on the 3rd and 7th days post lithotripsy compared to the 15th day but does not reach prelithotripsy levels (Fig. 1). In patients with pelvic stones, excretory level of AAP elevates on the first day and reaches its highest activity on the 3rd day but fails to reach the prelithotripsy level even one week post lithotripsy (I6/83 ± 4/9u/gr of Cr) (Table 3).

In cases with lower and middle calix stones AAP excretion is greatest on the first day postlithotripsy which is significantly different from control group ($P < 0.05$) (Table 3). In the first group, this difference exists at 3, 7 days postlithotripsy as well (Table 3).

In patients with 0-1 cm stones, AAP urinary excretion is considerable on the first day postlithotripsy and is statistically significant to control group ($P < 0.05$) but it diminishes on the 3rd day and reaches prelithotripsy level after one week (Fig. 3). Urinary excretion of AAP in cases with 1-2 stones fails to show significant changes but when stone size increases (> 2 cm) it elevates to its greatest level on the 3rd day postlithotripsy (26/28 b/7 U/gr of cr) and fails to show any considerable decline even one week post lithotripsy (Fig. 3).

This difference in comparison with the control group is statistically significant ($P < 0.003$).

Comparison between three groups of patients with different stone sizes revealed that urinary activity of this enzyme correlates positively with stone size (Fig. 3).

Table 3. Correlation between urinary AAP and NAG activities and stone location in patients who underwent lithotripsy*

| Location	Enzyme	Control	Before Lithotripsy	After Lithotripsy		
				1 Day	3 Days	7 Days
Pelvic	NAG	3.84 ± 0.25	8.16 ± 0.91	9.78 ± 1.69	10.59 ± 2.52	9.72 ± 1.33
	AAp	10.84 ± 0.5	12.41 ± 1.49	14.26 ± 2.15	16.83 ± 4.08	11.91 ± 1.35
Lower Calix	NAG	3.84 ± 0.25	7.69 ± 1.98	9.75 ± 3.29	8.17 ± 1.64	14.80 ± 4.13
	AAp	10.84 ± 0.5	13.50 ± 3.25	23.65 ± 10.48	15.09 ± 3.26	21.25 ± 5.73
Middle Calix	NAG	3.84 ± 0.25	10.65 ± 3.75	7.76 ± 2.27	6.93 ± 2.86	8.67 ± 3.43
	AAp	10.84 ± 0.5	19.10 ± 6.09	13.91 ± 3.79	10.66 ± 3.01	14.02 ± 3.88

*Data are given as mean ± S.E.M.
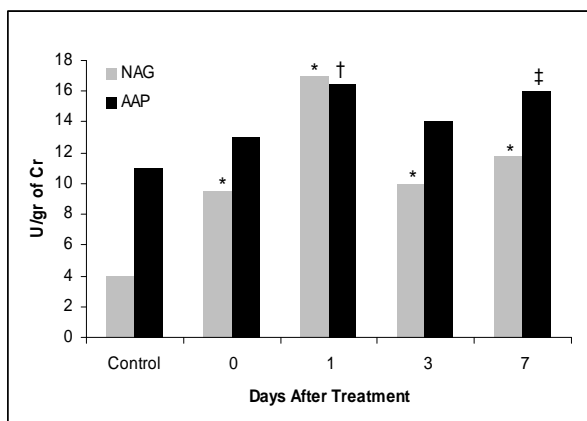
Fig. 1. Urinary NAG and AAP excretion in patients with nephrolithiasis treated with ESWL. *, $P < 0.02$; †, $P < 0.05$; ‡, $P < 0.01$; compared to control group.
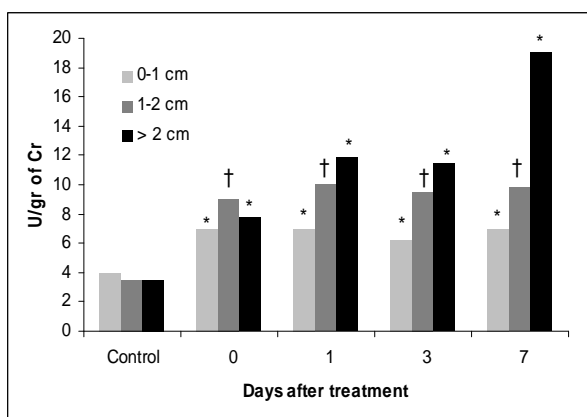

Fig. 2. Correlation between urinary AAP excretion and stone size. *, $P < 0.01$; †, $P < 0.001$; compared to control group.
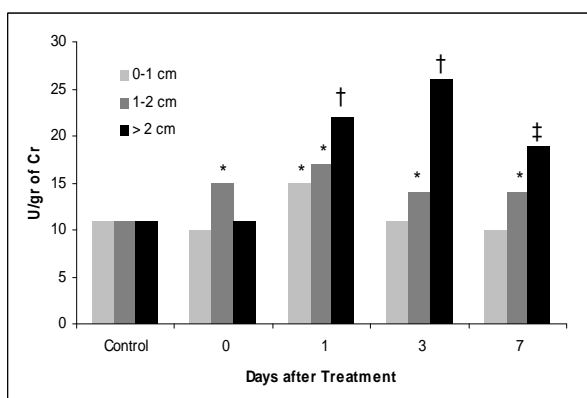

Fig. 3. Correlation between urinary NAG activities and stone size. *, $P < 0.05$; †, $P < 0.001$; ‡, $P < 0.003$; compared to control group.

DISCUSSION

Although ESWL in nephrolithiasis is currently considered as the preferred procedure compared to open surgery, there are evidences which indicate that some parenchymal and functional injuries will occur after ESWL. However absence of scar formation in this procedure and its easy performance without any problem makes the compliance in patients (5). Urinary enzyme testing has been used by many investigators to diagnose and monitor various types of renal injury and it has been frequently shown that these tests are more valuable than markers including measurable changes in glomerular filtration or tubular function (3).

The shock wave number or power in cases treated with ESWL as well as the number of renal puncture in PCN group did not show any significant changes in post treatment NAG levels in comparison with patients who were treated, but the later group had significant and prolonged increase of postoperative NAG levels (6).

NAG (a lysosomal enzyme) is basically found in renal tissue and its greatest activity is in this organ. Although it exists in blood circulation, its presence in urine originates from the kidney since its high molecular weight does not let it pass through the glomerulus even in pathological conditions of increased glomerular permeability. The urinary activity of this enzyme correlates with severity of renal injury; hence it is used as a marker for assessment of renal injury due to nephrotoxic drugs, secondly renal complications of some chronic diseases, nephrotoxic heavy metals and in cases of kidney transplantation, to warn patients who are in danger of rejection and also ESWL (1, 3, 4, 7).

AAP and gamma glutamyl transpeptidase (GGT) are two other appropriate markers for the evaluation of renal injuries which have high activity in renal tissues particularly the membranous brush border of tubular cells. In this study we used the urinary activity of the first one. Like NAG, AAP is also an enzyme with high molecular weight which cannot pass through glomerular membrane. Hence, its presence in urine denotes its renal origin as it was mentioned in the previous part, urinary activity of these two enzymes were higher before lithotripsy

compared to control group and in case of NAG this difference was statistically significant ($P < 0.001$). These findings are similar to results of Karlin (1) and Assimos *et al.* (3). Our study shows that urinary activity of NAG and AAP is high during the first 24 hours after lithotripsy. These results are consistent with what Kaplan *et al.* found in their study with GGT (the enzyme of the membranous brush border of tubular cells that its excretion is similar to AAP) and NAG. Karlin (1) and Sens *et al.* (8) in their investigation on the effects of ESWL on kidney parenchymal damage used a number of markers including NAG and demonstrated that urinary excretion of this enzyme elevated significantly after ESWL. However, Karlin's study revealed that this increment does not last for more than 7 days and both concluded that the resulted damage is of limited magnitude and causes no deterioration in renal function. Similar results were acquired by other authors (9, 10). Akdas *et al.* in their study on short term bioeffects of ESWL on 32 patients evaluated 8 different markers including NAG and concluded that because of transient glomerular and tubular damage after ESWL, the minimum interval between two shockwave treatments should be at least 7 days (11).

There was significant correlation between urinary NAG and the number of pulses in third by after ESWL. Tubular damages and that the tubular damages might last longer than 7 days, although these functional changes recovered within one week. The changes were related to the energy level of shock wave and the degree of renal damage would increase when energy level was above 12.5 kV and 2500 pulses (12). Surprisingly, our study revealed that the severity of kidney parenchymal injury evaluated by urinary activity of the mentioned enzymes correlated with the size of stone. There were some patients in this study who didn't show any significant changes in urinary excretion of NAG and AAP in spite of having kidney stones as large as 1.5-2 cm. It could be interpreted in two ways: first urinary concentration of the enzymes produced by the damaged kidney can be diluted by the urine yield of the other kidney, and second; the damaged kidney may produce less urine, compared with the other kidney, because of relative obstruction. In

conclusion, we would like to emphasize that patients with larger stones (> 2 cm) show higher urinary enzyme activity and they are at greater risk for kidney parenchymal damages. Considering our findings that urinary excretion of these enzymes sometimes does not reach normal even 7 days after lithotripsy, it is highly recommended that for the next lithotripsy, at least one week after previous one, the urinary levels of these enzymes be assessed and in case being higher than normal, the procedure should probably be postponed to avoid further damage to the kidney parenchyma.

Conflict of interests

We have no conflict of interests.

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