Leucine aminopeptidase enzymuria: Quantification of renal tubular damage following extracorporeal shock wave lithotripsy

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To evaluate the extend of renal parenchymal injury following high energy shock wave (HESW) application we measured the urine levels of two renal tubulus brush border enzymes: Leucine Aminopeptidase (LAP) and Gama-Glutamyltranspeptidase. The measurements were taken the day before, 24-hours and 7 days after extracorporeal shock wave lithotripsy (ESWL) in 23 patients. All patients had caliceal stones and treated with Dornier MPL 9000 lithotriptor under sedoanalgesia. Creatinine concentration of each sample together with Total Shock Wave Effect (TSWE) values were also assessed. Our results indicated a statistically significant rise in urinary excretion of both enzymes after 24-hours following lithotripsy ($p<0.05$). All these values returned to normal limits within 7 days after ESWL ($p>0.05$). Transient tubular damage due to HESW was found to be related to TSWE values assessed in our group. [Turk J Med Res 1996; 14(1): 16-18]

Key Words: ESWL, Tubulopathy, Leucine aminopeptidase, y-Glutamyltranspeptidase

Extracorporeal shock wave lithotripsy (ESWL) has become the preferred treatment modality for the majority of urinary calculi with its effective and successful results (1). However increasing experience in this field has shown that the rapid adoption and acceptance of ESWL was facilitated by its perception as an entirely safe treatment devoid of side effects. Now, however there is abundant evidence to show that ESWL can cause some serious side effects on the function (2-5) and morphology of treated kidneys (5). Although the target of shock waves is the stone located in different parts of kidney, the surrounding renal parenchyma is also subjected to trauma during ESWL treatment. Apart from gross hematuria, intraparenchymal-perirenal hemorrhage and loss of corticomedullary junction, alteration in effective renal plasma flow and glomerular filtration rates have been reported in a number of studies.

Taking the limited damage to the focused area into account, early diagnosis of the extend of this damage can only be assessed by changes of very sensitive indicators such as excretion of urinary enzymes (4,6-8). Related with this subject oxyreductases such as lactic dehydrogenase (LDH), its isoenzymes, transferases, peptidases particularly aminopeptidases and a variety of lysosomal glycosidases including p-glucronidase, p-galactosidase and N-acetyl-p-D-glucosaminidase are highly evaluated as urinary markers in the literature (8,9). Leucine aminopeptidase (LAP) being located in the brush border membranes of proximal tubuli, has a molecular weight of 300.000 dalton and has found not be filtered from glomerules. Studies concerning its excretion in urine revealed that under some pathologic conditions such as toxic renal damage, acute inflammatory disease, hypoxic renal damage and tumors of kidney, excretion rate of this enzyme considerably increases (10,11).

In this present prospective study, to quantitate the extend of acute parenchymal injury following high energy shock wave application (HESW), urine levels of LAP and Y-dutamyltranspeptidase have been assessed the day before, one day and one week after ESWL.

MATERIALS AND METHODS

23 patients with caliceal calculi (16 men, 7 women) were treated in our ESWL unit between October 1993 and March 1994. Age of the patients ranged from 16 to 66 years with an avarage value of 45.5 years. All of the treatments were performed with Dornier MPL 9000

Received: Aug. 15,1995 Accepted: Dec. 16,1995

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lithotriptor under sedoanalgesia. Stone sizes was less than 25 mm in all patients.

Following a standart preparation and application of ESWL procedure; based on plain abdominal X-ray film and renal ultrasound findings, we observed no obstructed renal unit by fragmented stone particles after 24- to 48 hours post-lithotripsy. No urethral catheterization was performed. Assessment of LAP in urine was performed in 24-hours collected urine specimens, collected the day before, one day and one week after ESWL procedure. Three patients were excluded from the study because of macroscopic hematuria (hemoglobin >20 mg/dl in urine) in two patients and menstruation in one patient, following ESWL.

Measurements of LAP (3.4.11.2) and y-Glutamyltranspeptidase (2.3.2.2) were performed in the second morning-mid stream urine samples. Urine samples were centrifugated (3000xg, 10 min) and dialyzed against tap water for 8 hours at 4°C. Urinary LAP levels were determined as described by Patterson E.K. with SIGMA® Diagnostics Leucine Aminopeptidase Kit (procedure no:251) (6). Gamma-Glutamyl transpeptidase levels were measured by the method described by Szazs et al (12). On the other hand, creatinine concentration of each urine sample was also determined in order to comparatively evaluate variations caused by changes in urine flow.

Statistical significance evaluation was made with Wilcoxon Rank test.

RESULTS

I. ESWL Parameteres

Number of shock waves ranged from 1754 to 2859 with an avarage value of 1986. Again, electrical discharge (Kv) was between 18-21 kv (mean 19.4 kv). On the other hand total shock wave effect (TSWE) for our lithotriptor was calculated as No. of shock waves X Electrical discharge value for each patient. Evaluation of ESWL parameters and TSWE are presented in Table 1.

II. Urinary Enzyme Excretion

Evaluation of our results indicated a significant alteration with respect to urinary enzyme excretion 24-hours after lithotripsy ($p<0.05$). However, comparative evaluation of LAP and y-GGT values revealed no statistically significant alteration after 7 days following shock wave application ($p>0.05$) (Table 2). Evident increase in urinary levels of both enzymes has been observed parallel to the increase in TSWE in our group.

DISCUSSION

Although ESWL procedure has become the first treatment choice in the non-invasive management of urinary stones, its biological side effects on functioning renal units are not fully known and have to be evaluated in both clinical and experimental studies. Currently, radiosonographic evaluation has not been found to be sufficiently sensitive in order to detect the extend of focal parenchymal injury after high energy shock wave (HESW) application (13,14). Studies in this field revealed that measurement of urinary enzyme levels, as very sensitive indicators of renal parenchymal damage, may be helpful in the quantification of such injuries. Several urinary proteins and specific enzymes have been evaluated as an injury marker following HESW application (6,7,12-15). Among these enzymes N-Acettyl-p-D-Glucosaminidase, y-Glutamyl-transpeptidase (GGT), p-galactosidase and Lactate dehydrogenase (LDH) have been the most commonly subjected ones (8).

Table 1. ESWL parameters

No of shock waves	1754-2859 (mean: 1986)
KVvalue	18-21 KV (mean: 19.1)
Total shock wave effect	21016-44098 (mean: 33669)

Table 2. Evaluation of urinary enzyme activity before and after ESWL

	Before	24-hours	7-days
LAP (Ul/ml/Cre)	5.116+2.17	6.26±2.34	3.840±1.035
Gama-GGT (Ul/ml/Cre)	18.86±6.45	24.65+5.49	16.85±4.05
P*		<0.05	0.05

•Wilcoxon Rank Test

However conflicting data have been reported concerning urinary enzyme levels before and after shock wave application. While some authors have found shock wave application to cause increase in urinary enzyme levels (2-5), Jung et al were not able to show any change in the output of urinary enzymes but that of LDH (15).

Leucine Aminopeptidase (LAP) has been found to be located in the brush border membranes of proximal tubulus and its activity in urine has been extensively studied both in clinical and experimental studies (11). Under some definite pathologic conditions, such as toxic renal damage (mercuric chloride or snake venoms), drug induced renal injury, renal infections and pregnancy itself, urinary excretion of LAP has been found to be increased (10). Johnstone et al were able to show increased LAP excretion in 14 of 35 hypertensive cases (40%), on the other hand they observed such increase in only 6 of 66 cases without renal disease (8%) (16).

In our present study we aimed to quantificate the extend of renal tubular injury by measuring the urinary

levels of two different enzymes such as LAP and GGT. Our results indicated an transient increase in the urinary out-put of both enzymes which returned to normal range in 7 days after ESWL Increase in the urinary enzymes has been found to be more significant during 0-24 hour follow-up evaluation after ESWL. On the other hand by measuring the same enzyme levels some authors did not find any alteration (17). Taking the localization of both enzymes in proximal tubulus into account, GGT is localized more deeply in the brush border membrane and is more sensitive to toxic or inflammatory events than LAP (9). For that reason, GGT levels increased more prominently than LAP despite minimal TSWE degree. Parallel to the findings of some studies (18,19) in our study also the number of shock waves and output voltage value in other words TSWE degree has been found to be effective in the excretion rate of both enzymes. However Karlsen et a), were able to show no significant effect of these factors on the excretion of N-Acetyl-Glycosaminoglycan and Alkaline phosphatase in ESWL subjected patients (4).

In the lights of our findings, selective urinary excretion of some enzymes in individuals undergoing ESWL can be used during monitorization of shock wave induced renal injury. Every patients must be well monitorized on this aspect in order to evaluate the exact changes in renal morphology and function. Urinary excretion of y-GGT and LAP on this aspect showed a transient alteration in tubular function in our study group. However we believe that with larger series, including other definite parameters are certainly needed In order to get more discrete findings regarding adverse effects of high energy shock waves.

Lözin aminopeptidaz enzimüri:
Ekstrakorporeal litotripsi sonrası
renal tübüler hasarın değerlendirilmesi

Ekstrakorporeal şok dalgası Htotripsisi (ESWL) uygulanımı sonrası oluşabilecek renal parankima! hasarın boyutunu değerlendirebilmek amacıyla 2 renal tübülüs fırçamsı kenar enzim (Lözin aminopeptidaz ve Gama-glutamiltranspeptidaz) ölçümleri yapıldı. Bu ölçümler ESWL uygulanan 23 hastadan, ESWL'den 1 gün önce, 24 saat sonra ve 7 gün sonra olmak üzere yapıldı. Tüm hastalarda kaliks taşları mevcut idi ve hepsinin tedavileri Dornier MPL 9000 litotriptörü ile sedoanaljezi altında yapıldı. Kreatinin konsantrasyonları ve Total Şok Dalgası Etkisi (TŞDE) değerleri enzimüri seviyeleri ile birlikte değerlendirildi. Her iki enzimin üriner ekskresyonları ESWL'den 24 saat sonra istatistiksel olarak anlamlı düzeyde artmış olarak bulundu (p<0.05). Tüm enzim değerleri ESWL'den 7 gün sonra normal seviyelere dönmüş olarak bulundu (p>0.05). Bizim hasta grubumuzda ESWL sonrası oluşan geçici tübüler hasarın TŞDE değerleri ile İlişkili olduğu kanısına varıldı.
[Türk J Med Res 1996; 14(1):16-18]

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