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Evaluating ESWL related alterations in renal pelvis and proximal ureter by analayting of tissue hydroxyproline levels

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ABSTRACT

The aim of the study is to evaluate extracorporeal shock wave lithotripsy (ESWL) related alterations in renal pelvis and proximal ureter by using histopathological methods and analayting hydroxy-proline levels. Twelve New-Zealand rabbits were allocated into two groups (n=6). Right sites of control group (CG, n=6) were harvested without any intervention. In ESWL group (EG), right kidneys of subjects were exposed to 3000 shock waves (14 kV) by using electro-hydraulic type ESWL device three times. Rabbits in EG were sacrificed on day 7. Tissues were examined histopathologically for presence of edema, inflammation, congestion, hemorrhage, fibrosis, vascularization and biochemically for hydroxyproline concentrations. Histopathologically, tissue edema was increased in renal pelvises and inflammation was increased in ureters in the EG compared to that in the CG ($p<0.5$). There was no difference in other parameters between the groups ($p>0.05$). Tissue collagen density did not show any significant difference ($p>0.05$). There was no difference in the tissue hydroxyl-proline levels of ureter samples ($p>0.05$). Tissue hydroxyproline levels were significantly higher in EG than CG in renal pelvis ($p<0.05$). In conclusion, although no major histopathological alteration due to ESWL was detected in renal pelvis and proximal ureter, increased hydroxyproline levels in the renal pelvis can be suggested as a finding of tissue injury in collecting system.

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1. Introduction

Since extracorporeal shock wave lithotripsy (ESWL) was introduced for the treatment of urolithiasis in 1980, it has been used as the first choice of treatment in pediatric renal and proximal ureter stones (Chaussy et al., 1984; Skolarikos et al., 2006; Minevich, 2010; Gnessin et al., 2012). ESWL is known as a non-invasive and effective method with high success rate (Skolarikos et al., 2006). However, ESWL is not considered as a completely safe procedure (Skolarikos et al., 2006). Several studies demonstrated the deleterious effects of shock waves to the renal and extra-renal tissues (Skolarikos et al., 2006; Gnessin et al., 2012; Goktas et al., 2012). The shock waves have been reported to cause traumatic vascular injury, ischemic renal injury and intraparenchymal hemorrhage

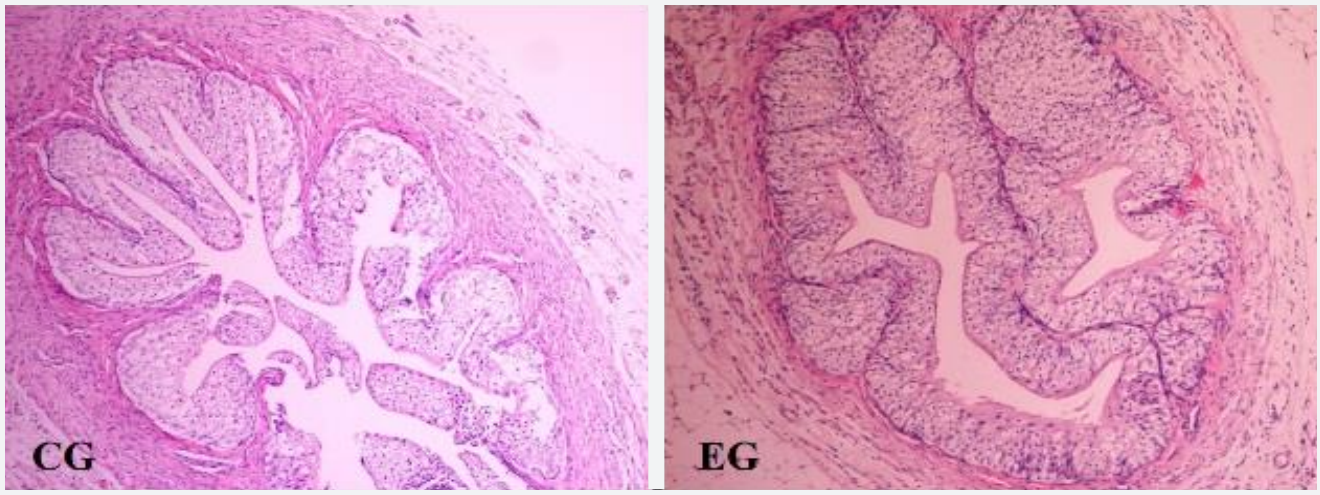

Fig. 1. Histopathologic evaluation of renal pelvis samples (p>0.05). (H&E, x 100, **CG:** Control group; **EG:** ESWL group)

(Skolarikos et al., 2006; Goktas et al., 2012). The injuries to endothelium of vessels and glomerular capillaries, nephrons, and tubules have been demonstrated and these effects were attributed to ESWL related hypertension and renal function loss as a long term sequel of ESWL (Skolarikos et al., 2006).

Although effects of ESWL on renal parenchyma are well known, its effect on extrarenal collecting system has not been evaluated in detail previously. There are a few studies regarding urinary flow obstruction causing low levels of stone-free rate after ESWL (Skolarikos et al., 2006). It was also reported in a clinical study that ureteral stricture after several sessions of ESWL may be seen (Finter et al., 2007).

An experimental study was carried out to evaluate the ESWL related alterations in renal pelvis and proximal ureter by histopathologic methods and hydroxyproline (OH-proline) levels.

2. Materials and methods

The experiments were performed in adherence to the Declaration of Helsinki and by approval of the Local Ethical Committee of Kırıkkale University (2011/11).

Twelve New Zealand rabbits weighting 2500-3000 grams were allocated to two groups (n=6). After fasting overnight, rabbits were anesthetized with intramuscular ketamine hydrochloride (50 mg/kg, Ketalar, Eczacıbaşı, İstanbul, Turkey). Right renal pelvis and proximal ureter of control group (CG, n=6) were harvested without any intervention. In the ESWL group (EG), right kidneys of subjects were exposed to 3000 shock waves at 14 kV energy by using electro-hydraulic type, 3rd generation Stonelith V5 ESWL device (PCK, Ankara, Turkey) three times every other day with a total 6 day treatment period. The ESWL was performed by the same person each time. Ultrasonographic probing was performed to localize the right kidney. During this treatment period the rabbits were kept warm and in same conditions with standard feeding. Rabbits in EG, were sacrificed on day 7 and renal pelvis and proximal ureter were harvested.

Tissues were examined histopathologically for the presence of edema, inflammation, congestion, hemorrhage, fibrosis and vascularization. Tissue collagen levels were also compared between groups. Biochemical analysis of tissue samples were performed for OH-proline concentrations.

The data obtained from the experiments were analyzed with Mann Whitney U test (SPSS 15.0). The p values lower than 0.05 were considered as significant.

Histopathological evaluations

The preserved samples were fixed in 10% formalin. All segments were embedded in paraffin block after tissue processing. Tissues were sectioned in 4-5 μm pieces and stained with routine hematoxyline-eosine stain. The specimens were examined under a light microscope (Leica, Wetzlar, Germany) by the same pathologist who was blind to the study.

Tissues were stained with Masson's Trichrome stain in order to examine the tissue collagen density.

Histopathologic findings were graded semiquantitively for each parameter separately as following: Grade 0, normal; grade 1; mild, grade 2; moderate and grade 3; severe.

Determination of hydroxyproline levels

All samples were preserved in a deep freezer (-80°C) and washed with 0.9% NaCl. The tissues were homogenized (Labor Technique, Müllheim, Germany) with 0.9% NaCl solution. The homogenized samples were centrifuged at 1,500 g for 10 minutes at 4°C. Proteins were determined with supernatants. The OH-proline levels in tissue samples were measured by the spectrophotometric method as defined by Bergman and Loxley (Bergman and Loxley, 1970). The results were expressed in μg/mg protein.

Table 1. The median values of the histopathological grades (inter-quartile range within brackets).

Groups	Edema	Inflammation	Congestion	Hemorrhage	Fibrosis	Vascularization
CG-Renal pelvis	0 * (0-1)	1 (0.75-1)	1 (1-1)	0 (0-0.25)	0 (0-0)	0 (0-0)
EG-Renal pelvis	1 * (0-1.5)	1 (0.75-3)	1 (1-1)	0 (0-0.25)	0 (0-0.25)	0 (0-0)
CG-Ureter	0 (0-0)	0 β (0-0.25)	1 (1-1)	0 (0-0.25)	0 (0-0)	0 (0-0)
EG- Ureter	0 (0-0.25)	1 β (0-1)	1 (0.75-1)	0 (0-0)	0 (0-0)	0 (0-0)

***;** p<0.05; **β;** p<0.05; **EG:** ESWL group; **CG:** Control group

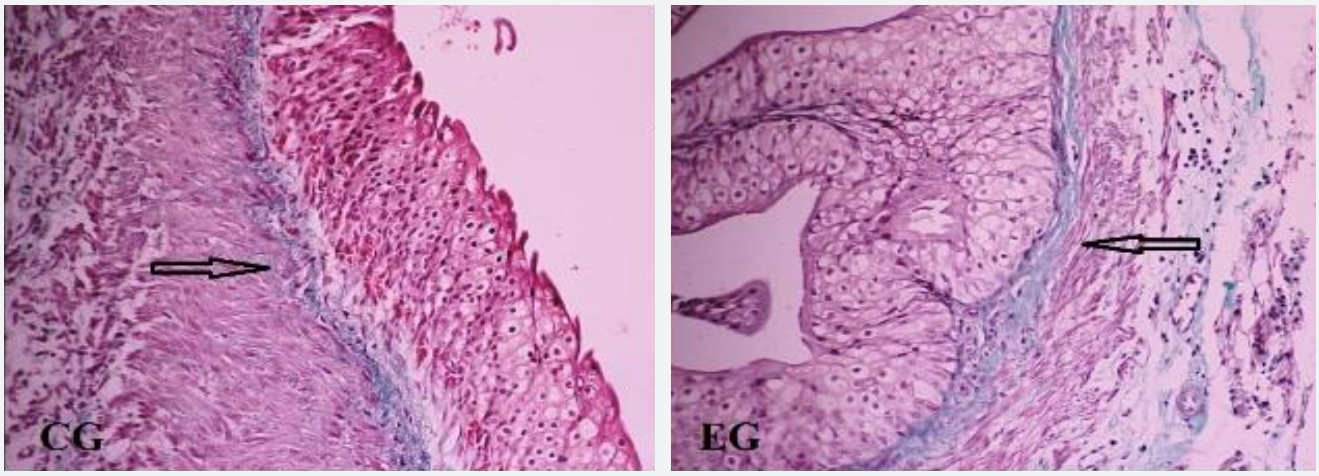

Fig. 2. Histopathologic evaluation of renal pelvis samples for tissue collagen density (p>0.05). (Masson's Trichrome, x 200; **CG:** Control group; **EG:** ESWL group; **Arrows:** Collagen deposition)

3. Results

The median values of the histopathological grades were given in Table 1. Histopathological evaluations in both renal pelvis and ureter (Fig. 1) showed no difference between groups for congestion, hemorrhage, fibrosis and vascularization grades (p>0.05). However, tissue edema was found increased in renal pelvis and inflammation was increased in ureters in EG compared to that in CG (p<0.05). The tissue collagen density did not show any significant difference (p>0.05) (Fig. 2).

The median of tissue hydroxyproline levels of ureters was 0.39 μg/mg protein (0.33-0.45) in CG and 0.37 μg/mg protein (0.32-0.44) in EG. The median of tissue hydroxyproline levels of pelvises was 0.33 μg/mg protein (0.32-0.37) in CG and 0.39 μg/mg protein (0.34-0.47) in EG. When tissue hydroxyproline levels were compared, no difference was found in ureter samples (p>0.05). However, tissue hydroxyproline levels in renal pelvis were significantly higher in EG than CG (p<0.05) (Fig. 3). Any complication or death due to ESWL procedure was not been observed in the experiments.

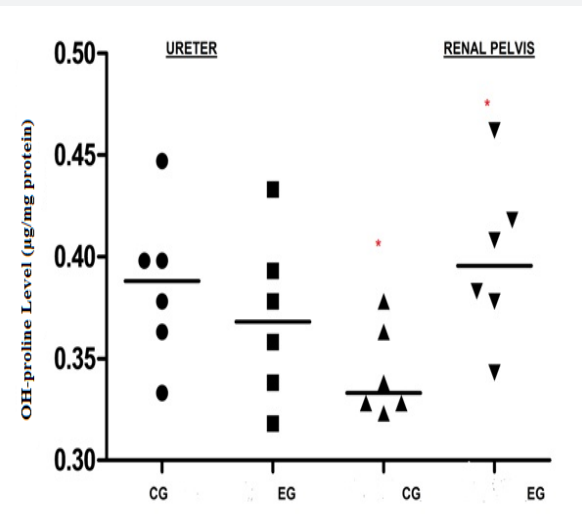

Fig. 3. The comparison of tissue hydroxyproline levels in proximal ureter and renal pelvis samples (* p<0.05).

4. Discussion

Although ESWL is the first choice treatment modality in renal and proximal ureter stones, it has several deleterious effects to the renal and extra-renal tissues showed in many studies (Skolarikos et al., 2006; Minevich, 2010; Gnessin et al., 2012; Goktas et al., 2012). The shock-wave induced renal parenchymal and extra-renal tissue injuries have been studied before, and are all well-known. However, the effects of shock waves to the extra-renal collecting system have not been studied before except a few clinical studies revealing ureteral structure and urinary flow obstruction after ESWL treatment (Skolarikos et al., 2006; Finter et al., 2007).

The shock-wave induced complications are related to the damage to thin-walled vessels resulting hemorrhage in the parenchyma of kidneys and adjacent tissues (Skolarikos et al., 2006). Another responsible mechanism is thought to be the oxidative damage and free radical formation (Serel et al., 2004; Clark et al., 2009). The shock-waves cause hemolytic cleavage of molecules and increases free radical formation causing damage to the cells (Serel et al., 2004). Also, after ESWL renal vasoconstriction takes place resulting ischemic and hypoxic damage to the renal parenchyma (Clark et al., 2009). The inflammatory response to all this process takes place and causes chronic functional loss and scar formation which is responsible for the long-term consequences of ESWL (Skolarikos et al., 2006). Although it is well known that microvasculature, renal tubules and vessels are more vulnerable to energy discharge after ESWL, the effect of shock waves to extra-renal collecting system is not well known (Skolarikos et al., 2006). The calculi localized in mid to distal ureter have been avoided to be managed with ESWL because of lower success rate and possible complications related to sacroiliac joints and gonads (Minevich 2010). However, complications related to extra-renal collecting system have not been pronounced for management of mid to distal ureteral calculi.

Although there is no consensus about the ESWL dosage, there are some studies in the literature revealing that more than one session of ESWL (up to 3 sessions), each of which is consisting about 1800 to 2000 shock waves (up to 4000) may be needed for a better stone-free rate (McAdams and Shulka, 2010; Minevich, 2010; Gnessin et al., 2012). Therefore, we performed ESWL in 3000 shock waves for three times in the present study. Although it seems that it is a too large dose to be performed, the results suggest it was just sufficient to cause histopathological changes.

We examined the ESWL related alterations in renal pelvis and proximal ureter by histopathologic methods in the present study. We could not detect any significant difference in renal pelvis and ureter histopathologically between groups except tissue edema and inflammation. We also examined the collagen deposition in extra-renal collecting system. The collagen, the main component of the extracellular matrix, is also a key point in wound healing (Somuncu et al., 2006). The OH-proline is a constitutional component of collagen. The measurement of OH-proline levels is an important way to examine collagen turnover (Klinge et al., 2000; Somuncu et al., 2006). Thus, we also measured the tissue OH-proline levels in the present study. Although we could not detect any significant difference regarding to collagen deposition, we found that OH-proline levels in renal pelvis was significantly higher in EG. The measurement of OH-proline levels of urethral tissue in strictured urethra has been studied by Baskin et al (Baskin et al., 1993), but no significant difference in total collagen count could be detected. This is the first study that OH-proline levels were investigated in ureter and renal pelvis after ESWL.

The increased level of OH-proline levels in renal pelvis in EG may be an indicator of shock-wave induced tissue injury in our study. The reason of the inability to confirm this injury with histopathologic examination may be the close time between shock wave exposure and tissue sampling. Some studies showed that the initial renal damage resolves over days to months (Williams et al., 1998; Carvalho et al., 2009). Since we took the samples at 7th day we would be late to show any histopathologic alteration in tissues. The increased level of OH-proline in renal pelvis shows that damage had been occurred after ESWL and the healing process started to take place by increased collagen turnover. However, the effect of this result to the long-term consequences of ESWL should be examined in future studies.

In conclusion, although no major histopathologic alteration due to ESWL was detected in renal pelvis and proximal ureter, increased OH-proline levels in renal pelvis can be suggested as a finding of tissue injury in the collecting system.

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