



## HEAVILY SCARRED REFLUXING RENAL UNITS HAVE SIGNIFICANTLY DIFFERENT PATHOLOGIC FEATURES AT THE DISTAL END OF THE RELATED URETER COMPARED TO THE URETERS OF LESS SCARRED RENAL UNITS

### AĞIR SKARLI BÖBREKLERİN ÜRETER ALT UÇ PATOLOJİK ÖZELLİKLERİ AZ SKARLI TARAFLA KARŞILAŞTIRILDIĞINDA BELİRGİN FARKLILIK GÖSTERMEKTEDİR

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#### Abstract

**Objective:** This study aims to describe the pathological features of the distal end of ureters and their correlation with scar formation patterns in the kidney.

**Methods:** The study includes 83 children (49 male, 34 female), who underwent ureteroneocystostomy (UNC) operations from 2008-2010. The distal end of ureters (approximately 3-4 mm) were resected and 110 ureter specimens from 83 patients were available for pathological examination. The presentation age, clinical presentation pattern, reflux grade and scar patterns on dimercaptosuccinic acid (DMSA) scan were obtained from record and correlation with histopathological findings were investigated. Scar patterns were defined as presence of no scar (NS), diffuse scar (DS) or focal cortical scars (FS). General structural changes were also investigated histopathologically including inflammatory changes graded for intensity (G1-G3), extracellular matrix and smooth muscle content, ureteric diameter and wall thickness.

**Results:** Histological examination of the ureterorenal units showed that there were 35/110 (33%), 30/110 (27%) and 45/110 (40%) in the NS, DS and FS groups, respectively. When scar rates were investigated by grade of reflux (G1-2 low grade vs. G3-5 high grade reflux) the scar rates were 39.5% and 90.3% respectively. High grade inflammation (G3) was found in 66%, 28.9% and 36.4% in kidneys in the DS, FS and NS scar groups respectively ( $p < 0.05$ ).

**Conclusion:** This investigation shows that there are significant differences of histologic structure of the distal end of the ureters when they are classified by kidney scarring. There is more intense inflammation, more collagen deposition, less smooth muscle content and increased ureteric wall thickness in the ureters of the kidneys with diffuse scar when compared to others with less or no scar.

**Keywords:** Vesicoureteral reflux, kidney scar, ureter

#### Öz

**Amaç:** Bu çalışma, üreterlerin distal ucunun patolojik özelliklerinin böbrekteki skar oluşum patternleri ile olan ilişkisini araştırmayı amaçlamaktadır.

**Yöntem:** VUR tanısıyla takip edilen ve 2008-2010 yılları arası bilateral ve ünilateral üreteroneocistostomi (UNC) ameliyatı yapılan 83 çocuk hasta (49 erkek, 34 kız) değerlendirildi. UNC yapılan taraf üreter alt ucu rezeke edildi ve 83 hastanın toplam 110 adet üreter alt ucu spesimeni, mevcut klinik ve patolojik bulgularla beraber değerlendirildi. Hasta yaşı, klinik patterni, reflü derecesi, DMSA'daki skar patterni üreter alt ucu histopatolojik yapısıyla birlikte değerlendirildi. Skar patternleri skarsız, diffüz skar, fokal skar olmak üzere ayrıldı. Histopatolojik değerlendirme sırasında inflamasyon Grade 1-3 olarak derecelendirildi. Ekstrasellüler matriks, düz kas oranı ve üreter çapı ve duvar kalınlığı değerlendirildi.

**Bulgular:** Toplam 110 üreterorenal ünitenin otuz beşinde (%33) skar yoktu. Otuzunda (%27) diffüz skar ve 45 ünite (%40) fokal skar tespit edildi. Skar oranları düşük dereceli reflü grubu (G1-2) ve yüksek dereceli reflü grubunda (G3-4-5) sırasıyla %39,5 ve %90,3 olarak bulundu. Skar oranları reflü derecesine (G1-2 düşük vs. G 3-5 yüksek) göre değerlendirildiğinde skar oranları sırasıyla %39,5 ve %90,3 olarak bulundu. İnflamasyonun en yüksek olarak değerlendirildiği grade 3 inflamasyon diffüz skar, fokal skar ve skarın olmadığı grupta sırasıyla %66, %28,9 ve %36,4 olarak bulundu ( $p < 0,05$ ).

**Sonuç:** Çalışmamızda gruplardaki hastalar skar derecesine göre sınıflandığında üreter alt uç patolojileri arasında belirgin fark gözlenmiştir. Diffüz skarı bulunan grupta inflamasyon fokal skarı bulunan ve skarı bulunmayan gruba göre fazladır. Aynı zamanda bu grupta kollajen miktarının, üreter duvar kalınlığının, inflamasyon şiddetinin fazlalığı görülmüştür. Buna bağlı olduğu düşünülen kollajen depozisyonunun artışı ve mevcut olan düz kas miktarının belirgin olarak azaldığı belirlenmiştir.

**Anahtar Kelimeler:** Vesikoureteral reflü, renal skar, üreter

## Introduction

Vesicoureteral reflux (VUR) is reflux of urine from the bladder to the upper urinary tract. VUR is seen in 1% of children.<sup>1</sup> Although the etiology and natural history of VUR is not clear it is a common risk factor for febrile urinary tract infections (UTI) and kidney damage. Renal scarring and hypertension leading to renal failure have been described as the classical pathophysiological complications of VUR.<sup>2</sup> The structure of the ureterovesical junction (UVJ) has an important association with VUR and has thus been the focus of much research.<sup>3-7</sup> Studies in kidneys with VUR have investigated the replacement of extracellular matrix with smooth muscle, disorganization of smooth muscle bundles,<sup>4</sup> metabolic changes such as accumulation of phagocytes and impaired vascularization,<sup>5</sup> loss of neural elements, ganglionic cells and gap junctions,<sup>8</sup> micro vessel density and the expression of vascular endothelial growth factor (VEGF),<sup>7</sup> the role of cytokines in promoting fibrosis and tissue destruction<sup>9</sup> and prenatal and postnatal neuromuscular developmental changes<sup>10</sup> at the distal end of the ureters. However, there is no study of the correlation between pathological features of the distal end of ureters with respect to scar formation patterns in the upper urinary tract. Thus, this study was designed to provide an insight into the developmental changes of the whole refluxing renal unit by investigating the pathological features of the distal end of ureters and correlation with scar formation patterns in the kidney.

## Methods

### Patients

Institutional ethical committee approval (approval number 2008/013) for the study was granted. The study included 83 patients who underwent unilateral or bilateral ureteroneocystostomy (UNC) operations at Hacettepe University Department of Urology between 2008-2010. All patients had been evaluated with ultrasound (US), voiding cystourethrography (VCU) and diuretic renal scan using dimercaptosuccinic acid (DMSA). The distal ends of ureters, measuring approximately 3-4 mm, were resected during the reimplantation operation. The final number of ureteral units available for histopathological examination was 110. Presentation age, clinical presentation patterns, reflux grade, and scar formation patterns on DMSA were obtained from records and correlation with the histopathological examinations was investigated.

### Scar formation patterns

DMSA was performed in all patients. Scar formation patterns were defined as: a presence of a focal scar (FS) (Figure 1), diffuse scar (DS) (Figure 2) or no scar (NS).

### Pathologic examination of distal end of ureters

A Ureteral endings excised from the patients were placed in 10% formalin and sent to the Pathology Laboratory. After 24-hour formalin fixation and following routine tissue processing, specimens were sectioned (5 µm thickness) and examined by an experienced histopathologist. Ureteral wall thickness, mucosal loop thickness, ureteric diameter, presence of oedema or inflammation/inflammatory cells, thickness of the muscular layer, presence or absence of hypertrophy in the muscular layer, and the presence of increase in collagen fibers were evaluated histologically (Figure 3).

Ureteral wall thickness, ureteral loop thickness and ureteric diameter were measured using the SPOT software program.<sup>11</sup> Inflammation was separated into three grades depending on the degree of inflammatory reaction:

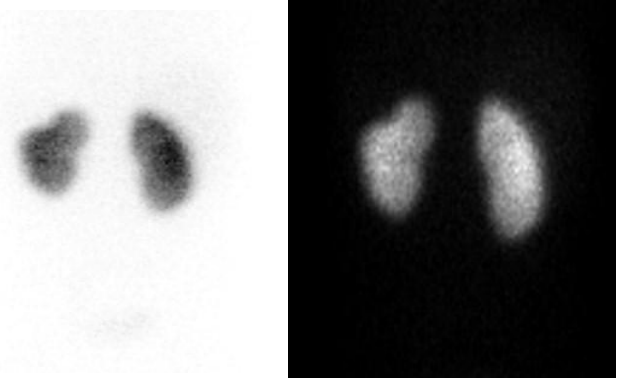
Grade 1: There is no inflammatory reaction or there are only very few lymphocytes (Figure 4)

Grade 2: Perivascular, scattered lymphocytes (Figure 5)

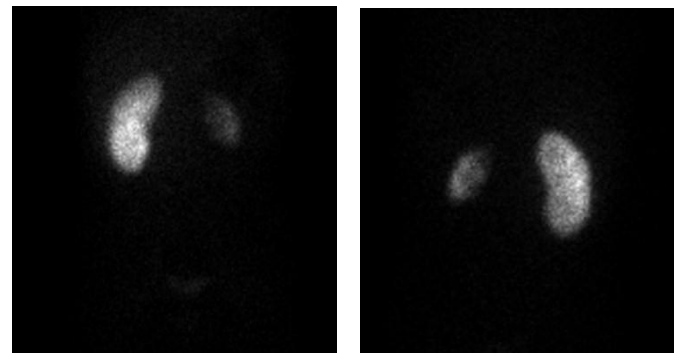
Grade 3: Scattered and moderate number of lymphocytes (Figure 6).

Inflammation was then classified as mild (Grade 1) or severe (Grade 2+3). The presence of lymphoid follicles was noted as a separate criterion.

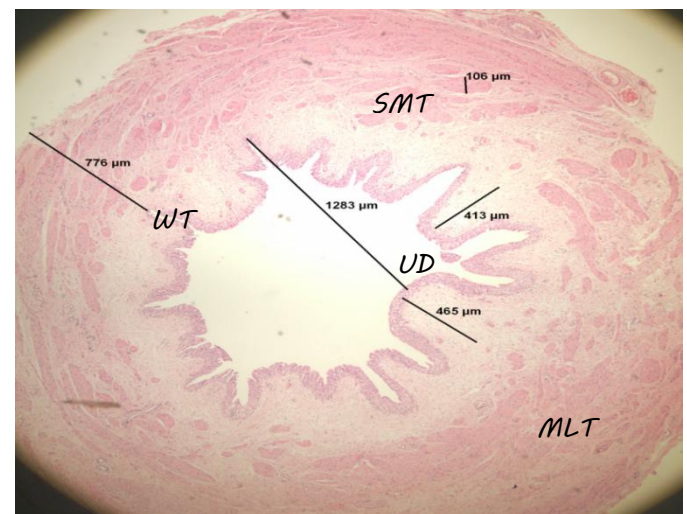
Collagen deposition and muscular hypertrophy are shown in Figure 7 and Figure 8, respectively.



**Figure 1.** Focal renal scar on the top of a kidney (DMSA scan).

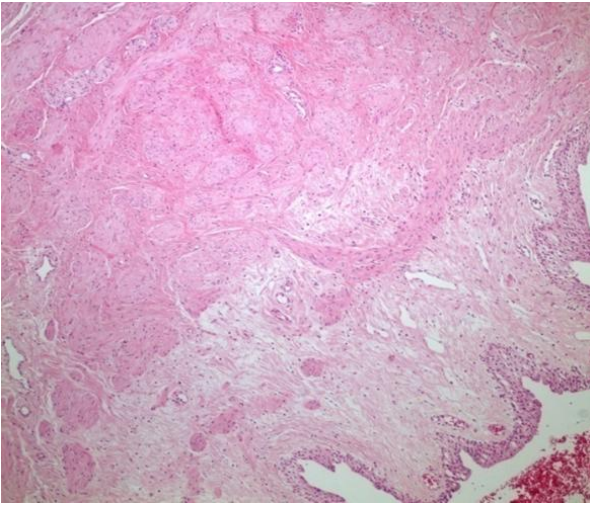


**Figure 2.** Diffuse renal scarring in kidney (DMSA scan).

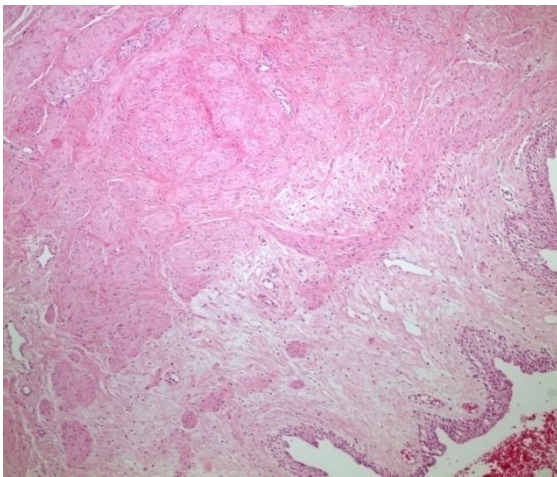


WT: Wall thickness; UD: ureteric diameter; MLT: Mucosal loop thickness; SMT: Smooth muscle thickness

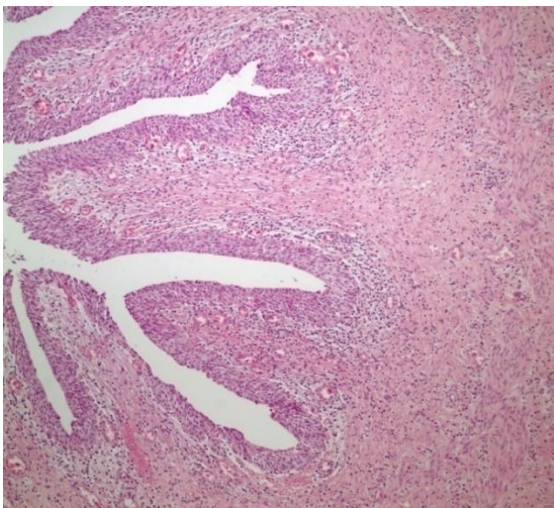
**Figure 3.** Distal end of ureter showing associated structures and the measurement parameters used in the study (Haematoxylin and eosin stain; original magnification X10).



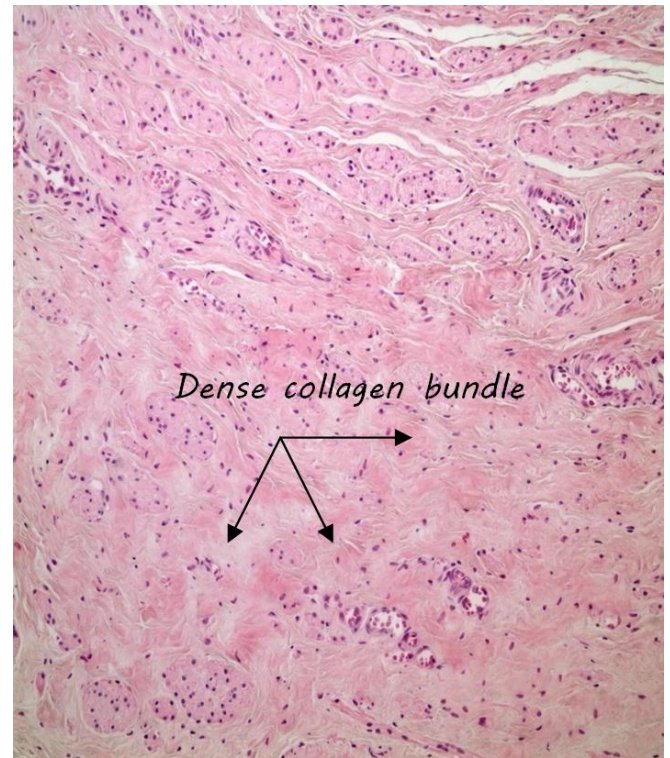
**Figure 4.** An example of Grade 1 inflammation. No inflammatory reaction and/or very few lymphocytes are present (haematoxylin and eosin stain; original magnification X40).



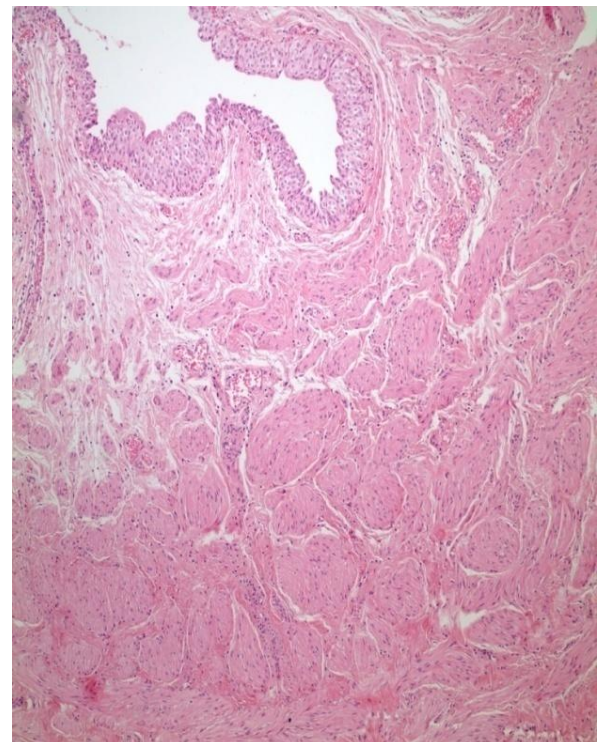
**Figure 5.** An example of Grade 2 inflammation. Perivascular, scattered lymphocytes are present (haematoxylin and eosin stain; original magnification X100).



**Figure 6.** An example of Grade 3 inflammation. An increase in the number of lymphocytes is evident (haematoxylin and eosin stain; original magnification X40).



**Figure 7.** Collagen deposition (X100).



**Figure 8.** Muscular hypertrophy (X40).

#### Statistical Analysis

Continuous variables, presented as the mean ( $\pm$ ) SD if normally distributed and as the median (IQR) if abnormally distributed, were analyzed using the t-test and the Mann-Whitney U test, respectively. Categorical variables were analyzed by the chi-square test. A value of  $p < 0.05$  was considered statistically significant for all tests. All statistical

analysis was performed using SPSS (version 17.0 SPSS, Chicago, Ill., USA) was used.

## Results

The enrolled patients consisted of 49 (59%) male and 34 (41%) female patients. Median age of the patients was 28 (12-192) months. Renal scar and inflammation groups were compared for all 110 units. Patient demographics and clinical details are shown in Table 1. The grades of VUR in this study group and scar rates for all reflux grades of patients are shown in Table 2. The proportions of scar formation patterns are shown in Table 3.

**Table 1.** Patient demographics and clinical details.

	n	%
Number	83	-
Age (range) in months	28 (12-192)	-
Gender	49M/34F	61/39
Ureteral unit	110	-
Recurrent UTI*	21	25
Febrile UTI	21	27
Prenatal hydronephrosis	19	23
Lower urinary tract infection	13	15
Other signs (Stomach, vomiting).	7	10

\* UTI: Urinary tract infection

Grade 1, Grade 2 and Grade 3 inflammation at the distal end of a ureter associated with a focally scarred kidney (n=45) was 48.9%, 22.2% and 28.9%, respectively ( $p<0.05$ ).

**Table 2.** Distribution of patients according to VUR grades.

Reflux grade	n total	Scarred unit n (%)
Grade 1	8	2 (25)
Grade 2	11	5 (45)
Grade 3	25	17 (68)
Grade 4	44	32 (72)
Grade 5	22	19 (86)
Total	110	75 (68)

**Table 3.** The number and proportions of scar formation patterns for all ureteral samples (n=110).

Scar pattern	n	%
Diffuse scarring	30	27
Focal scarring	45	40
No scarring	35	33

Similarly, in ureters associated with a diffusely scarred kidney, inflammation Grade 1, 2 and 3 was found in 30%, 3.3% and 66.7% respectively ( $p<0.05$ ). There was a significantly higher proportion of Grade 3 inflammation in ureters from the DS group compared to the FS group (see Table 4).

Comparison of the presence of increased collagen deposition in ureters stratified by grade of inflammation is shown in Table 5. There was a significant difference between the groups based on degree of inflammation present ( $p<0.01$ ).

The association between collagen deposition and scar type was also evaluated and was shown to be significantly different between the NS, DS and FS groups (see Table 6).

The diameter of the distal end of the ureter, wall thickness and muscular thickness were compared based on the presence of mild (Grade 1) or severe (Grades 2+3) inflammation. The results of this comparison are shown in

Table 7. There was a significantly increased mean wall thickness in ureters with severe inflammation present ( $p<0.05$ ).

**Table 4.** Comparison of the existing scar types and inflammation grade in the ureterorenal unit.

Scar		Inflammation			p value
		Grade 1	Grade 2	Grade 3	
Focal	n	22	10	13	<0.05*
	%	48.9%	22.2%	28.9%	
Diffuse	n	9	1	20	
	%	30.0%	3.3%	66.7%	
No scar	n	14	7	12	
	%	42.4%	21.2%	36.4%	

\*Grade 3 inflammation: Diffuse scar group >No scar group >Focal scar group

\*Grade 1 inflammation: Focal scar group >Diffuse scar group

**Table 5.** Association of the degree of inflammation with collagen.

Inflammation		Collagen		p value
		Present	Absent	
Grade 1	n	10	37	<0.01*
	%	21.3%	78.7%	
Grade 2	n	8	10	
	%	44.4%	55.6%	
Grade 3	n	30	15	
	%	66.6%	33.3%	

\*Existence of collagen: Grade 3 Group > Grade 2 Group > Grade 1 Group

**Table 6.** Association of the scar type with collagen.

Scar		Collagen		p value
		Present	Absent	
Focal	n	14	31	<0.05*
	%	31%	69%	
Diffuse	n	19	11	
	%	63%	37%	
Normal	n	15	18	
	%	45%	55%	

\*Existence of collagen: Diffuse scar group >Focal scar group

**Table 7.** Association of ureteral inflammation with ureteral diameter, wall thickness and muscular thickness.

	Inflammation	n	%	Mean measurement (µm)	p Value
	Grade 1(mild)	33	45	3211.5	
	Grade 2 + 3	42	57	3765.0	
Wall thickness	Grade 1	31	43	2230.0	<0.05
	Grade 2 + 3	41	45	340.0	
Muscular thickness	Grade 1	33	55	380.0	0.33

There were 74 ureteral samples where the thickest muscle bundle at the distal end of the ureter was histologically assessed as normal (see Table 8). These 74 samples were then assessed according to the type of scarring present in the associated kidney and the amount of collagen present. For ureters with no kidney scarring present and only a small amount of collagen present (n=15) the mean thickness of the muscle was 432 µm. In ureters with diffuse scarring of the associated kidney and dense collagen deposits (n=19) this thickness was 290 µm which represents a 33% muscle loss and was significantly less when compared to the first group ( $p<0.05$ ).

**Table 8.** Association of muscular thickness with scar and amount of collagen in specimens judged to have normal muscle bundle structure (n=74).

Scar and amount of collagen	n	Mean muscular thickness (µm)	Degree of muscle thickness (%)	P value
Diffuse scar, collagen+ve	19	290.00	67	
No scar, collagen-ve	15	432.00	100	<0.05*
Lower end of ureter all ureteral units	74	357.00	82	

\*Average muscular thickness: No scar without collagen>Diffuse scar with collagen

## Discussion

Kidney scarring may occur as a result of either congenital changes or as a secondary process following urinary tract infection and the scar formation mechanism may differ between these two processes.<sup>12</sup> In addition, it has been reported that there is an increased probability of diffusely scarred kidneys in patients when an antenatal diagnosis of dysmorphic kidney has been made.<sup>13</sup> In these infants with diffuse scarring, even in the absence of infection, VUR is much more likely.<sup>14</sup> The most important cause of morbidity in VUR is the presence of renal scarring and there is a strong association between recurrent febrile UTI and new renal damage, particularly in girls.<sup>15</sup> In our study diffuse scarring was present in 27% and focal scarring was present in 40% of samples. This scar rate is consistent with previous reports where 64% of patients with repeated UTI and VUR had renal scarring.<sup>16</sup> Furthermore, there is a direct relation between the degree of reflux and nephropathy.<sup>17</sup> In our study sample the proportion of scarred kidneys in the patients with Grade 3, 4 and 5 reflux were 88%, 96% and 86% respectively. Nephrectomy samples have shown that diffuse scarring combined with reflux is often associated with dysplasia and cortical loss.<sup>18</sup> Substantial similarities at the distal end of ureters of renal units with similar scarring patterns have also been reported.<sup>4</sup>

The results of this study showed that there is a significant difference in inflammation intensity present when the renal scarring is of the diffuse or focal types. These findings suggest that the mechanism leading to scarring in the upper system depended on whether congenital or acquired factors were at play and gives some clues as to the development pathways of both mechanisms separately. The finding that Grade 3 inflammation at the distal end of ureter was more common in ureters associated with diffusely scarred kidneys suggests that a more severe dysplastic process in the kidney has a greater deleterious effect on the distal end of the ureter.

Wound healing occurs by the formation of collagen, intermediate material, proteins and glycoproteins, all of which are synthesized mainly by fibroblasts.<sup>19</sup> In wound healing collagen synthesis is the process which completes the critical step. Thus the finding of a high proportion of intense collagen bundles (66%) in the group having Grade 3 inflammation at the distal end of ureter and Grade 3 inflammation progression on the side of the diffusely scarred kidney (66.7%) is not surprising. The replacement of ureteral muscle, which plays a major role in antireflux mechanisms, with collagen suggests one possible mechanism whereby diffuse scarring of the kidney may occur.<sup>6,20</sup>

The mechanism of smooth muscle, neural and vascular cell loss at the distal end of a ureter may be due to production of

profibrotic cytokines and an increase in extracellular matrix proteins in earlier studies of the histopathology of the distal end of the ureter in VUR. 6-8 It has been suggested that these metabolic abnormalities may lead to reflux because of collagen deposition which results in fibrosis.<sup>7,9</sup> Comparison of the distal ends of ureters associated with either diffuse or focal scarring in the kidney in this study showed that those associated with diffuse scarring were more likely to be affected by severe inflammation and were more likely to have a higher degree of collagen deposition compared to ureters of focally scarred kidneys.

In this study the ureteral diameter, ureteral wall thickness, mucosal loop thickness and the thickness of the muscle bundles were measured. A combination of these measurements together with the intensity of inflammation were evaluated. It was found that the ureter wall thickness of kidneys with diffuse scarring and Grade 3 inflammation were significantly thicker than that of kidneys having focal scarring. The difference may be due to both the intensity of inflammation and the persistence of dense collagen deposition. When the association of the inflammation and scar type with muscular thickness was examined it was shown that there was a significantly greater decrease in muscle thickness in distal ureters associated with intense inflammatory changes. Even in ureters where muscle structure was judged histologically to be normal, there was a mean 33% loss of muscle in ureters associated with diffuse scarring compared to 18% in all specimens judged to have normal muscle structure. In addition, the smallest degree of muscle loss was associated with ureters with the least collagen deposition and no evidence of kidney scarring on DMSA scan.

Loss of muscle density in the ureters of diffusely scarred kidney is thought to be a secondary effect of acquired infection, and thus the presence of this muscle density loss is not surprising. It is also possible that the pronounced differences in the ureters from the dysplastic side reported here, with the associated intensity of inflammation and increased and persistent collagen deposition may result from the same etiology.

## Conclusion

This investigation showed that there are significant differences of histological structure of the distal end of the ureters when they are classified by the presence or absence of scarring and the type of scarring of the associated kidney. There was more intense inflammation, more collagen deposition, less smooth muscle content and increased ureteric wall thickness in the ureters of kidneys with diffuse scarring compared to ureters of kidneys with less or no scar. The presence of diffuse scar formation in the kidney was reflected throughout the whole renal unit and we hypothesize that this is probably due to a developmental pathology.

## Conflict of Interest

None.

## Financial Support

None.

## Author Contributions

ÖK: Project development, manuscript writing, data collection, data analysis, SA: Project development, manuscript writing, data analysis, AKU: Manuscript writing

and editing, DEB: Project development, pathological evaluation, ST: Project development, manuscript writing.

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