

THE EFFECTS OF UNILATERAL URETHRAL OBSTRUCTION ON TUBULAR CELLS APOPTOSIS: An Experimental Study in Rabbits

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ABSTRACT

To explain effects of unilateral urethral obstruction on tubular cells apoptosis as the mechanism of renal dysfunction. Forty-eight rabbits (Oryctolagus cuniculus) underwent distal unilateral urethral obstruction. The experimental animals were divided into 6 groups: 2 groups had sham operation, which served as control, 2 groups had left urethral partial obstruction, 2 groups had left urethral total obstruction. Tubular cells apoptosis of the ipsilateral kidney were analyzed 7 days and 14 days after obstruction by means of immunohistochemical method. T analysis revealed no different tubular cells apoptosis of the ipsilateral kidney between partial urethral obstruction groups and control groups at 7 and 14 days ($p = 0.085$ and $p = 0.252$), but there were differences in total urethral obstruction groups at 7 and 14 days. ANOVA analysis revealed that in unilateral urethral obstruction there were different tubular cells apoptosis of the ipsilateral kidney in control, partial and total urethral obstruction groups at 14 days ($p = 0.0001$). There were different tubular cells apoptosis of the ipsilateral kidney between control and partial urethral obstruction groups, control and total urethral obstruction groups ($p = 0.0001$) at 7 days. Tubular cells apoptosis of the ipsilateral kidney in total urethral obstruction groups were different at 7 and 14 days ($p = 0.012$), and not different in partial urethral obstruction groups at 7 and 14 days ($p = 0.252$). There were no different tubular cells apoptosis of the ipsilateral kidney in partial and total urethral obstruction at 7 days but the difference was found at 14 days.

Keywords: Unilateral urethral obstruction, tubular cells apoptosis, ipsilateral kidney

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INTRODUCTION

Obstructive uropathy is the most commonly found urologic cases in daily practice. In the United States, majority of the cases result from urethral stones and the incidence is 1 per 1,000 populations, while the bilateral obstruction had an incidence of 1 per 1,000 populations. Urine obstruction in the ureter may have causes in the upper or lower part, and it may be acute or chronic, complete or incomplete, unilateral or bilateral (Soebadi 2002). Obstructive uropathy may result from congenital or acquired abnormalities and can be intra- or extraluminally (Young et al. 1999). Each cause may have typical characteristics, although all may have similar results to renal function and disorder. Renal function may decrease, either reversibly or irreversibly (Tanago 1992). In acute obstruction, a sudden increase of pressure in urethra and kidney may occur in positive correlation with prevailing diuretic status. The increase is forwarded back to tubular lumen. The increase is only transient, and the pressure will reduce gradually (O'Reilly 2001). The reduction of Glomerular Filtration

Rate (GFR) and Renal Blood Flow (RBF) in acute and transient obstruction are generally temporary and reversible. Rat's kidney may tolerate total urethral obstruction for 4 - 7 days before impairment in nephron occurs. Renal function may fully recover if total obstruction can be removed before that period (O'Reilly 2001). A study on dog showed direct correlation between the duration of obstruction and severity of renal function abnormality. It can be completely recovered if the obstruction can be removed within 7 days (Vaughan & Gillenwater 1992). Total obstruction in prolonged period may result in progressive nephron loss, leading to atrophy in medulla and cortex (Vaughan & Gillenwater 1992). The degree of obstruction is one of critical factors in the occurrence of renal disorder (Rao Sameet 2001).

An experiment on rats subjected to unilateral urethral obstruction revealed an increased renal cells proliferation and apoptosis (Chevalier et al. 1999). Another experiment suggested that urethral obstruction resulted in renal parenchyma, presenting as tubular cell

reduction and cell proliferation (Nguyen et al. 2000). Urethral obstruction may induce renal pathological and functional changes, so that early detection and management is imperative to maintain renal function from irreversible renal tissue obstruction (Power et al. 2004).

Correlation between renal tubular cells apoptosis and the duration of obstruction should be identified to find the severity of obstruction due to urethral obstruction. The identification is necessary to determine actions for preventing renal damage after the obstruction occurs. Because such study is highly invasive, it is not applicable to human beings. Therefore, this study was carried out to rabbits (*Oryctolagus cuniculus*) as they were easily available and had a relatively large ureter. The authors attempted to explain the influence of the duration and severity of experimental unilateral distal urethral obstruction on apoptotic tubular cell count in rabbits' (*Oryctolagus cuniculus*) ipsilateral kidney.

MATERIALS AND METHODS

This was an experimental study involving rabbits (*Oryctolagus cuniculus*) as experimental animals, and used posttest control group design, in which variables measurement was carried out after treatment. Samples comprised 48 male rabbits randomly allocated into 6 groups. Each groups consisted of 8 rabbits. Those groups were control group 7 days, control group 14 days, partial urethral obstruction group 7 days, partial urethral obstruction group 14 days, total urethral obstruction group 7 days, and total urethral obstruction group 14 days. The dependent variable was apoptotic tubular cell count in ipsilateral kidney, while the independent variables were severity and duration of obstruction.

Partial urethral obstruction was carried out by ligating the ureter with silk thread 4.0. The ureter had been blocked in its extraluminal part with string measuring 50% of its diameter. The string was slowly pulled, so that partial obstruction was induced. Total urethral obstruction was induced by ligating the ureter totally with silk thread 4.0. Control groups were subjected to sham operation. Ipsilateral nephrectomy was done at day 7 for three groups (control, partial, and total obstruction) and at day 14 for 3 other groups (control, partial, and total obstruction). Furthermore, apoptotic renal tubular cell examination was conducted using ApopTag produced by Intergen Company with code S 7101.

Obtained data were statistically tabulated. Data were presented descriptively and analyzed using t test and ANOVA to compare the effects of partial/total ligation and the duration of obstruction on the apoptotic renal tubular cell count. The significance level was $\alpha = 0.05$.

RESULTS

Rabbits' bodyweight

Rabbits' (*Oryctolagus cuniculus*) bodyweight was control variable, so that variance homogeneity analysis among 6 groups had to be conducted to determine whether the change occurred only as the result of the treatment, i.e., unilateral urethral obstruction for 7 and 14 days. The results of homogeneity test of rabbits' bodyweight in 6 groups using one-way ANOVA analysis are presented in the following table:

Table 1. Bodyweight Homogeneity Test (gram) between Treatment Groups

Groups	Bodyweight (gram) (X \pm SD)	ANOVA		Note
		F value	P value	
Control 7 days	1687.5 \pm 44.32	0.366	0.869	NS
Control 14 days	1687.5 \pm 44.328			
Partial obstruction 7 days	1693.75 \pm 49.55			
Partial obstruction 14 days	1706 \pm 49.55			
Total obstruction 7 days	1700 \pm 53.45			
Total obstruction 14 days	1712 \pm 4432			

Above table shows that rabbits' bodyweight in this study is not significantly different ($p > 0.05$). Therefore, any

change in the dependent variable must have resulted from the treatment of urethral obstruction in this study.

Normal distribution test on data

To determine the type of hypothesis used in this study, normal distribution test was carried out using One

Sample Kolmogorov-Smirnov (one sample K-S), with the following results:

Table 2. Normality Test of Apoptotic Cell Count in Each Group

Groups	P value	Note
Control 7 days	0.884	NS
Control 14 days	0.998	NS
Partial obstruction 7 days	0.766	NS
Partial obstruction 14 days	0.813	NS
Total obstruction 7 days	0.992	NS
Total obstruction 14 days	0.995	NS

Above table shows that data on apoptotic cell count in this study had normal distribution ($p > 0.05$). Therefore, hypothetical test that would be used to perform analysis was parametric statistical test.

Discriminant test to the time between observations

Discriminant test was done on the variables of apoptotic tubular cell count in kidney. According to the results of

normality test and data scale of the variables, discriminant test used to analyze the difference between treatment groups during observation was t test and ANOVA. t test was used to find the difference of apoptotic cell count in renal tubule in each group during observations for 7 and 14 days. The results are presented in the following table:

Table 3. The Difference of Apoptotic Cell Count in Chronic Obstructive Renal Cells in Day 7 and 14

Groups	Apoptotic cell count in kidney		T test		Note
	Observation Day 7	Observation Day 14	F value	P value	
Control	1.875 ± 0.607	1.9250 ± 0.740	- 0.148	0.885	NS
Partial obstruction	11.813 ± 2.426	10.213 ± 2.907	1.195	0.252	NS
Total obstruction	12.000 ± 0.767	15.525 ± 2.954	- 3.267	0.012	S

Above table shows no significant difference in apoptotic tubular cell count in kidney in control groups between observation at day 7 and 14 ($p = 0.885$). Similar finding was also found in group receiving partial obstructive treatment ($p = 0.252$). In group receiving total obstruction, we found significant difference in apoptotic tubular cell count in kidney between observations at day 7 and 14 ($p = 0.012$).

Discriminant test between treatment groups

ANOVA was used to assess the difference of apoptotic tubular cell count in kidney in each observation time

between different treatment groups. The results can be seen in Table 4.

It can be seen that there is a significant difference between control, groups receiving partial obstructive and groups receiving total obstructive treatment at observation day 14 ($p = 0.0001$). This is also indicated by the difference of superscript (a, b, c) in those three treatment groups. At observation day 7, there was significant difference between control group and partial obstructive group; and control group and total obstructive group ($p = 0.0001$). The difference was also confirmed by the difference in superscript (a, b) at observation day 7.

Table 4. The Difference of Apoptotic Cell Count in Chronic Obstructive Renal Cells between Treatment Groups in Observation Day 7 and 14

Observation	Treatment Groups			ANOVA		Note
	Control	Partial Obstruction	Total Obstruction	F Value	P Value	
Day 7	1.875 ± 0.607 ^a	11.813 ± 2.426 ^b	12.000 ± 0.767 ^b	117.664	0.0001	S
Day 14	1.925 ± 0.740 ^a	10.213 ± 2.907 ^b	15.525 ± 2.954 ^c	63.617	0.0001	S

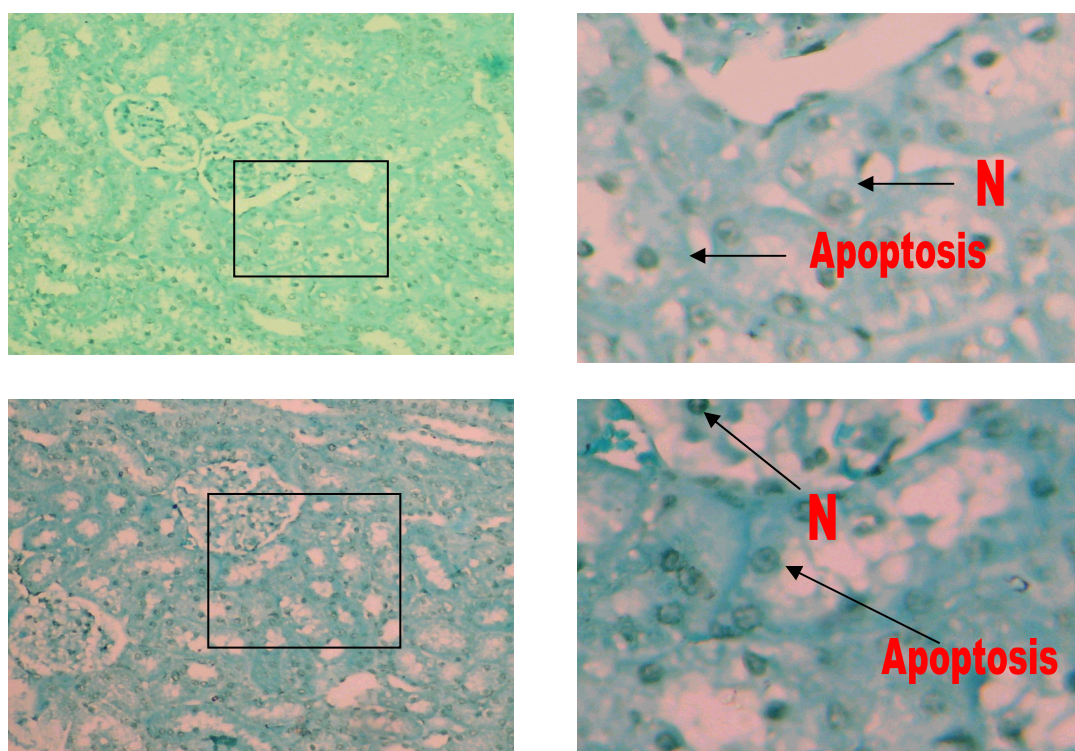


Figure 1. Apoptosis in control group at week 1 with magnification 100 x (upper left) and 400 x (upper right), and control group at week 2 with magnification 100 x (lower left) and 400 x (lower right).

DISCUSSION

As seen in Table 3, the results of t test revealed that there was no significant difference in apoptotic tubular cell count in ipsilateral kidney in control group at observation day 7 and 14 ($p = 0.885$). No significant difference was also found in partial unilateral distal obstructive treatment ($p = 0.252$). However, significant difference was found in total unilateral distal obstructive treatment ($p = 0.012$). This indicates that apoptotic ipsilateral renal tubular cells in control group and those in group receiving partial unilateral distal urethral

ligation were not different between observation times, while in group receiving unilateral total distal urethral ligation, there was difference between observation times.

In a study using mice subjected to left urethral ligation and control group, and observation was done at day 4, 7, 15, 20 and 30, the apoptotic renal tubular cells count reached the peak at day 4, remained steadily until day 15, and then reduced, while in control and contralateral renal groups the apoptosis was minimal (Truong et al. 2001). This finding was similar to that in this study (at observation day 7 dan 14).

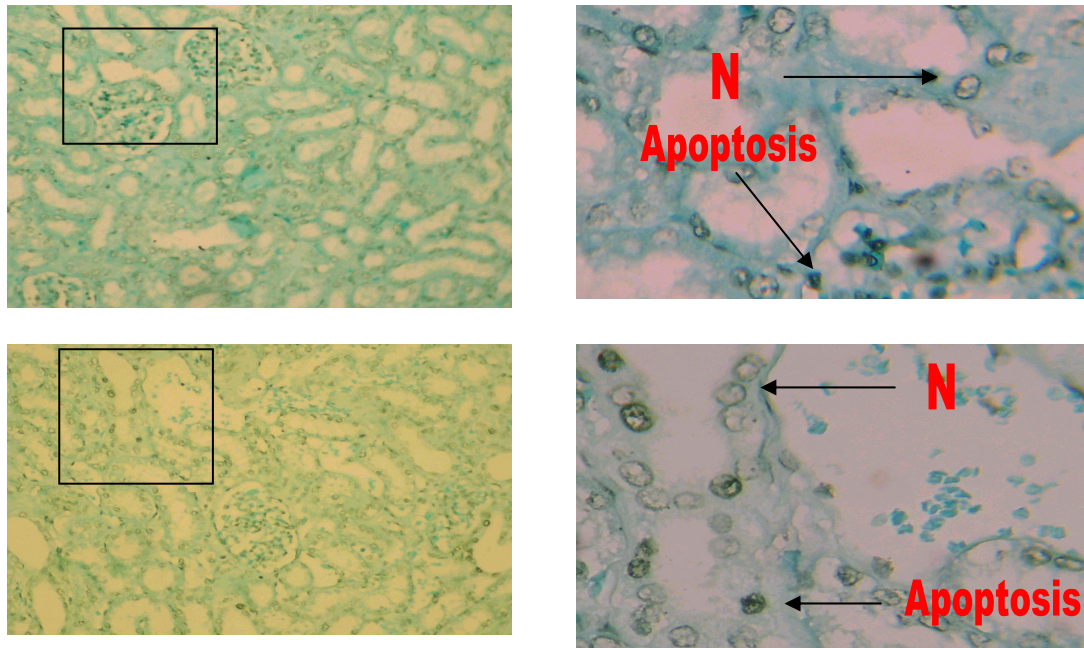


Figure 2. Apoptosis in partial obstructive group at week 1 with magnification 100 x (upper left) and 400 x (upper right), and partial obstructive group at week 2 with magnification 100 x (lower left) and 400 x (lower right).

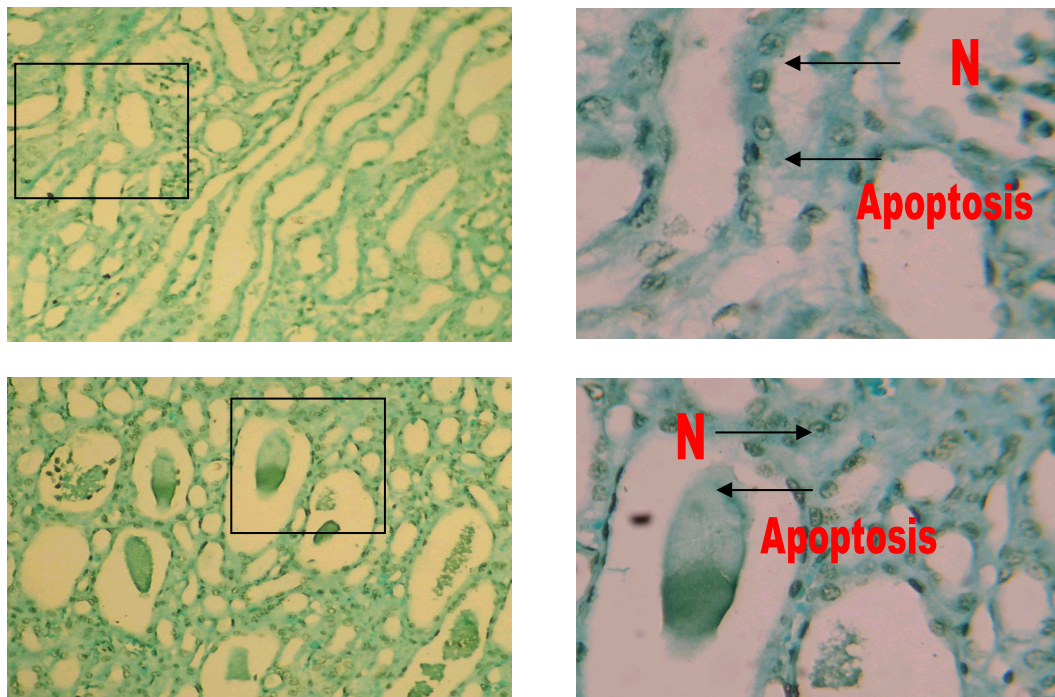


Figure 3. Apoptosis in total obstructive group at week 1 with magnification 100 x (upper left) and 400 x (upper right), and total obstructive group at week 2 with magnification 100 x (lower left) and 400 x (lower right).

In Table 4, ANOVA test used to assess the difference of apoptotic tubular cells count in ipsilateral kidney revealed that there was significant difference between control, and groups receiving partial as well as total unilateral distal obstruction at observation day 14 ($p = 0.0001$) and day 7 ($p = 0.0001$). It indicated that apoptotic tubular cells in ipsilateral kidney in control group, group receiving partial unilateral distal urethral ligation and that receiving total unilateral distal urethral ligation were different between treatment groups.

In a study using rabbits receiving treatment of partial left urethral ligation, total left urethral ligation, and control group (sham operation), in which each group comprised 12 rabbits and observed for 2 hours, 1 week, 4 weeks, and 12 weeks, there was significant difference between obstructive and control groups ($p = 0.02$) in the observation of apoptotic tubular cells in ipsilateral kidney (Erbagci et al. 2002). This finding is also confirmed by this study.

CONCLUSIONS

1. Apoptosis in tubular cells in ipsilateral kidney at day 14 was higher than that at day 7 in rabbits (*Oryctolagus cuniculus*) groups subjected to total unilateral distal urethral obstruction ($p = 0.012$), while groups receiving partial unilateral distal urethral obstruction showed no significant difference ($p = 0.252$).
2. Apoptosis in tubular cells in ipsilateral kidney in rabbits (*Oryctolagus cuniculus*) subjected to partial and total unilateral distal obstruction was higher than that in control group at day 7 and 14. No significant difference was found in apoptotic tubular cells in ipsilateral kidney in group subjected to partial and total unilateral distal urethral obstruction at day 7, but the significant difference was found at group receiving partial and total unilateral distal urethral obstruction at day 14.

It is suggested that the results of this study can be used for the basis in clinical practice to predict the prognosis of patients with unilateral distal urethral obstruction. It is recommended to immediately conduct desobstruction in patients with uropathy obstruction as it may result in better prognosis. Further studies involving larger sample, longer obstruction time and more variations in obstruction should be carried out. Studies in human using non-invasive parameters are also suggested.

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