The Dynamics of Ureter Changes After Medial and Distal Unilateral Ureteral Obstruction in Rat

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Abstract. Unilateral ureteral obstruction is an abnormality that often occurs in the urinary tract. Obstruction can be placed proximal, medial or distal ureter. The physiological response that occurs depends on the severity, duration, location and type of obstruction. The aim of this study was to look at the macroscopic and histopathological changes of the ureter also Hydronephrosis Hydroureter Index after medial and distal unilateral ureteral obstruction. Thirty-six white rats were divided into 3 groups and each group consist of 12 individuals. Group I as a control got a laparotomy without ureter ligation. Group II laparotomy with medial right ureteral ligation and group III laparotomy with distal right ureter ligation. Three rats in each group were randomly collected at weeks 1, 2, 3 and 4 after treatment. The kidneys and ureters were removed from the abdominal cavity to measure the diameter of the ureter and weigh. After the accumulated liquid was taken, the weighing was done again. An ureter sample was taken for histopathological preparations. The results of the macroscopic and histopathological examination of the ureter were discussed qualitatively. Measurement of the diameter and thickness of the ureteric wall was analyzed using statistical analysis of factorial pattern variations 3x4. The accumulation of fluid in the ureter causing changes in the shape, color, size and structure of the ipsilateral ureter in both ligation groups. Histopathological observations showed depletion, alteration and damage to the structure of the ipsilateral ureteric wall structure. Unilateral ureteral obstruction affected ureter diameter increase, thinning of the ureteral wall and ipsilateral IHH (P <0.05). The presence of unilateral ureteral obstruction had no impact on the contralateral ureter (P> 0.05).

Keywords: ureteral obstruction, unilateral, macroscopic, histopathologic, Hydronephrosis Hydroureter Index

1. Introduction

The kidneys and urinary tract play an important role in excrete the metabolic waste products, regulate the volume and composition of extracellular fluid in the body [15]. Disease that often occur in the urinary tract are obstruction. It has potential to cause death if not handled properly. In the young animals, obstruction is caused by congenital factors such as atresia or stenosis of the urinary tract. In the older animals it is usually caused by tumors, neoplasia and calculi [12; 20]. Ureteral obstruction can occur in one ureter called an unilateral ureteral obstruction and in both ureters called bilateral ureteral obstruction.
The location of ureteral obstruction can occur at the ureteropelvis junction (proximal ureter), medial, and ureterovesicul
asar junction (distal ureter) [4; 16]. The prevalence of proximal urinary tract obstruction in dogs and cats caused by urolithiasis is 5%, whereas in distal urinary tract is 95% [19].

The physiological response because of ureteral obstruction is complex and depends on the species, age of the animal, the severity of the obstruction, duration of obstruction, type of obstruction (partial or total, unilateral or bilateral), and the location of the blockage [6]. When obstruction occurs, the ureter will develop local inflammation, swelling, and smooth muscle spasm due to abnormal peristalsis [18]. The urine will be blocked and returned to the kidneys. Accumulation the urine cause dilatation between renal pelvis and proximal calix and it called hydrenephrosis [23]. Prolonged obstruction can cause ureters swell and distension called hydroureter [5]. Changes and damages to the ureter will clearly occur in cases of ureteral obstruction in the medial and distal part. More urine will accumulate in the ureteral lumen from the renal pelvis to the location of the obstruction in the ureter. The aim of this study was to look at the macroscopic and histopathological changes of the ureter also Hydrenephrosis Hydroureter Index after medial and distal unilateral ureteral obstruction.

2. Research Methods

Thirty-six white rats were divided into 3 groups of 12 individuals. Group I as a control get a laparotomy without ureter ligation. Group II get a laparotomy and right ureter ligation in the medial part. Group III get a laparotomy and right ureter ligation in the distal part.

2.1. Surgical Procedure

Rats were fasted for 8 hours before performed a surgery. Anesthetic combination of ketamine at a dose of 90 mg / Kg BW and xylazin HCl at a dose of 2 mg / Kg BB is injected intramuscularly. After being anesthetized, the rats were positioned dorsally on the surgical table. The hair in the abdomen shaved clean and the skin is sterilized used 70% alcohol and 10% povidone iodine solution. The surgery begin by made the incision on the part midline of the abdominal skin and the subcutaneous layer. After that, make an incision on the the linea alba until the peritoneum opens. The intestine and visceral organs were shift to the left lateral to expose the kidneys and ureters. Right ureter ligated with silk suture 3/0 in the medial or distal part. After the right ureter ligated, the visceral organs is restored in the normal position. The abdominal cavity lavage use 0.9% physiological NaCl. The linea alba and sub cutaneous sutured with catgut chromic a 3/0 and the skin sutured use silk 3/0.

2.2. Macroscopic Observation and Measurement of the Ureter Outer Diameter

Three rats in each group were randomly euthanized at weeks 1, 2, 3 and 4 after treatment. The kidneys, ureters and bladder removed from the abdominal cavity. Macroscopic observation and measurement of ureter diameter using calipers.

2.3. Hydroureter-Hydrenephrosis (HHI) Index

Ipsilateral and contralateral kidneys-ureters are weighed after removed from the abdominal cavity. The urine that accumulates in the kidneys-ureters is taken using a 1 ml syringe dan it re-weigh. Hydroureter-Hydrenephrosis Index (HHI) is performed by calculating the difference in weight before and after removal the fluid inside [2].

2.4. Sampling for histopathological preparations

Ipsilateral ureter samples were taken before ligation and contralateral ureters were taken medially. Histopathological preparations stained using Hematoxilin Eosin (HE) staining.
2.5 Measurement of Ureter Wall Thickness

Measurement of ureteral wall thickness is calculated from the lumen mucosal wall to the outer of tunica muscularis. Histopathology slide photographed using a microscope with camera. Measurement of the wall thickness used a software Image Raster®. Thickness will be stated in micrometers (µm).

2.6 Data Analysis

Macroscopic and histopathological observations were analyzed descriptive comparative by looking at changes in the ureter. The measurement results of ureter wall thickness and Hydroureter-Hydronephrosis Index were analyzed statistically using a 3x4 factorial design.

3. Result and Discussion

3.1 Macroscopic Observation of Ureters and Measurement of Ureteric Diameter

Ipsilateral kidney and ureters in both ligation groups (Figure 1) change in shape, color, size and structure. Hydronephrosis and hydroureter have occurred since first week after treatment. Hydroureter characterized by fluid accumulation in the lumen from the site of ligation up to the proximal ureter (pelvis renis). The ureter diameter of the ligation groups increases continuously until the fourth week. The ureter diameter of group II greater than group III. Reddish yellow liquid is clearly visible from outside the kidney and ureter. Obstruction causes the ureteral lumen to become inflamed, swollen, and spasm of the smooth muscle because peristalsis cannot occur normally [18]. Urine accumulation that occurs in the ureteral lumen will then cause ureter dilation [10]. When the ureter is dilated, the opposite occurs in the thickness of the ureteric wall. Wall thinning makes ureter looks more transparent. Based on statistical results (Table 1), ureteral obstruction had a significant effect on increasing ipsilateral ureter diameter (P <0.05) compared to the control group. However, the difference in ipsilateral ureteral diameter between groups II and III from weeks 1 to 4 was not significant (P> 0.05).

The contralateral kidney in both ligation groups are still reddish brown with a smooth structure and are shaped like kidney beans. Ureter and pelvis renis have not dilatation or macroscopic changes. The similar conditions in both kidneys and ureter of groups I is characterized by the absence of changes in size, color and structure. Contralateral ureteral diameter in all groups did not change (Table 2). The difference in treatment and measurement time did not have a significant effect on the contralateral ureteral diameter of the three groups (P> 0.05).
Figure 1. Macroscopic observation of the kidneys and ureters in all groups 1-4 weeks post-treatment. Group II (medial ureteral ligation) (A, B, C, D). Group III (distal ureteral ligation) (E, F, G, and H). Group I (without ureteral ligation) (I, J, K, L). Yellow arrow = Right ligated ureter.

Table 1. Measurement of ipsilateral ureteral diameter of group I, II, III (mm).

|       | Group I (± SD)     | Group II (± SD) | Group III (± SD) |
|-------|--------------------|-----------------|------------------|
| Week I| 0.200 ± 0.000<sup>ai</sup> | 1.933 ± 0.115<sup>bi</sup> | 1.566 ± 0.321<sup>bi</sup> |
| Week II| 0.200 ± 0.000<sup>ai</sup> | 2.133 ± 0.231<sup>bi</sup> | 2.033 ± 0.152<sup>bi</sup> |
| Week III| 0.2333 ± 0.577<sup>ai</sup> | 2.500 ± 0.458<sup>bi</sup> | 2.433 ± 0.208<sup>bi</sup> |
| Week IV| 0.2333 ± 0.039<sup>aii</sup> | 3.633 ± 0.603<sup>biili</sup> | 3.500 ± 0.458<sup>bi</sup> |

*) The similar letter in one row shows no significant difference in treatment and the similar roman numerals in one column shows no significant difference in the time of sampling.
Table 2. Measurement of the contralateral ureter diameter of group I, II, III (mm).

|       | Group I (± SD) | Group II (± SD) | Group III (± SD) |
|-------|---------------|----------------|-----------------|
| Week I| 0.200 ± 0.000 | 0.200 ± 0.000  | 0.200 ± 0.000   |
| Week II| 0.233 ± 0.577 | 0.233 ± 0.577  | 0.233 ± 0.577   |
| Week III| 0.200 ± 0.000 | 0.233 ± 0.577  | 0.266 ± 0.577   |
| Week IV| 0.233 ± 0.577 | 0.300 ± 0.000  | 0.267 ± 0.577   |

*) The similar letter in one row shows no significant difference in treatment and the similar roman numerals in one column shows no significant difference in the time of sampling.

3.2. Histopathological Observation and Measurement of The Ureter Wall Thickness

Contralateral ureter in both of ligation groups did not experience histopathological changes in the 1st to 4th weeks after treatment (Figure 2; Figure 3). The ureteral lumen remains in the form of a fold that indicates the absence of a liquid pile until just before the rat dies. Contralateral ureter in cases of total unilateral obstruction from 1-42 days after treatment does not show any changes and still looks normal histopathologically [2; 3]. The normal ureteral lumen is a stellate shape consisting of 4-8 folds that appear when the ureter condition is empty and without contraction of the muscular layer [11]. The transitional epithelium layer appears to be composed of squamous, columner and cuboid cells. Squamus cells are on the surface of the ureteral lumen and underneath are column cells. The lamina propria appears clearly demarcated with cuboidal cells in the epithelial layer and is more transparent in color than the tunica muscularis and epithelium layer. Collagen fibers and fibroblasts appear as constituents of lamina propria. Blood capillaries are seen in the area of the lamina propria which shows good circulation along the ureter and as an indication that no damage occurs along the ureter. Some lymphocytes are also seen scattered in lamina propria. The muscular tunica which is directly adjacent to the lamina propria is seen in the form of a longitudinal section and on the outside is circular in shape. In the peripheral part of the ureter also appears to be wrapped by tunica adventitia which carries blood vessels and nerve fibers towards the ureter [7]. The measurement results (Table 4) show that the difference in treatment and the difference in sampling time did not affect the change in contralateral ureteral wall thickness (P> 0.05).
On the first week, the both of the ligation groups (Figure 2; Figure 3) dilated and the lumen walls was also flat with no folds as in normal conditions. The transitional epithelial layer is thinning due to the accumulation of fluid in the lumen. The ipsilateral ureteral epithelial layer of group III is relatively thicker than group II. The lamina propria is also seen thinning, but collagen fibers are still clearly visible and some blood capillaries can still be observed and the presence of lymphocyte infiltration which is a marker of inflammation. Depletion of the lamina propria causes the folds in the ureteral lumen to not form. The tunica muscularis of the ligation groups still appears to have almost the similar thickness when compared to the contralateral ureter in the different group. This is a mechanism to expand the lumen as compensation for the accumulation of fluid that cannot pass through the ureteral lumen due to obstruction. At the beginning of the obstruction, the ureter will increase peristalsis which aims to increase the pressure on the proximal area of the blockage and relax the area of the blockage and the area after the blockage. The aim is to push the obstruction so that it can be removed from the ureter. However, when the blockage cannot be pushed, the longer it will cause damage to the ureter [8].

At the end, maximal ureteral dilatation was seen in both ligation groups. The ureteral wall looks thinner. The most obvious layer to thin due to fluid accumulation is the epithelial layer. Changes in the epithelial
lining and the structure of the ureter reflect the internal condition of the ureter in the case of ureteral obstruction [2]. The transitional epithelium layer actually appears as a pseudocomplex epithelium. In the empty condition, the epithelial layer looks like a pile of cuboid and polygonal cells [1]. When filled with fluid it will distend so that it will appear as a complex squamus cell. This tissue is accommodated to be able to stretch when the lumen contains urine. This serves to prevent the transfer of fluid from the connective tissue space to the lumen caused by urine that is hypertonic. When the urine accumulated in the ureter, the changes of the epithelial layer thickness will be very clearly visible. The lamina propria looks so thin it is almost invisible. Collagen fibers, blood capillaries and lymphocytes are completely invisible. Tunica muscularis are also getting thinner because of the maximum stretch. It is responsible for changes in the size and volume of the lumen of the tubular organ and facilitates the movement of material inside the organ [1]. Longitudinal smooth muscle functions to regulate the shortening of the ureter and play a role in the movement of urine. Circular smooth muscle plays a role in the contraction of the ureteral wall and the process of dilatating and contracting to give urine a boost to the distal ureter [22]. Ureteral muscular layer thinning is the effort of this layer to increase the volume of the ureteral lumen. Decreased thickness of this layer can also caused myositis are damaged and reduce in the ability of the ureter in the peristalsis to urine from the proximal to the distal part. Obstruction of the proximal urinary tract causes ureter dilatation, weakness of the ureteral wall and interferes with the transport of urinary boluses [14]. The prolonged of accumulation fluid in the lumen causing oxidative stress. This cause damage to cell structures and tissue of the ureteral wall and medium damage due to ischemia [2].
The change in thickness of the ureteral wall is affected by the thickness of each of its constituent layers. The thickness of the ureteral wall is measured from the wall of the ureteral lumen to the tunica muscularis. Tunica adventitia as the outer layer of the ureter that serves to link the ureter to the abdominal wall will often be damaged or even lost in the process of sampling the ureter. The results of statistical calculations (Table 3) show that obstruction influences changes in ipsilateral ureteral wall thickness compared to group I (P <0.05). The difference between groups II and III is not significant (P >0.05). The sampling time also did not affect the change in ipsilateral ureteral wall thickness (P <0.05).

Table 3. Results of ipsilateral ureteral wall thickness measurements of group I, II, and III rats from 1-4 weeks post-treatment histopathologically (µm).

|                | Group I (± SD)          | Group II (± SD)          | Group III (± SD)         |
|----------------|------------------------|-------------------------|-------------------------|
| Week I         | 58,840 ± 2,373 ai      | 38,923 ± 1,937 bi       | 41,756 ± 2,897 bi       |
| Week II        | 57,163 ± 5,430 ai      | 32,400 ± 0,279 bi       | 34,003 ± 0,384 bi       |
| Week III       | 56,280 ± 12,099 ai     | 21,453 ± 0,350 bi       | 24,047 ± 0,349 bi       |
| Week IV        | 57,850 ± 7,795 ai      | 11,670 ± 0,310 bi       | 16,856 ± 0,310 bi       |

*) The similar letter in one row shows no significant difference in treatment and the similar roman numerals in one column shows no significant difference in the time of sampling.

Table 4. The results of measurements of the contralateral ureteral wall thickness of group I rats, II, and III from 1-4 weeks post-treatment histopathologically (µm).

|                | Group I (± SD)          | Group II (± SD)          | Group III (± SD)         |
|----------------|------------------------|-------------------------|-------------------------|
| Week I         | 52.497 ± 9.130 ai      | 58.827 ± 11.259 ai      | 57.653 ± 9.492 ai       |
| Sunday II      | 57.507 ± 11.429 ai     | 54.646 ± 9.492 ai       | 55.937 ± 6.879 ai       |
| Sunday III     | 55.253 ± 10,380 ai     | 56.953 ± 4,093 ai       | 56.893 ± 4,093 ai       |
| Sunday IV      | 59,293 ± 12,171 ai     | 59,667 ± 16,675 ai      | 58,843 ± 16,675 ai      |
*) The similar letter in one row shows no significant difference in treatment and the similar roman numerals in one column shows no significant difference in the time of sampling.

3.3. Hydroureter-Hydronephrosis Index (HHI)

The results of the Hydroureter Hydnefrosis Index (HHI) of both ligation groups experienced a significant increase compared to the control group (P, 0.05) (Table 5). Changes in HHI between the medial and distal ligation groups were not significant (P> 0.05). The difference in sampling time did not significantly influence the change in HHI (P <0.05). The increase in HHI is due to the continued accumulation of urine and changes in the structure of the kidneys and ureters due to obstruction. Urine that should be removed will be blocked and will certainly return and accumulate to the kidneys which cause toxic uremia and progressive kidney damage [17]. The increase in kidney weight occurs along with the severity of the kidney’s obstruction of hydronephrosis. Significant increase in ipsilateral kidney weight occurs from the 7th day after obstruction. HHI ipsilateral 1-14 days after obstruction ranged from 0.3 to 2.1 [2]. Hydronephrosis due to ureteral obstruction causes atrophy, tubular apoptosis, and necrosis. In the case of severe hydronephrosis the kidney is dominated by fluid that fills the vacuole. The longer time of hydronephrosis will increase overall kidney weight [21]. At the beginning of hydronephrosis kidney weight increased to 34% from the initial weight until the 35th day the renal weight was dominated by hydronephrosis (76-81.7%) [24]. HHI of all contralateral groups under normal conditions. According to Chuang et al. (1995), contralateral HHI ranged from 0.01-0.04. There was no difference between groups I, II and III (P> 0.05).

Table 5. Measurement results of the ipsilateral Hydroureter-Hydronephrosis Index

|               | Group I (± SD) | Group II (± SD) | Group III (± SD) |
|---------------|---------------|----------------|-----------------|
| Week I        | 0.037 ± 0.015 *a | 0.067 ± 0.066  *b | 0.653 ± 0.311  *b |
| Week II       | 0.040 ± 0.010 *a | 1.280 ± 0.252  *b | 1.050 ± 0.390  *b |
| Week III      | 0.037 ± 0.015 *a | 2.140 ± 0.064  *b | 1.713 ± 0.064  *b |
| Week IV       | 0.043 ± 0.020 *a | 3.140 ± 0.826  *b | 2.447 ± 0.826  *b |

*) The similar letter in one row shows no significant difference in treatment and the similar roman numerals in one column shows no significant difference in the time of sampling.
Table 6. Measurement results of the contralateral Hydroureter-Hydronephrosis Index

| Week   | Group I (± SD)       | Group II (± SD)      | Group III (± SD)     |
|--------|----------------------|----------------------|----------------------|
| I      | 0.037 ± 0.015 A¹     | 0.047 ± 0.005 A¹     | 0.040 ± 0.010 A¹     |
| II     | 0.057 ± 0.020 A¹     | 0.043 ± 0.025 A¹     | 0.033 ± 0.005 A¹     |
| III    | 0.037 ± 0.005 a¹     | 0.043 ± 0.011 a¹     | 0.043 ± 0.005 a¹     |
| IV     | 0.043 ± 0.015 a¹     | 0.047 ± 0.015 a¹     | 0.037 ± 0.011 a¹     |

*) The similar letter in one row shows no significant difference in treatment and the similar roman numerals in one column shows no significant difference in the time of sampling.

4. Conclusion
Medial and distal unilateral ureteral obstruction caused hydronephrosis and hydroureter in both ligation groups. Ureter changed in shape, color, size and structure. Histopathological observation showed depletion, alteration and damage to the structure of the ipsilateral ureteral wall structure. Unilateral ureteral obstruction caused the increase of ureter diameter, thinning of the ureteral wall and increasing the HHI (P <0.05). The presence of unilateral ureteral obstruction did not affect the contralateral ureter (P > 0.05).

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