EFFECT OF ARTIFICIAL UNILATERAL URETER TOTAL OBSTRUCTION ON SERUM CYSTATIN-C LEVELS IN ORYCTALAGUS CUNICULUS

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ABSTRACT

The objective of this study was to determine the effect of artificial unilateral ureter total obstruction on serum Cystatin C levels in Oryctalagus cuniculus. Cystatin-C is a non-glycosylated protein with low molecular-weight (13.36 kD) derived from cystatin super-family of cysteine protease inhibitor. This substance is synthesized constantly by all nucleated cells and not influenced by inflammatory processes, infectious diseases, lean body mass, fever, age, gender or dietary factors. Twenty-two Oryctalagus cuniculus were divided into two groups, 11 were performed artificial unilateral ureter total obstruction and others were performed sham operation as control group. Data of serum cystatin-C were collected on pre-operation, day 7 and day 14 from each sample. Serum Cystatin C levels were analyzed with Sandwich-ELISA. The data were statistically analyzed with t test. Results showed that the mean serum Cystatin C levels of the male, 3-month old Oryctalagus cuniculus was 0.00671 ± 0.00558 mg/L, day 7 after intervention was 0.01051 ± 0.00536 mg/L and day 14 was 0.019 ± 0.01005 mg/L as examined with Sandwich-ELISA. In conclusion, there was no significant increase of serum Cystatin C on day 7, but by the 14th day a significant increase of serum Cystatin C was observed. There was no significant difference in the increase of serum Cystatin C on day 7 in the intervention group compared to the control group, but there was a significant difference in the increase of serum Cystatin C on the day 14.

Keywords: serum cystatin-C level, artificial unilateral ureter total obstruction, orictalagus cuniculus

INTRODUCTION

Urologic emergency that drives a patient to search for medical intervention is colic and acute urine retention. Both conditions often lead to unbearable severe pain. Acute urine retention is an obstructive uropathy of lower urinary tract, while obstructive uropathy of upper urinary tract is often ignored by the patients and clinicians. The patients who are visiting are referred to clinicians have often been in advanced obstructive condition (Soebadi 1994). Such condition may result in the reduction of renal function, either reversible or irreversible. Renal function is one important factor to be considered by the urologists to make decision in determining definitive therapy for a case. The most reliable global index to identify renal function is glomerular filtration rate (GFR). Several methods have been invented by the experts to measure GFR. Inuline clearance is a gold standard for measuring renal function. In further development, clearance from several radioisotopes, such as Chromium 51-EDTA or Technetium 99-DTPA, Iothalamate or Iohexol, have accuracy close to that of inuline clearance, so that they can be used as a substitute of inuline. However, those examinations need more time and higher cost, rendering them to be impractical and less ideal for routine or massive clinical application (Orlando 2002). Today, endogenous marker commonly used in clinical practice is serum creatinine, either alone or in combination with 24-hour urine pool for determining creatinine clearance. There are several factors that limit or affect the accuracy of the marker in GFR measurement, such as the accuracy of measuring 24-hour urine, the effect of body mass on endogenous creatinine production, creatinine secretion and reabsorption in the tubule, diet type, age and analytic problem of assay method for creatinine. Based on these obstacles, the experts continuously develop studies to find more accurate endogenous marker for measuring GFR.

Cystatin-C is a non-glycosylated protein with low molecular weight (13.36 kD), a derivate of cystatin superfamily from cysteine protease inhibitor. The substance is produced stable by all nucleated cells. Endogenous production level of cystatin-C is constant and not affected by inflammatory process, body mass, fever, age, or sex. Cystatin-C is filtrated freely by glomerulus and rapidly metabolized (neither secreted
nor reabsorbed) in proximal tubule (Herget-Rosenthal 2000; Reed 2000). Cystatin-C examination does not require urine sample, the level is not affected by drugs or several metabolites, such as bilirubin and ketone. The result of cystatin-C examination can be immediately obtained. After all, cystatin-C meets various criteria as an ideal marker of endogenous GFR. Based on this superiority, the experts underwent researches, and some of current studies suggest that cystatin-C has more superior capability than creatinine clearance in measuring GFR.

The intention of this study was to find the serum cystatin-C level in experimental rabbits subjected to artificial unilateral ureter total obstruction for 7 and 14 days, so that in daily clinical practice it can be used for predicting renal function and prognosis more practically, rapid, and accurate. In this study, the authors measured serum cystatin-C level in experimental rabbits with artificial unilateral total urethral obstruction. In general, this study attempted to prove the increase of serum cystatin-C level in those rabbits and in particular to prove such increase in day 7 and 14, and to prove the difference in the increase of serum cystatin-C level in those rabbits according to the length of obstruction.

**MATERIALS AND METHODS**

This was an experimental study using pre- and post-test control group design. This study involved rabbits (*Oryctalagus cuniculus*) that met the criteria as follows: male, aged 13-17 weeks, bodyweight 1250-1750 grams, healthy, without abnormalities. The rabbits were treated with unilateral total urethral ligation for 7 and 14 days. Prior to and after treatment, the cystatin-C level was measured and control group was also involved in this study. As many as 22 male rabbits were divided randomly (Steel & Torrie 1994) into 2 groups, each comprising 11 rabbits. Both groups were subjected to bodyweight measurement prior to the treatment. Samples were excluded if the experimental animals died during the study. There were 6 rabbits that died during this study (2 in control group at the first week, and 1 at the second week, while in treatment group there were 3 rabbits that died during the first week). Group I: Rabbits were subjected to sham operation at day 0. Blood sample was taken for cystatin-C examination before operation, at day 7 and 14 post-operation. Group II: Rabbits were subjected to operation and unilateral total urethral ligation. Blood sample was taken for cystatin-C examination before operation, at day 7 and 14 post-operation.

The healthy male rabbits were adapted within the cage for three days. Operative procedure for total ligation in one side ureter and sham operation were done at day 0. Before operation, 5 ml blood sample was taken from blood vessel on the ear of each rabbit in both groups. The rabbits were also fasted (except from drinking) before operation for 5-6 hours. They were given with 100 mg/kg BW ampicillin prior to the operation. Half an hour before being anesthetized, 1-3 mg/kg BW atropine was given intramuscularly. Subsequently, 40 mg/kg BW ketamine was injected intramuscularly, combined with 0.5 mg/kg BW parafldehyde in order to prolong the anesthetic effect. After being anesthetized, the rabbits were positioned supine. Aseptic procedure was done on and around the operation field using 10% povidone iodine, and operation field was covered with sterile cloth. Approximately 3 cm suprapubic incision was made, followed with deeper incision layer by layer. Ureter identification was then undertaken, and in group II total urethral ligation was carried out by ligating the ureter using silk thread 4-0, and group I was subjected to sham operation. Bleeding was treated and operation wound was closed layer by layer. After regained their consciousness, the rabbits were given with 200 mg ampicillin intravenously through the auricle of the ear. Rabbits were treated as required and in groups I and II blood sample was taken at day 7 and 14.

Method for examining cystatin-C serum level was Sandwich-ELISA/Double Antibody Sandwich-ELISA/Direct-Sandwich ELISA, carried out at the Virology and Immunology Laboratory, Department of Veterinary Microbiology, Airlangga University School of Veterinary Medicine, Surabaya (non-commercial). To obtain optimum validity, we undertook chequerboard titration, both for coating antibody or antigen and conjugate (Handojo 2003). Data were recorded and processed with personal computer and analyzed using SPSS program. In this study, statistical test used was independent t test with the assumption that data distribution was normal and sample characteristic was homogeneous with significance limit of a < 0.05.

**RESULTS**

Table 1 shows that the data on bodyweight and serum cystatin-C level in *Oryctalagus cuniculus* pre-operatively, at day 7, and day 14 had normal distribution (p > 0.005). Therefore, the further analysis in this study used parametric statistical test. Before statistical analysis was done on the variables, we attempted to find the bodyweight and serum cystatin-C level of the animals before treatment in each group, since the difference of bodyweight and serum cystatin-C level may affect the difference in the variable. For that purpose, a homogeneity test was carried out, whose results can be seen in Table 2. Table 2 shows that...
bodyweight and serum cystatin-C level in the rabbits pre-operatively was not significantly different \( (p > 0.005) \). Therefore, if there is a change in serum cystatin-C level, it must result from the artificial unilateral total urethral obstruction. The level of cystatin-C of *Oryctalagus cuniculus* in treatment group increased at day 7 and 14, while in control group it increased at day 7 and reduced back at day 14 (Figure 1). Table 3 shows that in treatment group there is no significant increase in serum cystatin-C level pre-operatively and at day 7 \( (p = 0.302) \). There is a significant increase of serum cystatin-C level in pre-operative and day 14 observation \( (p = 0.0029) \), and at observation day 7 and 14 there was no significant increase in serum cystatin-C level \( (p = 0.067) \). Table 4 shows that in control group there was no significant increase of serum cystatin-C pre-operatively and day 7 \( (p = 0.905) \), day 7 and 14 \( (p = 0.109) \), pre-operatively and day 14 \( (p = 0.071) \).

**Table 1. Normality test of the variables**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Treatment (mean ± SD)</th>
<th>p value</th>
<th>Control (mean ± SD)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (gram)</td>
<td>1406.25±178.16</td>
<td>0.992</td>
<td>1475.00±128.17</td>
<td>0.899</td>
</tr>
<tr>
<td>Cystatin C pre op(mg/L)</td>
<td>0.0067±0.00558</td>
<td>0.085</td>
<td>0.00397±0.0016</td>
<td>0.130</td>
</tr>
<tr>
<td>Cystatin C day 7(mg/L)</td>
<td>0.0105±0.00536</td>
<td>0.436</td>
<td>0.00758±0.0049</td>
<td>0.569</td>
</tr>
<tr>
<td>Cystatin C day 14(mg/L)</td>
<td>0.0190±0.01005</td>
<td>0.180</td>
<td>0.00390±0.0005</td>
<td>0.207</td>
</tr>
</tbody>
</table>

Note: Data had normal distribution if \( p > 0.005 \)

**Table 2. Homogeneity test on BW and serum cystatin-C level pre-operatively in *Oryctalagus cuniculus***

<table>
<thead>
<tr>
<th>Variables</th>
<th>Treatment (mean ± SD)</th>
<th>Control (mean ± SD)</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (gram)</td>
<td>1406.25±178.16</td>
<td>1475.00±128.17</td>
<td>-0.886</td>
<td>0.391</td>
</tr>
<tr>
<td>Cystatin C pre op(mg/L)</td>
<td>0.0067±0.00558</td>
<td>0.00397±0.0016</td>
<td>1.323</td>
<td>0.222</td>
</tr>
</tbody>
</table>

Note: Data were homogeneous if \( p > 0.005 \)

**Figure 1. Pre-operative mean cystatin-C level at day 7 and 14 in *Oryctalagus cuniculus***

**Table 3. The change of pre-operative serum cystatin-C level at day 7 and 14 in treatment group**
Paired t test

<table>
<thead>
<tr>
<th>Time of observation (day)</th>
<th>Δ (mg/L)</th>
<th>% Δ</th>
<th>Paired t test t value</th>
<th>p value</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-operation to day 7</td>
<td>0.00381±0.00966</td>
<td>56.87</td>
<td>1.114</td>
<td>0.302</td>
<td>NS</td>
</tr>
<tr>
<td>Pre-operation to day 14</td>
<td>0.01230±0.01266</td>
<td>195.24</td>
<td>2.747</td>
<td>0.029</td>
<td>S</td>
</tr>
<tr>
<td>Day 7 to 14</td>
<td>0.00849±0.01109</td>
<td>80.86</td>
<td>2.167</td>
<td>0.067</td>
<td>NS</td>
</tr>
</tbody>
</table>

Note: Significant if p < 0.005

Table 4. The change of serum cystatin-C level preoperatively, at day 7 and 14 in control group

<table>
<thead>
<tr>
<th>Time of observation (day)</th>
<th>Δ (mg/L)</th>
<th>% Δ</th>
<th>Paired t test t value</th>
<th>p value</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-operation to day 7</td>
<td>0.00360±0.00554</td>
<td>90.45</td>
<td>1.833</td>
<td>0.109</td>
<td>NS</td>
</tr>
<tr>
<td>Pre-operation to day 14</td>
<td>-0.00008±0.00178</td>
<td>-1.96</td>
<td>-0.124</td>
<td>0.905</td>
<td>NS</td>
</tr>
<tr>
<td>Day 7 to 14</td>
<td>-0.00370±0.00489</td>
<td>-48.81</td>
<td>-2.129</td>
<td>0.071</td>
<td>NS</td>
</tr>
</tbody>
</table>

Note: significant if p < 0.005

Table 5. Difference in the change of serum cystatin-C level in treatment and control group

<table>
<thead>
<tr>
<th>Time of observation (day)</th>
<th>Treatment (rerata±SD.mg/L)</th>
<th>Control (rerata±SD.mg/L)</th>
<th>2 sample t test t value</th>
<th>p value</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre op to 7</td>
<td>0.00381±0.00966</td>
<td>0.00360±0.00554</td>
<td>0.052</td>
<td>0.959</td>
<td>NS</td>
</tr>
<tr>
<td>Pre op to 14</td>
<td>0.01230±0.01266</td>
<td>-0.00008±0.00178</td>
<td>2.737</td>
<td>0.028</td>
<td>S</td>
</tr>
<tr>
<td>7 to 14</td>
<td>0.00849±0.01109</td>
<td>-0.00370±0.00489</td>
<td>2.841</td>
<td>0.018</td>
<td>S</td>
</tr>
</tbody>
</table>

Note: Significant if p < 0.005

Table 5 shows that there is no significant difference in the increase of serum cystatin-C in pre-operative observation and observation at day 7 between treatment and control group (p = 0.959). There was significant difference in the increase of serum cystatin-C in pre-operative observation and observation at day 14 between treatment and control group (p = 0.028). There was significant difference in the increase of serum cystatin-C in observation day 7 and 14 between treatment and control group (p = 0.018).

DISCUSSION

Using Sandwich-ELISA method, this study revealed that in male Oryctolagus cuniculus aged 3 months with average bodyweight of 1406.25 ± 178.16 grams subjected to artificial unilateral total obstruction, serum cystatin-c level preoperatively was 0.00671 ± 0.00558 mg/L; at day 7 was 0.01051 ± 0.00536 mg/L and at day 14 it was 0.01900 ± 0.01005 mg/L. Sandwich-ELISA method could detect serum cystatin-C level until one thousandth, while other methods, such as PENIA (Particle-enhance-nephelometric immunoassay) (Behring Nephelometer), showed detection limit to 0.17 mg/L with an average length of examination of 6 minutes. PETIA (Particle-enhance turbidimetric immunoassay) (Cobas Mira) method has detection limit of 0.15 mg/L and length of examination of 7 minutes, PETIA (Particle-enhance turbidimetric immunoassay) (Monarc 2000) method has detection limit of 0.03 mg/L with length of examination of 5 minutes, and even the TRIF (Time-resolved fluorimmunoassay) method had detection limit of 0.001 mg/L, but with longer length of examination, 4 hours (Newman 2002).

Serum cystatin-C level at day 7 in treatment and control group increased, although statistically not significant, and there was no significant difference in the change of serum cystatin-C. Unilateral total urethral obstruction had apparently not caused the increase in serum cystatin-C level. Serum cystatin-C level at day 14 in treatment group increased significantly and the difference of change was significant. Unilateral total urethral obstruction had already resulted in the increase of serum cystatin-C level. The increase of the serum in both groups at day 7 might result from pre-operative procedure, i.e. blood sample taking as much as 5 ml, the provision of anesthesia, antibiotics, operative procedure, operative wound healing, which affected pre-renal condition that resulted in the reduction of renal function.
Cystatin-C could be used for early detection of mild reduction of renal function.

Several studies have proved the superiority of cystatin-C against serum creatinine in detecting the reduction of renal function. Meta analysis of 46 research articles comparing correlations between cystatin-C and serum creatinine with GFR measurement standard, such as inulin clearance, 51 Cr-EDTA, 99Tm-DTPA, thalhamate or iohexol, revealed that cystatin C had stronger correlation (r = 0.816 p < 0.001) compared to serum creatinine (r = 0.742 p < 0.001) (Dharmidharka 2002). Other authors who used 99mTc-DTPA as the standard of GFR measurement found that cystatin-C had a stronger correlation (r = 0.91) than serum creatinine (r = 0.89) (Randers et al. 1998). Coll et al. (2000) in their study using 125 I-ICI as GFR standard measurement also found that cystatin-C had stronger correlation (r = 0.77 p < 0.0001) compared to serum creatinine (r = 0.73 p < 0.0001) or creatinine clearance (r = 0.74 p < 0.0001).

Patients with reduced renal function showed significant correlation between cystatin-C and DTPA Renogram (r = 0.839 with p= 0.01) and creatinine clearance with DTPA Renogram (r = 0.605 with p = 0.037). However, cystatin-C had stronger correlation and higher significance with DTPA Renogram compared to creatinine clearance with DTPA Renogram (Halilintar 2004). In patients with mild reduction of renal function, there was significant and stronger correlation between cystatin-C with DTPA Renogram (r = 0.724 with p = 0.027), while creatinine clearance had no significant correlation with DTPA Renogram (Halilintar 2004). In various conditions, either in normal, reduced or mildly reduced renal function, cystatin-C had stronger correlation with higher significance level compared to creatinine clearance. The strongest correlation between cystatin-C and DTPA Renogram was found in patients with reduced renal function (Halilintar 2004).

Some studies showed that mild reduction of renal function can be more easily detected by cystatin-C than serum creatinine. Coll et al. (2000) in their study found that serum cystatin-C started to increase above normal value in GFR 88 ml/minutes, while serum creatinine started to increase above normal if GFR has reduced to 75 minutes. In mild reduction of renal function (GFR between 50 and 83 ml/minutes), serum cystatin-C increases in all (100%) patients, while serum creatinine increased only in 75% of the patients. Newman et al. in their study on 469 patients concluded that in addition to be a better estimator than serum creatinine, cystatin-C was also a more sensitive marker of even minute changes in GFR. In mild reduction of renal function, cystatin C increased in 71.4% patients, while serum creatinine increased only in 52.4% of the patients (Laterza 2002).

The results of this study confirmed the previous ones. Rat’s kidney could tolerate 4-7-day total obstruction before nephron loss occurred. Renal function would recover completely if total obstruction was eliminated before the time limit, if there was infection or previous renal disease. In animals, total obstruction for more than 4-6 weeks result in only minimal recovery of glomerulus function (O’Reilly 2001). In acute obstruction, initially, the glomerular filtration rate (GFR) did not change, but some time thereafter increasing blood pressure reduced filtration fraction and pressure. Subsequently, RBF reduced and filtration fraction increased and filtration fraction had a light increase. However, such compensation is incomplete, so that, in general, the filtration was reducing steadily. Therefore, in several hours GFR decreased. After 1-week obstruction, GFR reduced to 10-20% from pre-obstruction level (Fitzpatrick 1999), in which the contralateral side increased to 165% of the pre-obstruction level (O’Reilly 2001).

Histopathologically, atrophy is a sign of damage resulting from renal obstruction, which in 7 days occurs in distal nephron. After 14 days atrophy occurs in renal cortex, resulting in glomerulus damage (Fitzpatrick 1999). Another author found that apoptotic number in ipsilateral renal tubular cells at day 14 was higher than that at day 7, and there was a significant difference in the apoptosis of renal tubule due to artificial unilateral ureter total obstruction as compared to control (Daryanto 2005). A study by Adi (2005) found that in 7-day artificial total urethral obstruction there was no significant difference in the proportion of renal tubular cell apoptosis between contralateral and control group, but at day 14 the significant difference was found, while tubular cell and fibroblast proliferation in interstitial showed significant difference even at day 7 or 14.

CONCLUSION

In conclusion, at day 7 there is no significant increase of serum cystatin-C level, but at day 14 there is a significant increase of serum cystatin-C. There is no significant different increase of serum cystatin-C level pre-operatively until day 7 between treatment and control groups. There is significant different increase of serum cystatin-C level pre-operatively until day 14, and from day 7 to day 14 between treatment and control group. Since there is a significant increase of serum cystatin-C level, in unilateral total urethral obstruction it is suggested to provide therapy for freeing from the obstruction as early as possible. Serum cystatin-C level
can be used as a basis for predicting the prognosis of patients with unilateral ureter total obstruction.

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