**Research Article**

# URINARY Γ - GLUTAMYL TRANSFERASE AND URINE PROTEIN - TO- CREATININE RATIO ESTIMATION AS PROGNOSTIC RENAL MARKERS IN PYOMETRA AFFECTED DOGS

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**A B S T R A C T**

Twenty four cases of pyometra (confirmed by ultrasonography) were classified into three groups I, II and III, treated under different treatment regimens. The animals were presented at the university veterinary hospitals of Kerala Veterinary and Animal Sciences University, Pookode, Wayanad. Detailed clinico-gynaecological and ultrasonographic examination of the reproductive tract, haemato-biochemical tests and urine samples were collected on the day of admission (day 0) and subsequently on day 5,10 and 20 for the estimation of haemato-biochemical parameters and kidney function for case diagnosis and prognostic evaluation. The dogs were subjected to urine catheterization for examining the biochemical parameters such as Urine protein – to - creatinine (UPCR) ratio and Urinary γ - glutamyl transferase (UGGT) estimation for prognostic evaluation. Urine protein – to – creatinine ratio values ranged from $0.05 \pm 0.01$ to $0.71 \pm 0.36$ and statistically no significant ($P>0.05$) difference was noticed between the three groups on different days of observation. Urinary γ - glutamyl transferase estimation had no statistically significant difference observed between the groups ($P>0.05$) on different days of observation. However, a highly significant ($P<0.01$) decrease in the values ranging from $31.25 \pm 0.49$ to $12.75 \pm 0.86$ (IU/L) observed within all the three groups I, II and III from day 0 to day 20.

## INTRODUCTION

Canine breeding is considered as one of the fastest and profitable industry with tremendous demand for pure bred or pedigree dog and the reproductive performance is vital for producing good pedigree dogs. The pet population has increased to three-fold and 0.6 million pets being adopted every year with turn-over of 800 million dollar of pet industry (Singh *et al.,* 2020). Ovariohysterectomy is considered to be safe and effective solution for pyometra. However, the animal loses its breedability. Canine pyometra is often accompanied by glomerular or renal tubular dysfunctions or both. Proteinuria is a feature associated with renal dysfunction as proteins such as albumin is not filtered by glomerulus and is reabsorbed by tubular cells. Hence, urine protein -to-creatinine ratio (UPCR) is performed to assess the involvement of tubular cell lesions in pyometra and to evaluate renal injury before developing azotemia. Urinary γ - glutamyl transferase (UGGT) is considered as a urinary biomarker as it an enzyme located on proximal tubule membrane and the levels will be increased in acute renal failure cases and early detection helps for the prediction of prognosis of the disease.

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## MATERIALS AND METHODS

Twenty four clinical cases of open cervix canine pyometra presented to TVCC, Pookode were selected for the present study. Detailed clinico-gynaecological and ultrasonographic examination of the reproductive tract and haemato-biochemical tests were carried out for the confirmatory diagnosis of pyometra. The animals were randomly divided into three groups of eight animals each (Group I to III) and subjected to different treatment regimen. The response to the treatment and prognostic evaluation tests were carried out.

The selected animals will be subjected to three treatment groups comprising of eight animals in each group (n=8) as follows, group I was subjected to @ Dinoprost tromethamine 0.2 mg/kg body weight, s/c, bid, 5 days, group II was subjected to Mifepristone@ 2.5 mg/kg body weight, po, bid, 5 days and Group III was subjected to Dinoprost and Mifepristone @Dinoprost- 0.2 mg/kg body weight, s/c, bid, alternate days, thrice. Mifepristone- 2.5 mg/kg body weight, po, bid, 5 days. All the animals in the treatment groups were administered with antibiotic combination of amoxicillin and clavulanate at the dose rate of 12.5 mg/kg body weight daily intravenously for five days along with supportive therapy using intravenous fluids.

Urine samples was collected subsequently on day 5, 10 and 20 kidney function tests for the prognostic evaluation. Results were assessed by Analysis of Variance (ANOVA). All the parameters estimated were placed in three groups with each group consisting of different treatment regimen and performed on days 0, 5, 10 and 20. As there was comparison between three groups and between the days in each individual group, one-way ANOVA was used for statistical interpretation.

## RESULTS AND DISCUSSION

Urine samples were collected on the day of admission (day 0) and subsequently on day 5,10 and 20 for the estimation of kidney function for case diagnosis and prognostic evaluation.

**Table 1** (Mean± SE) Urine Protein – to – Creatinine ratio on different days of observation in pyometra affected dogs with three different treatment protocols (n= 8)

| Days of observation | Urine Protein – to – Creatinine ratio | | | F-value (P-value) |
|---|---|---|---|---|
| | Group I | Group II | Group III | |
| 0 | $0.48 \pm 0.2$ | $0.18 \pm 0.12$ | $0.71 \pm 0.36$ | $1.174^{ns}$ (0.329) |
| 5 | $0.32 \pm 0.17$ | $0.12 \pm 0.07$ | $0.55 \pm 0.27$ | $1.249^{ns}$ (0.307) |
| 10 | $0.32 \pm 0.17$ | $0.12 \pm 0.07$ | $0.55 \pm 0.27$ | $1.23^{ns}$ (0.312) |
| 20 | $0.32 \pm 0.14$ | $0.05 \pm 0.01$ | $0.44 \pm 0.21$ | $1.874^{ns}$ (0.178) |
| F-value (P-value) | $1.000^{ns}$ (0.396) | $1.313^{ns}$ (0.290) | $2.810^{ns}$ (0.126) | |

**Group I:** Dinoprost tromethamine, **Group II:** Mifepristone, **Group III:** Dinoprost + Mifepristone
*ns- Non-Significant (P>0.05)*

**Table 2** (Mean± SE) Urinary γ - glutamyl transferase on different days of observation in pyometra affected dogs with three different treatment protocols (n= 8)

| Days of observation | Urinary γ - glutamyl transferase (IU/L) | | | F-value (P-value) |
|---|---|---|---|---|
| | Group I | Group II | Group III | |
| 0 | $31.25 \pm 0.49^{d}$ | $29.88 \pm 0.69^{d}$ | $31.00 \pm 2.63^{d}$ | $0.21^{ns}$ (0.81) |
| 5 | $23.63 \pm 1.36^{c}$ | $23.63 \pm 1.25^{c}$ | $25.63 \pm 1.21^{c}$ | $0.82^{ns}$ (0.46) |
| 10 | $17.50 \pm 0.63^{b}$ | $17.88 \pm 1.41^{b}$ | $18.50 \pm 1.02^{b}$ | $0.23^{ns}$ (0.80) |
| 20 | $13.13 \pm 0.77^{a}$ | $12.75 \pm 0.86^{a}$ | $13.25 \pm 0.70^{a}$ | $0.11^{ns}$ (0.89) |
| F-value (P-value) | $75.20^{**}$ (<0.01) | $35.91^{**}$ (<0.01) | $27.76^{**}$ (<0.01) | |

**Group I:** Dinoprost tromethamine, **Group II:** Mifepristone, **Group III:** Dinoprost + Mifepristone
*ns- Non-Significant (P>0.05), ** Significant at 0.01 level,*
*Means having different letter as superscript differ significantly within a column*

### Urine Protein – to – Creatinine ratio

Urine protein – to – creatinine ratio values ranged from $0.05 \pm 0.01$ to $0.71 \pm 0.36$ and statistically no significant (P>0.05) difference was noticed between the three groups on different days of observation. Urine protein -to- creatinine ratio (UPCR) is performed to assess the involvement of tubular cell lesions in pyometra and to evaluate renal injury before developing azotemia (Heiene *et al.,* 2001). Similar results were observed by Sant' Anna *et al.* (2019) who evaluated protein – to – creatinine ratio in 44 dogs affected with pyometra to assess renal injury and reported UPCR values ranged between 0.02 – 5.53 and 0.52 -3.02 for non azotemic and azotemic cases, respectively.

### Urinary γ - Glutamyl Transferase

Urinary γ - glutamyl transferase estimation had no statistically significant difference observed between the groups (P>0.05) on different days of observation. However, a highly significant (P<0.01) decrease in the values ranging from $31.25 \pm 0.49$ to

$12.75 \pm 0.86$ (IU/L) observed within all the three groups I, II and III from day 0 to day 20. UGGT is considered as a urinary biomarker as it an enzyme located on proximal tubule membrane and the levels will be increased in acute renal failure (De Loor *et al.*, 2013). They further explained that tubular enzymes such as alkaline phosphatase (AP) and gamma glutamyl transferase (GGT) were limited to lysosomes or cytoplasm of proximal tubular cells. The magnitude of urinary AP and urinary GGT-to-creatinine ratio was found to be associated with severity of lesions in proximal tubular cells of dogs affected with pyometra. Heiene *et al.* (2001) also reported that in dogs affected with pyometra, enzymes from proximal renal tubules were excreted in urine in cases of acute renal injury and high levels of urinary enzymes UGGT and NAG values indicated damage to renal tubules. The results were in accordance with Yogeshpriya *et al.* (2014) who opined that in normal urine, γ - glutamyl transpeptidase were found in low levels (< 2.5 IU m mol $^{-1}$ creatinine). They further opined that UGGT values <10 IU m mol $^{-1}$ creatinine indicated as low level injury to renal tubules, 10 - 20 m mol $^{-1}$ creatinine as intermediate and >20 IU mmol $^{-1}$ creatinine as higher levels of injury to renal tubules. On the contrary, Mrudula *et al.* (2005), reported that the mean ALP and GGT values in dogs with renal failure were $15.34 \pm 2.05$ and $12.25 \pm 2.16$ U/m mol creatinine, respectively as compared to a normal level of $3.67 \pm 1.27$ and $2.5 \pm 0.36$ U/m mol creatinine. However, there is limited published data available with respect to research works specifically related with Urinary γ - glutamyl transferase in pyometra affected dogs.

## CONCLUSION

Twenty four cases of pyometra (confirmed by ultrasonography) were classified into three groups I, II and III, treated under different treatment regimens. The dogs were subjected to urine catheterization for examining the biochemical parameters such as Urine protein – to - creatinine (UPCR) ratio and Urinary γ - glutamyl transferase (UGGT) estimation for prognostic evaluation. Canine pyometra is often accompanied by glomerular or renal tubular dysfunctions or both. Urine protein -to- creatinine ratio (UPCR) is performed to assess the involvement of tubular cell lesions in pyometra and to evaluate renal injury before developing azotemia. Urinary γ - glutamyl transferase (UGGT) is considered as a urinary biomarker as it an enzyme located on proximal tubule membrane and the levels will be increased in acute renal failure cases and early detection helps for the prediction of prognosis of the disease. In the study, it was found that, UPCR values ranged from $0.05 \pm 0.01$ to $0.71 \pm 0.36$ and statistically no significant (P>0.05) difference was noticed between the three groups on different days of observation. Urinary γ - glutamyl transferase estimation had no statistically significant difference observed between the groups (P>0.05) on different days of observation. However, a highly significant (P<0.01) decrease in the values ranging from $31.25 \pm 0.49$ to $12.75 \pm 0.86$ (IU/L) observed within all the three groups I, II and III from day 0 to day 20. Feasibility of prognostic renal markers can be considered as a tool for diagnostic and prognostic renal evaluation of canine pyometra and needs further studies involving larger group animals

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