ORIGINAL ARTICLE

https://doi.org/10.21708/avb.2023.17.3.10357

Urine specific gravity as an indicator for the determination of urinary GGT in dogs with visceral leishmaniasis

Densidade urinária como um indicador para determinação da GGT urinária em cães com leishmaniose visceral

Gustavo Gomes de Oliveira[1]*, Juliana Rosa de Oliveira Maia[1], Fabiano de Oliveira Frazilio[1], Polliana Alves Franco[2], Alda Izabel de Souza[1]

ABSTRACT: The urinary Gama-Glutamyl Transpeptidase to urinary Creatinine ratio (uGGT/uCR) is a laboratory marker that may signal renal tubular lesion in dogs with visceral leishmaniasis (VL). In this study, our goal was to determine whether urine specific gravity (USG) can accurately indicate an elevated uGGT/uCR and, consequently, tubular injury. Twenty-eight animals with VL and five healthy animals (control group) were included in the study. The diseased animals were classified as azotemic (n = 6) and non-azotemic (n = 22). The difference between all groups was tested with the Kruskal-Wallis test for the USG, uGGT/uCR, and urine protein: creatinine ratio (UPC). Sensitivity, specificity, positive and negative predictive value and the accuracy of using USG as an indicator of tubular injury were calculated with the Receiver Operating Characteristic (ROC curve). The probability of an animal with suboptimal USG presenting tubular injury was obtained by calculating the prevalence ratio. The Kruskal-Wallis test revealed differences between groups for all analyzed parameters (p< 0.05). The chosen USG cut-off value, determined by the ROC curve was 1.030, with an accuracy of 78.09%. The probability of a dog with LV and USG below 1.030 having a high uGGT/uCR ratio was 4.95 (p <0.01, 95% CI - 1.32-18.48) when compared to individuals with ideal DU. In conclusion, dogs positive for Leishmania sp. with low USG (<1.030) should have their uGGT/uCR ratio measured to verify the presence of tubular injury even before the onset of azotemia.

KEYWORDS: Tubular injury, Gama-glutamyl transpeptidase, Tubular function.

RESUMO: A razão gama-glutamil transpeptidase urinária/creatinina urinária (uGGT/uCR) é um marcador laboratorial que pode sinalizar lesão tubular renal em cães com leishmaniose visceral (LV). O objetivo deste trabalho foi determinar se a densidade urinária (DU) pode indicar com precisão a elevação da uGGT/uCR e, consequentemente, lesão tubular. Vinte e oito animais com LV e cinco animais saudáveis (grupo controle) foram incluídos no estudo. Os animais doentes foram classificados em azotêmicos (n = 6) e não azotêmicos (n = 22). A diferença entre todos os grupos foi testada com o teste de Kruskal-Wallis para DU, uGGT/uCR e razão proteína/creatinina urinária (UPC). A sensibilidade, especificidade, valor preditivo positivo e negativo e a acurácia do uso da DU como indicador de lesão tubular foram calculadas por meio da *Receiver Operating Characteristic* (curva ROC). A probabilidade de um animal com DU abaixo do ideal apresentar lesão tubular foi obtida pelo cálculo da razão de prevalência. O teste de Kruskal-Wallis revelou diferenças entre os grupos para todos os parâmetros analisados (p< 0,05). O valor de corte escolhido para DU pelo cálculo da curva ROC foi 1,030, com precisão de 78,09%. A probabilidade de um cão com LV e DU abaixo de 1,030 apresentar razão uGGT/uCR elevada foi de 4,95(p <0,01, IC95% - 1,32-18,48) quando comparado com indivíduos apresentando DU ideal. Conclui-se que cães positivos para *Leishmania* sp. com DU abaixo do ideal (<1,030) devem ter sua relação uGGT/uCR medida para verificar a presença de lesão tubular antes mesmo do início da azotemia.

PALAVRAS-CHAVE: Injuria tubular, Gama-glutamil transpeptidase, Função tubular.

[1]Universidade Federal de Mato Grosso do Sul
[2]Vetanalisa
*Corresponding author: gustavo.gomes@ufms.br
Received: 14/03/2023. Accepted: 26/09/2023

INTRODUCTION

Dogs affected by visceral leishmaniasis (VL) often have chronic kidney disease (CKD), a complication that usually leads to death (Solano-Gallego *et al*., 2011). CDK initially develops due to the accumulation of immune complexes in the glomeruli, resulting in glomerulonephritis with the invasion of inflammatory cells, proliferation of the mesangial matrix, and partial loss of the selective permeability of the filtration barrier (Soares *et al*., 2005; Riella, 2014; Godoy *et al*., 2017). This injury, initially originating from glomerular disease, places an overload on the cells of the proximal convoluted tubule, causing injury and consequent loss of function in this part of the nephron (Riella, 2014).

Animals with tubular injury experience a more significant progression of CKD than animals with only glomerular injury, so identifying the tubular injury can assist in determining the appropriate therapeutic approach and evaluating the patient's prognosis (Ibba; Mangiagalli; Paltriniere 2016).

Gamma-Glutamyl Transpeptidase (GGT) is an enzyme synthesized by the proximal convoluted tubule cells in the nephrons. Its production increases in cases of injury to these cells, showing an increased concentration in the urine, as the increased production is excreted into the tubular lumen. Several authors describe urinary GGT as a suitable biomarker of acute tubular injury (Hart, 2005; Mancinelli; Shaw; Meredith, 2012; Kovarikova, 2015), although its significance in CKD is still discussed. According to Ibba; Mangiagalli; Paltriniere (2016), it is possible to use the urinary GGT - urinary creatinine ratio (uGGT/uCR) to determine the source of proteinuria in dogs with VL.

Urine specific gravity (USG) changes in cases of chronic kidney disease (CKD) due to the decreased response of tubule cells to the antidiuretic hormone (ADH) and the progressive cell death of nephrons (Riella, 2014). For this reason, USG serves as an essential information source on the renal tubules, in addition to being an easy-access and low-cost parameter (Hart, 2005).

Considering the USG is directly related to the tubular function of water reabsorption and that uGGT/uCR may indicate the presence of injury in this segment of the nephrons in individuals with proteinuria of renal origin, such as in cases of VL, the purpose of this study was to determine whether USG is an accurate predictor of increased uGGT/uCR and subsequent tubular injury in dogs with VL.

MATERIALS AND METHODS

This research project was approved by the Ethics Commission on Animal Use of the Universidade Federal de Mato Grosso do Sul (UFMS) under the protocol number 897/2017.

Dogs of both sexes, positive for *Leishmania* sp., in lymph node or bone marrow cytology, aged between seven months and 10 years, without a history of previous treatment or other concurrent diseases, living in households, without a preference any specific breed, and irrespective of their reproductive condition were included in the study. From December 2017 to June 2018, 28 dogs meeting these criteria were treated at the veterinary hospital of UFMS. Five dogs with no abnormalities and negative immunochromatographic tests for VL (DPP® Dog Leishmaniasis Test, Brazil), participating in other projects at the veterinary hospital, comprised the control group.

Urine samples of at least 5 mL were collected by cystocentesis or urinary catheter from all animals and immediately analyzed following the methods described by Chew; Dibartola; Schenck (2011). The samples were then centrifuged at 400g for 10min, and the supernatant was separated. Only urine supernatants without active sediment (Chew; Dibartola; Schenck, 2011) were used in this research to measure GGT, creatinine and protein levels. All measurements were conducted within 6 hours after sample collection. Urinary GGT and creatinine were measured using an automated analyzer using commercial kits (Roche®, USA). Urinary GGT values were divided by urinary creatinine to correct for urine dilution (Ibba; Mangiagalli; Paltriniere, 2016). USG was measured with a benchtop refractometer (Atago®, Japan). Urinary protein levels were determined using a semiautomatic analyzer using a specific commercial kit (Sensiprot Analisa®, Brazil). Similar to GGT, urinary protein was divided by urinary creatine to account for urine dilution (Stockham; Scott, 2011).

Next, the animals were classified in two different ways. Initially the patients were grouped based on the presence of azotemia (creatinine ≥1,4mg/dL) into a non-azotemic group (NAG) and an azotemic group (AG), following the recommendations of the IRIS (2023). The healthy animals comprised the control group (CG).

The UPC, uGGT/uCR, and USG were compared among the groups using the Kruskal-Wallis test. The Mann-Whitney test was used as a *post hoc* test for pairwise comparisons. A significance level of $p<0.05$ was used for the Kruskal-Wallis test, and to control the type I error, the significance level used in the Mann-Whitney test was 0.016 (0.05/3 – number of pairwise comparisons performed).

The optimal USG value cutoff as a predictor of uGGT/uCR elevation was determined using the Receiver Operating Characteristic (ROC curve). Sensitivity, specificity, positive and negative predictive value were calculated using low USG as a test for the presence elevated uGGT/uCR. The prevalence ratio, the probability of animals with low USG having high uGGT/uCR compared to animals with normal USG, was calculated and considered significant at $p < 0.05$.

All statistical tests were calculated following the methodology described by Zar (1999), while prevalence ratio, sensitivity, specificity, predictive values and ROC curve were performed according to Jekel; Kartiz; Elmore (2001).

RESULTS AND DISCUSSION

Out of the 33 dogs included in this study, 19 were males and 14 were females. The median age was 36 months (minimum of seven and maximum of 120 months). The NAG and AG consisted of 22 and 6 animals, respectively. Table 1 shows the comparisons between groups for the USG, UPC, and uGGT/uCR.

USG values were significantly lower in group AG (Table 1), which is consistent with findings by Cortadellas *et al.* (2008). With the onset of CKD during the progression of LV, the distal and collecting tubules of the nephrons lose their ability to respond to ADH (antidiuretic hormone), in addition to the progressive cell death in the proximal convoluted tubules, which play an important role in the dehydration of the ultrafiltrate (Chew; Dibartola; Schenck, 2011; Riella, 2014). With that, the efficiency of the kidneys in concentrating urine is considerably reduced. Although no significant difference was verified between groups CG and NAG, 50% of the animals in group NAG had USG values lower than 1.030, whereas none of the animals in group GC showed USG lower than 1.030. According to Whatson; Lefebvre; Elliott (2015), hydrated dogs with USG below 1.030 should be investigated for the presence of CKD even in the absence of azotemia, as there are indications of failed ADH response. There are currently tests that are earlier than creatinine to detect CKD, such as SDMA, (IRIS, 2023) but they were not performed in the present study.

The UPC varied among the three groups and showed an increase in values in group GA. (Table 1). Proteinuria of renal origin can result from glomerular or tubular damage, or even a combination of both (Ibba; Mangiagalli; Paltriniere, 2016). Due to the criteria used in the methodology, animals with non-renal proteinuria were excluded. In dogs with VL, glomerulonephritis caused by the disease places stress on the proximal convoluted tubule cells, which attempt to reabsorb the large quantity of proteins filtered, leading to cell stress and death (Grauer, 2005; Chew; Dibartola; Schenck, 2011; Solano-Gallego *et al.*, 2011), which may justify the results found in the present study.

Statistical differences were observed between CG and NAG for the uGGT/uCRA. The uGGT/uCR ratio has been used as a marker of tubular injury, since the enzyme GGT is secreted by the proximal convoluted tubule cells and released into the lumen in disordered situations (Dierickx, 1981; Hiene; Moe; Molmen, 2001; Hart, 2005; Chew; Dibartola; Schenck, 2011). Frazílio *et al.* (2018) observed a statistical difference between the uGGT/uCR of animals without and with proteinuria, which is similar to that observed between the GC and GNA groups in the present study. This reinforces the fact that the presence of proteinuria is related to the tubular lesion verified by the increase in the uGGT/uCR ratio.

Although no statistical difference regarding the uGGT/uCR ratio has been detected between group AG and the remaining groups, it was possible to observe a higher median of this marker in group AG. However, 1/3 of the animals in this group showed uGGT/uCR ratio below 0.3, increasing the range of variation of the values and hindering statistical differentiation. A low uGGT/uCR ratio in azotemic animals may be due to decreased renal cell mass in the later stages of CKD, to keep the remaining tubular cells from producing enough enzyme to demonstrate an increase in urine as occurs in cases of cirrhosis to serum enzymes (Stockham; Scott, 2011). However, enzymuria is related to tubular injuries and not to glomerular filtration dysfunction (Ibba; Mangiagalli; Paltriniere, 2016).

The ROC curve for calculating the optimal USG value cutoff line resulted in a value of 1.026, but with a minimal difference in accuracy for the value of 1.030, accuracy of 78.13% for the value of 1.026 and 78.09% for the value of 1.030). As the USG value of 1.030 was recommended by Whatson; Lefebvre; Elliott (2015) and is already used in the veterinary practice, it was recommended to keep this value as the cutoff line. Using the USG value of 1.030 as a cutoff line for the presence of elevated uGGT/uCR, therefore indicated a higher possibility of injury or stress in the proximal convoluted tubule, a sensitivity of 85%, specificity of 73%, positive predictive value of 70% and negative of 87% was calculated. The prevalence ratio of a dog with leishmaniasis and USG below 1.030 presenting tubule injury was 4.95 ($p<0.01$, IC 95% - 1.32-18.48).

The ideal for diagnostic tests is 100% accuracy, as well as perfect positive and negative predictive values. However, this

Table 1. Comparison of median values, interquartile range and statistical difference for the urinary density, urinary protein: creatinine ratio, and urinary GGT: creatinine ratio among negative dogs (CG) for visceral leishmaniasis and azotemic (AG) and non-azotemic (NAG) positive dogs.

	CG (med±IQR) n=5	NAG (med±IQR) n=22	AG (med±IQR) n=6	p - value (Kruskal-Wallis)
USG	1.050-1,044-1,050^{A}	1.028-1.021-1.044^{A}	1.016-1,015-1,020^{B}	0,002
UPC	0.11-0.06-0.14^{A}	1.00-0,50-1,63^{B}	2.92-2,63-4,27^{C}	<0,0001
uGGT/uCR	0.23-0.21-0,26^{A}	0.66-0,47-1,60^{B}	0.95-0,40-1,80^{AB}	0,03

CG – Control group, NAG – Non-azotemic group, AG- Azotemic group, med – median, ±IQR – interquartile range, USG – urine specific gravity, UPC - urinary protein: creatinine ratio, GGTu/CRu – urinary GGT: urinary creatinine ratio. Different letters in the same row indicate a significant difference by the Mann-Whitney test ($p< 0.016$).

situation is practically impossible, so for a result obtained in a routine and low-cost exam with USG, lower accuracy values are acceptable (Stralen, *et al.*, 2009). With a negative predictive value of 87%, USG above 1.030 demonstrates a small possibility of the patient with VL having a tubular lesion, which can help in the screening of these patients.

Ibba; Mangiagalli; Paltriniere (2016) suggested that the uGGT/uCR should be used to verify the onset of CKD in dogs with VL, corroborating the results found in the present study. These results allow extending the recommendation that positive animals for VL and with USG below 1.030 should have their uGGT/uCR ratio evaluated in order to consider the existence of a possible tubular injury and the need for nephroprotective treatment, even without azotemia.

The limitations of this study were not measuring symmetric dimethylarginine (SDMA) as an early marker of CKD and performing electrophoresis of the collected urine proteins.

CONCLUSION

It is concluded that positive dogs for *leishmania* sp. with low USG (<1.030) should have their uGGT/uCR ratio measured to verify the presence of tubular injury even before the onset of azotemia. Thus, the presence of tubular injury can be better evaluated, which would be of assistance in choosing the therapy for each patient.

ACKNOWLEDGMENT

This work was carried out with the support of Universidade Federal de Mato Grosso do Sul and Capes (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, código de financiamento 001).

REFERENCES

CHEW, D. J; DIBARTOLA, S. P; SCHENCK, P.A. **Urologia e Nefrologia do cão e do gato**. 2 ed. Rio de Janeiro: Elsevier,2011. 554 p.

CORTADELLAS, O. *et al*. Glomerular Filtration Rate in Dogs with Leishmaniasis and Chronic Kidney Disease. **Journal of Veterinary Internal Medicine,** v. 22, p. 293-300, 2008.

DIERICKX, P. J. Urinary Gamma-Glutamyl Transferase as an Indicador of Acute Nephorotoxicit in Rats. **Archives of Toxicology**. v.47, p.209-215, 1981.

FRAZILIO, F. O. *et al*. Biomarkers and renal arterial resistive index in dogs naturally infected with Leishmania infantum. **Journal of Parasitology Research**, v. 117, p. 3399-3045, 2018.

GODOY, K. C. S.; ANTUNES, T. R.; BRAZ, P. H. *et al*. Comportamento dos marcadores laboratoriais de injúria renal em cães com leishmaniose visceral. In: **Enisap**, 2017, Cuiabá. Anais ... Cuiabá: UFMT, 2017.

GRAUER, G. F. Early detection of renal damage and disease in dogs and cats. **Veterinary Clinics of North America: Small Animal Practice,** v. 35, n. 3, p. 581-596, 2005.

HART, S. G. E. Assessment of renal injury in vivo. **Journal of Pharmacological and Toxicological Methods,** v. 52, p. 30-35, 2005.

HIENE, R; MOE, L; MOLMEN, G. Calculation of urinary enzyme excretion, with renal structure and function in dogs with pyometra. **Research in Veterinary Science**, v. 70, p. 129-137, 2001.

IBBA, F.; MANGIAGALLI, G.; PALTRINIERE, S. Urinary gamma-glutamyl transferase (GGT) as a marker of tubular proteinuria in dogs with canine leishmaniasis, using sodium dodecyl sulphate (SDS) electrophoresis as a reference method. **The Veterinary Journal**, v. 210, p. 89-91, 2016.

INTERNATIONAL RENAL INTEREST SOCIETY (IRIS). 2023. Disponível em: <http:/www.iris-kidney.com/>. Acesso em: 14 jul. 2023.

JEKEL, J. F; KARTZ, D. L; ELMORE, J. G**. Epidemiologia, bioestatística e medicina preventiva**. 2 ed., Porto Alegre: Artmed, 2001. 432 p.

KOVARIKOVA, S. Urinary biomarkers of renal function in dogs and cats: a review. **Vet Med (Praha)**, v.60, n.11, p. 589-602, 2015.

MANCINELLI, E; SHAW, D. J; MEREDITH, A. L. γ-Glutamyl-transferase (GGT) activity in the urine of clinically healthy domestic rabbits (Oryctolagus cuniculus). **Veterinary Record**, v. 10, 2012.

RIELLA, M.C**. Princípios de nefrologia e distúrbios hidroeletrolíticos**. 5 ed, Rio de Janeiro: Guanabara Koogan, 2014. 1247 p.

SOARES, M. J. V; MORAES, J. R. E; BORGES, V. P. *et al*. Renal Involvement in Visceral Leishmaniasis Dogs. **Journal of Venomous Animals and Toxins including tropical**, v. 11, n. 4, p. 579-593, 2005.

Solano-Gallego, L. *et al*. LeishVet guidelines for the practical management of canine leishmaniosis. **Parasit Vectors**, v. 86, n.4, 2011.

STOCKHAM, S. L; SCOTT, M. A. **Fundamentos de Patologia Clínica Veterinária**. Rio de Janeiro: Guanabara, 2011.724 p.

STRALEN, K. J; STEL, V. S; REITSNA, J. B. *et al*. Diagnostic methods I: sensitivity, specificity, and other measures of accuracy. **ABC of epidemiology**, v. 75, n. 12, p. 1257-1263, 2009.

WHATSON, A.D. J**;** LEFEBVRE, H. P; ELLIOTT, J. **Using urine specific gravity**, 2015. Disponível em: < http:/www.iris-kidney.com/education/urine_specific_gravity.html>. Acesso em: 13 set. 2018.

ZAR, J. H**. Biostatistical Analysis.** 4 ed. New Jersey: Prentice-Hall, 1999. 663 p.