Short communication

Urinary gamma-glutamyl transferase (GGT) as a marker of tubular proteinuria in dogs with canine leishmaniasis, using sodium dodecylsulphate (SDS) electrophoresis as a reference method

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Abstract

In order to assess if urinary $\gamma$-glutamyl transferase (GGT) identified tubular proteinuria in leishmaniotic dogs, the GGT/urinary creatinine (UC) ratio was calculated in 39 leishmaniotic dogs. According to sodium dodecylsulphate–agarose gel electrophoresis, the dogs had albuminuria (A, $n=10$), glomerular (G, $n=3$), tubular (T, $n=4$) or mixed proteinuria (M, $n=22$). The median GGT/UC ratio was 0.3, 0.3, 2.2, 7.5, in groups G, A, M, and T, respectively. Statistically significant differences were found between groups G and M ($P=0.002$), G and T ($P<0.001$), A and M ($P<0.001$), and A and T ($P<0.001$). Median values were higher in dogs with tubular components of proteinuria (M/T, 2.5) than in dogs without tubular components of proteinuria (A/G, 0.3), and in dogs with tubular proteinuria (T, 7.1) than in dogs with non-tubular proteinuria (NT, 1.0). GGT/UC values $>0.81$ or $>2.64$ could identify dogs in the M/T or T groups. Therefore, GGT/UC might be useful for the management of leishmaniotic dogs.

Keywords: Canine leishmaniasis; Proteinuria; Renal tubular damage; Sodium dodecylsulphate electrophoresis (SDS); Urinary GGT
The activity of urinary $\gamma$-glutamyl transferase (GGT), a marker of tubular damage (Smets et al., 2010), increases in leishmaniotic dogs (Palacio et al., 1997), but no comparisons with a reference method suggestive of tubular injury have previously been published. Sodium dodecylsulphate (SDS) electrophoresis separates low molecular weight (MW) proteins of tubular origin, from high MW proteins of glomerular origin (Zini et al., 2004), and correlates well with the results of renal biopsy (Zini et al., 2004; Brown et al., 2010).

The aim of this study was to assess if urinary GGT could identify leishmaniotic dogs with tubular proteinuria, using sodium dodecylsulphate (SDS)–agarose gel electrophoresis (SDS-AGE) as a reference method.

Urine specimens from 39 dogs with leishmaniasis (age range, 1-12 years; median age, 6 years) were analysed. Further details of the study population are reported in Table 1. Leishmaniasis was diagnosed based on clinical or laboratory changes and on positive cytology or PCR, as recommended by current guidelines (Paltrinieri et al., 2010). Proteinuric dogs (urinary protein to creatinine [UPC] ratio $>0.2$) with chronic kidney disease of International Renal Interest Society (IRIS) classification stage 1 (serum creatinine $<1.4$ mg/dL, $n=30$), stage 2 (serum creatinine 1.4 - 2.0 mg/dL, $n=6$) or stage 3 (serum creatinine 2.1 - 5.0, $n=2$) were enrolled. Specimens were collected by cystocentesis for diagnostic purposes with the informed consent of the dog owner: hence, according to Italian regulations the approval of the Ethical Committee was not necessary.

Specimens were centrifuged and sediment was analysed as described by Giori et al. (2011) to exclude specimens with bacteriuria, haematuria ($>5$ erythrocytes/high power field,
hpf) or pyuria (>5 leukocytes/hpf). The presence of cellular or granular casts or epithelial cells indicative of tubular damage was recorded.

Urinary protein and urinary creatinine (UC) were measured in supernatants using a spectrophotometer (Mindray BC-120, Shenzen Mindray Biomedical) and pyrogallol red using a modified Jaffe method, respectively. GGT activity was measured immediately after specimen collection and centrifugation, on the same analyser, using the reagents produced by the manufacturer of the instrument, based on the use of γ-glutamyl-p-nitroamidine as a substrate. The intra- and inter-assay coefficient of variations were < 2%. The UPC ratio and the GGT/UC ratio were calculated and supernatants were frozen at -20°C for ≤6 months.

After thawing, SDS-AGE was performed using automated equipment (Hydrasis, Sebia Italia S.r.l.), as previously described (Giori et al., 2011). Dogs were defined as albuminuric (A) or affected by glomerular (G), mixed (M) or tubular (T) proteinuria (Fig. 1).

The majority of dogs had mixed proteinuria (n=22, one with granular casts and one with epithelial cells in the sediment, and therefore both likely affected by tubular damage), followed by albuminuria (n=10), glomerular proteinuria (n=3) or tubular proteinuria (n=4). Although albumin can also be present in the urine if tubular damage is present, in people and probably in dogs, microalbuminuria can also be an early sign of glomerulopathy because of reduced resorption of albumin that normally passes through the glomerular barrier (Smets et al., 2010). Therefore, in our study, we considered it very likely that dogs from groups A and G had glomerular damage. The high proportion of dogs from ‘non T’ groups (groups A, G and M) agrees with the results of a study which demonstrated that tubulointerstitial lesions (potentially responsible for mixed proteinuria) frequently complicated glomerular disease, and
were potentially responsible for glomerular proteinuria and albuminuria (Zatelli et al., 2003). Conversely, pure tubular proteinuria was rare in our study, confirming the results of a study that identified tubular bands only when free light chain (FLC) proteinuria was present (Bonfanti et al., 2004).

Since SDS-AGE can show positive bands in non proteinuric dogs with concentrated urine (Giori et al., 2011), to avoid false positive results, only dogs with UPC ratios > 0.2 were enrolled in our study. Five dogs were borderline proteinuric (UPC ratio, 0.36-0.42) according to the IRIS classification (Elliott and Watson, 2009), and had glomerular proteinuria (n=1), mixed proteinuria (n=1), or albuminuria (n=3). Thirty-four dogs were proteinuric (UPC ratio, 0.56-13.33) and mostly had mixed proteinuria (n=21), but albuminuria (n=7), tubular proteinuria (n=4) and glomerular proteinuria (n=2) were also documented.

Statistics were performed using commercially available software (v. 2.21, Analyse-it Software). A Kruskall Wallis test followed by a post-hoc comparison with a Bonferroni correction was used to compare the GGT/UC ratios in groups M, T, A and G. Based on the number of comparisons, the P value to indicate statistical significance was adjusted to P<0.008. The median GGT/UC ratios in groups M and T (2.2 and 7.5, respectively) were significantly higher than those in groups G (P=0.002 vs. M; P<0.001 vs. T) and A (0.3 for each; P<0.001 vs. M and T; Fig. 2).

The median GGT/UC ratio was higher (2.5) in dogs with tubular components of proteinuria (M/T) than in dogs without tubular components of proteinuria (A/G; 0.3), and in dogs with pure tubular proteinuria (7.1) than in all dogs with non-tubular proteinuria (NT; 1.0). The results of these subgroups, however, were not compared statistically, since it was
considered statistically inappropriate to perform multiple analyses on groups formed by combining the same data in different subgroups, especially since our group sizes were relatively low. Therefore, it would be interesting in the future to assess whether these differences are confirmed by the analysis of a larger caseload.

Receiver operating characteristic curves were plotted to assess the ability of GGT/UC to identify dogs in the T or M/T groups. For each observed value, sensitivity, specificity and positive likelihood ratio (LR+) were calculated, based on the number of true positive or false positive results and false negative or true negative results. The areas under the curves were 0.97 (M/T) and 0.86 (T; \( P < 0.001 \) for both). The optimal cut-off values for the detection of dogs in the M/T or T groups were 0.81 and 2.64, respectively (M/T sensitivity, specificity and LR+: 92.3%, 84.6%, 6.0, respectively; T sensitivity, specificity and LR+: 75.0%, 74.3%, 2.9, respectively).

In our study, GGT/UC differentiated dogs that, according to SDS-AGE, had mixed or tubular proteinuria. Although previously published work has reported the specificity of SDS-AGE tubular bands to identify tubular damage varies from 50% to 62.5%, its sensitivity is high (82% and 92%; Zini et al., 2004; Brown et al., 2010). Therefore, the detection of high GGT/UC values could be useful in the clinical management of leishmaniotic dogs, since tubular bands might indicate more advanced renal lesions (Zatelli et al., 2003), or could perhaps indicate FLC proteinuria (Bonfanti et al., 2004). Therefore, the presence of a tubular component could be a marker of disease progression. The GGT/UC ratio must be determined just after specimen collection to avoid storage artifacts (Flandrois et al., 1989) and could be a rapid, cheap tool to identify dogs that require further investigation using SDS-AGE or, if clinically appropriate, renal biopsy. Based on the LR+ calculated in our study, the probability
that a dog with GGT/UC > 0.81 or > 2.64 had mixed or tubular proteinuria, was six and three times higher than the probability that the dog did not have these changes on SDS-AGE, respectively. The lower LR+ for pure tubular proteinuria was probably related to the low number of cases in this study. Additionally, the individual variability shown in Fig. 1 creates overlapping results in groups T and M, thereby reducing the ability of GGT/UC to differentiate between the two conditions. However, as previously stated, tubular bands in isolation are associated with FLC proteinuria rather than with tubulointerstitial lesions (Bonfanti et al., 2004). Hence, the differentiation of dogs with tubular bands from those with mixed proteinuria due to tubulointerstitial damage is less relevant for clinical management.

The determination of GGT/UC in fresh urine specimens is a rapid and cheap tool to identify dogs that have tubular proteinuria on SDS-AGE analysis, consistent with advanced leishmaniasis, and should be used as an indicator to drive further diagnostic protocols in these animals.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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References


Table 1. Signalment and clinical signs

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Fig. 1. Sodium dodecylsulphate (SDS) gel electrophoresis. Glomerular proteinuria (G) was identified when bands corresponding to proteins with molecular weights (MW) higher than albumin were present. Tubular proteinuria (T) was identified when bands corresponding to proteins with MW lower than albumin were present. Mixed proteinuria (M) was identified when bands corresponding to proteins with MW higher and lower than albumin were present. Specimens with bands corresponding to the MW of albumin were considered albuminuric (A). Specimens with bands of MW equal to that of albumin and bands of MW higher or lower than that of albumin were classified as G or T, respectively. The first lane (ctrl) includes the MW marker, with bands corresponding to lisozime (LZM), triose phosphate isomerase (TPI), albumin (Alb) and immunoglobulin G (IgG).

Fig. 2. Distribution of results recorded in the dog groups and subgroups. (A) Comparison of all groups; A, albuminuric; G, glomerular proteinuria; M, mixed proteinuria; T, tubular proteinuria. (B) Comparisons of results for dogs with and without tubular involvement; A/G, albuminuric or glomerular proteinuria; M/T, mixed or tubular proteinuria. C, Comparison of results from dogs with pure tubular proteinuria and those from other groups; NT, non tubular proteinuria (e.g. albuminuric, glomerular or mixed proteinuria); T, tubular proteinuria.