Evaluation of snake envenomation induced renal dysfunction in dogs using early urinary biomarkers of nephrotoxicity

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Abstract

Renal dysfunction in dogs envenomed by snakes has mostly been evaluated using routine serum and urine renal markers. Generally, these are not able to detect the renal damage at an early stage and their sensitivity is affected by hemolysis, hematuria and pigmenturia. Selective use of urinary biomarkers can provide early information on severity and stage of renal injury caused by nephrotoxic substances before major decline in renal function occurs. Therefore, the aim of this study was to evaluate venom-induced renal damage using urinary markers of glomerular (urinary albumin (uAlb), immunoglubin G (IgG) and C-reactive protein (uCRP)) and proximal tubular dysfunction (urinary retinol binding protein (uRBP)) and compare these markers with routine renal markers (serum urea (BUN) and creatinine (sCr), urinary specific gravity (USG) and urinary protein to creatinine ratio (UPC)). Nineteen dogs envenomed by either neurotoxic or cytotoxic snakes and ten clinically healthy dogs were included in this study. Urinary markers were measured using previously validated commercially available ELISA kits. Among measured routine renal markers a significant difference between snake -envenomed and healthy dogs was noted only in UPC, but in the presence of hematuria and hemoglobinuria, differentiation between prerenal and renal proteinuria was not possible. The urinary biomarkers uAlb, uIgG and uRBP in snake-envenomed dogs were significantly increased ($P<0.05$) when compared to healthy dogs at admission, whereas 24 h after envenomation only uCRP was significantly elevated. Using urinary /markers, results of this study showed that snake venom evokes renal dysfunction at the glomerular and tubular region of the nephron.

Keywords: Envenomation; canine; glomerular marker; tubular marker; renal injury

Introduction

Snake envenomation in dogs is mostly reported in countries with high snake burden, namely Sub-Saharan Africa (Leisewitz et al., 2004; Lobetti and Joubert, 2004) and Australia (Heller et al., 2007). However, in the United States envenomation has been recently estimated to affect 150,000 dogs and cats per year (Gilliam and Brunker, 2011) and in the United Kingdom, 0.7% of the calls to Veterinary Poisonous Information Services were reported to be related to snake envenomation events (Sutton et al., 2011). Mortality of dogs secondary to snake envenomation in different parts of the world has been reported to be from 1 - 5% (Lervik et al., 2010; Gilliam and Brunker, 2011; Sutton et al., 2011) and as high as 30% (Gilliam and Brunker, 2011).

Tissue injury resulting from envenomation is usually a result of the enzymes, cytokines and polypeptide toxins in the venom (Kang et al., 2011). As a highly vascularised organ and also as a major elimination route of the snake venom, human and animal kidneys are very susceptible to nephrotoxic effects of envenomation (Sitprija, 2006; Kanjanabuch and Sitprija, 2008; Mello et al., 2010). Kidney injury after envenomation in humans, dogs and rats was reported to be caused either by a direct action of venom or an indirect action through inflammatory mediators, and nephrotoxic effects of myo- and haemoglobin, hypovolemia, disseminated intravascular coagulopathy (DIC) and renal ischemia (Sitprija, 2006; Mello et al., 2010; Goddard et al., 2011; Jacoby-Alner et al., 2011). Renal pathology inflicted by snake venom involves all renal structures, i.e. glomeruli, tubuli, renal interstitium and vasculature. The most commonly observed clinical manifestations of renal abnormalities after snake envenomation in humans and dogs are acute kidney injury (AKI), proteinuria and hematuria (Sitprija, 2006; Kanjanabuch and Sitprija, 2008; Goddard et al., 2011)

The majority of studies and reports of snake envenomation in dogs have focused on routine serum analyses and only to some extent on urinalysis (Heller et al., 2007; Lervik et al., 2010; Gilliam and Brunker, 2011; Sutton et al., 2011). Nevertheless, these parameters are nonspecific and insensitive as they are only altered at the point when about 50 – 75% of renal function is already lost (Finco, 1997). Data on the extent and regions of venom-induced renal damages in dogs have mostly been gathered on autopsy cases through histopathological examinations of renal tissue (Lewis, 1994; Puig et al., 1995; Jacoby-Alner et al., 2011). The latter provide static images on structural kidney lesions in dead animals, but give no insight into dynamics and functional changes ultimately leading to renal failure *in vivo*. Studies in dogs assessing functional changes in kidneys after snake envenomation are still missing.

A variety of urinary biomarkers have recently been introduced ranging from proteins of low (LMW) to high molecular weight (HMW) which are helpful in the assessment of the localisation, extent and progression of renal injuries (Price, 2002; Smets et al., 2010; Maddens et al., 2011) . Among these biomarkers, the HMW proteins, such as urinary albumin (uAlb), immunoglubin G (IgG) and C-reactive protein (uCRP) are usually associated with glomerular damage, whereas detection of urinary retinol binding protein (uRBP) typically reflects tubular damage (Bernard et al., 1987; Price, 2002; D'Amico and Bazzi, 2003). uAlb has been demonstrated to be an indicator of early glomerular damage which precedes more severe proteinuria (Price, 2002; D'Amico and Bazzi, 2003; Smets et al., 2010). Two other HMW proteins which are also associated with a damaged glomerular barrier are uIgG and uCRP (Price, 2002; D'Amico and Bazzi, 2003). Retinol binding protein (RBP) belongs to the group of LMW proteins and is freely filtered through the glomerulus but subsequently reabsorbed by epithelial cells of the proximal tubules (Marinó et al., 2001). An elevated concentration of

RBP in urine has been described in various kidney diseases in cats as well as in dogs (van Hoek et al., 2008; Smets et al., 2010; Maddens et al., 2010; Nabity et al., 2012).

As most routine markers measure the overall effect of toxin on kidneys, it was speculated that the determination of glomerular and tubular damage urinary markers could assist to determine the stage, severity and localisation of early renal damage in snake-envenomed dogs. Additionally, such measurements could be performed *in vivo* in an easy and non-invasive way. Therefore, the main objective of this study was to assess the localisation and extent of renal damage in dogs envenomed by snakes, using urinary markers for glomerular (uAlb, uIgG and uCRP) and proximal tubular dysfunction (uRBP) in comparison to the routine renal parameters (serum urea (BUN) and creatinine (sCr), urinary specific gravity (USG) and urinary protein to creatinine ratio (UPC)).

Materials and methods

Animals

This prospective clinical study included 19 client-owned dogs of various breeds, age and weight that were presented to the Onderstepoort Veterinary Academic Hospital (OVAH), University of Pretoria. Dogs were recruited if envenomed by cytotoxic or neurotoxic snakes. Clients witnessing the bite were asked to identify the snake either by an accurate description, or alternatively, from a picture. Presence of concurrent systemic diseases was excluded through: detailed anamnesis, physical examination, routine hematology including evaluation of a thin peripheral blood smear to exclude *Babesia spp.* and *Ehrlichia spp*., serum biochemistry and a urinalysis including bacterial culture. At the presentation routine hematology and serum biochemistry were performed in all dogs, whereas 24 h after admission only routine hematology, BUN and sCr were measured. Upon presentation to the hospital and

after collection of samples, the snake envenomed dogs received appropriate symptomatic treatment, which included treatment with polyvalent antivenom in the majority of patients.

The control group consisted of 10 client-owned dogs of all ages, breeds and both sexes from the same geographical area as the patients. All dogs were determined to be healthy on the basis of their history and the results of a physical examination, routine hematology and urinalysis (including negative bacteriological culture and a UPC < 0.5). In addition in all control dogs BUN and sCr were measured as well. Client consent was obtained for all dogs included in this study and the study protocol was approved by the Animal Use and Care Committee of the University of Pretoria (Protocol no. V058-10).

Evaluation of serum CRP (sCRP) concentration

The sCRP concentration was measured using an automated turbidometric immunoassay (TIA) for human CRP (Randox CRP-assay), previously validated for use in dogs (Kjelgaard-Hansen et al., 2003). The assay was calibrated with commercially available purified canine CRP (Life Diagnostics) to ensure species-specific measurement of CRP concentration with the heterologous assay. The intra-assay coefficient of variation for the CRP method was calculated as 0.1%. The detection range for the assay was 5.1 to 163.3 mg/L. Internal controls, consisting of pooled serum samples from 5 healthy dogs, were routinely run with the batch of samples. Samples were analyzed as a batch to avoid inter-assay variability, and outliers were immediately reanalyzed to confirm results.

Sample handling and submission

Upon presentation and 24 h post-envenomation urine samples were collected by cystocentesis. Collection of urine by sterile urinary catheterization or voiding was used only

in dogs with suspected coagulopathies. Urinalysis included visual evaluation (clarity, colour) of urine, evaluation of urine specific gravity (USG) with a refractometer, routine urine sediment analysis, routine dipstick analysis (Combur 9 Test kit, Roche), UPC and bacteriological culture. After collection, urine was centrifuged (447 x *g*, 3 min) and aliquoted. Urinalysis was performed at the Clinical Pathology Laboratory of the OVAH within 30 min of collection. Remaining aliquots were stored at -80 °C until analysis. The stored urine samples were transported to the Department of Pharmacology, Toxicology and Biochemistry, University of Ghent, Faculty of Veterinary Medicine, on dry ice, and transit time for the shipment was < 24 h.

Evaluation of urinary markers

Concentrations of uAlb, uCRP and uIgG were determined using commercial canine-specific sandwich enzyme-linked immunosorbent assay (ELISA) kits (Canine Alb ELISA, Dog CRP ELISA and Dog IgG ELISA, Immunology Consultants Laboratory) previously validated by our group (Smets et al., 2010; Maddens et al., 2010). Human sandwich ELISA kit (RBP ELISA, Immundiagnostiek AG) was used to quantify the relative amount of canine uRBP as previously validated by our group (Maddens et al., 2010).

Colorimetric measurements were performed at a wavelength of 450 nm with 650 nm as a reference using an ELISA plate reader Multiskan MS (Labsystems Thermo Fisher Scientific). A 4-parameter logistic curve fitting program (Deltasoft JV, Biometallics Incorporated) was used to generate the standard curve and to calculate the concentrations of uAlb, uIgG, uCRP, and uRBP. Finally, results were indexed to urinary creatinine concentrations (c) and expressed as ratios.

Statistical analysis

Data were analyzed using a commercial software package (GraphPad Prism version 5.00 for Windows, GraphPad Software). The nonparametric Mann-Whitney *U* test was applied to compare the results from the serum biochemistry, hematology, sCRP concentration, urinalysis and urinary biomarkers concentration between the snake-envenomed dogs and the control group. The same variables at the time of presentation and at 24 h post-envenomation were compared using the Wilcoxon signed-rank test for paired samples. When the normal distribution assumption did not hold, the Wilcoxon rank sum test was used. The level of significance was assigned with values of $P<0.05$.

Results

Study population

Dogs enrolled were envenomed by either cytotoxic (n=11) or neurotoxic snakes (n=8). Median age of snake-envenomed dogs was not significantly different from the median age of control dogs ($P=0.909$). There was also no significant difference between body weight of snake-envenomed dogs and control dogs ($P=0.630$) (Table 1). Breeds included were five smooth-haired dachshund, three crossbreeds, two Jack Russell terriers and boerboels and one rottweiler, beagle, Staffordshire bull terrier, fox terrier, bullmastiff, Yorkshire terrier and French poodle. Breeds in the control group included three beagles, two border collies and one Staffordshire bull terrier, English bull terrier, smooth-haired dachshund, German short-haired pointer and small crossbreed.

Results of hematology in control dogs and snake-envenomed dogs at presentation (T0) and 24 h post-envenomation (T1) are shown in Table 1. Comparing to control dogs at T0 and at T1 a significant decrease in number of platelets and a significant neutrophilia, and at T1

also a significantly decreased packed cell volume (PCV) and elevated white blood cells (WBC) were noted. No significant abnormalities were observed in routine biochemical variables at T0 (table not shown).

At T0 the sCRP concentration was measured in 14 and at T1 in 17 snake-envenomed dogs. At T0 and at T1 sCRP were 9.4 (range, BDL – 50.6) and 55.4 (range, BDL – 121.9) and in control group 5.6 (range, BDL – 11.1). sCRP was not significantly different between the T0 and the control group (P=0.417) but was significantly higher than in controls at T1 ($P <$ 0.0004). More importantly, significant elevation of sCRP concentration was also noted between T0 and T1 (P<0.02).

Macroscopic evaluation of urine at T0 showed 4 cloudy urine samples, and at T1 5 cloudy urine samples. At T0 a urine dipstick analysis revealed a proteinuria in 17 out of 19 and at T1 in 12 out of 15 dogs. At T0 9 out of 19 dogs had hemoglobinuria, which was severe (4+) in 4 dogs. At T1 hemoglobinuria was present in 12 of 15 snake-envenomed dogs and was considered severe (4+) in 11 of these dogs. At T0 glucosuria was present in 3 snake-envenomed dogs. At T1 3 dogs had bilirubinuria, from these it was considered severe (3+) in 1 dog. Urine cultures were performed in 18 snake-envenomed and 10 control dogs. Positive urine cultures were found in 2 snake-envenomed dogs and 1 control.

Routine serum and urinary renal markers

Results of routine serum and urinary markers in control dogs and snake-envenomed dogs at T0 and T1 are shown in Table 1. At T0 and T1 mean BUN and sCr were within the normal range. At T0 and at T1 compared to control dogs BUN concentration did not differ significantly (P=0.429 and P=0.249), as well as between T0 and T1 (P=0.076). Similarly, at

T0 and T1 comparing to control dogs no significant difference was observed in sCr concentration (*P*=0.099 and *P*=0.387), however at T0 sCr concentration was significantly higher than at T1 (*P*<0.003). No significant difference in USG comparing to control dogs was found either at T0 and T1 (*P*=0.547 and *P*=0.674), as well as between T0 and T1 (*P*=0.414). Both at T0 and at T1 compared to the control group, significant elevation in UPC was noted. No significant difference in UPC was observed between T0 and T1 (*P*=0.685). Evaluation of the magnitude of proteinuria based on UPC at T0 resulted in 8/19 dogs (42.1%) and at T1 3/15 dogs (20%) considered as proteinuric (UPC > 0.5).

At T0, cellular casts were observed in 2 (10.5%), granular casts in 4 (21%) and hyaline casts in 1 dog (5.2%), tubular epithelial (RTE) cells in 5 (26.3%) and bladder epithelial cells in 5 dogs (26.3%). At T1, cellular casts were noted in 4 dogs (26.6%), granular casts in 4 dogs (26.6%), RTE in 7 dogs (46.6%) and bladder epithelial cells in 4 dogs (26.6%). At T0, hematuria was present in 3 dogs (15.7%) and was severe in only 1 dog, whereas at T1 hematuria was noted in 12 dogs (80%) and was severe in 7 dogs. In the sediment of control dogs granular casts were found in 1 (10%) and small amount of bladder epithelial cells in 5 dogs (50%).

Urinary biomarkers

Results of uAlb/Cr, uIgG/Cr and uRBP/Cr are presented in Figure 1. At T0, all snake-envenomed dogs demonstrated a significant increase (*P*<0.05) in uAlb/Cr, uIgG/Cr and uRBP/Cr compared to control dogs. At T0, no significant difference (*P*=0.280) was found for uCRP/Cr which was below the detection limit (BDL) in all control dogs and was barely quantifiable but still detectable only in 3 snake-envenomed dogs. At T1 comparing to control dogs a significant increase in both, uIgG/Cr (*P*<0.01) and uCRP/Cr (0.0312 mg/dl (range,

BDL – 1.2; *P*< 0.001) was noted. Moreover, uCRP/Cr at T1 was also significantly higher (*P*<0.001) than uCRP/Cr at T0. However, at T1 no significant differences were noted in uRBP/Cr and uAlb/Cr (*P*=0.357 and *P*=0.108) when compared to control dogs. Furthermore, no significant differences were found between uAlb/Cr, uIgG/Cr and uRBP/Cr between T0 and at T1 (*P*=0.903, *P*=0.761 and *P*=0.791, respectively).

Results of the urinary markers analysis were also not affected by the presence of hemoglobinuria as previously shown by our group (Defauw et al., 2012).

Discussion

Snake-envenomed dogs in this study showed a considerable increase in concentration of four measured urinary markers, namely uAlb, uIgG, uCRP and uRBP, indicating dysfunction at the glomerular and tubular regions of the nephron. Conversely, routine renal markers, with the exception of UPC in this study failed to demonstrate early renal damage inflicted by snake venom.

BUN and sCr concentrations in snake-envenomed dogs measured at T0 were within the normal reference range, although it should be remarked that the serum renal markers in dogs are usually only altered when about 75% of functioning nephrons are lost (Finco, 1997). In this view routine urinary marker assays are generally known to be better indicators of renal damage as the loss of urine concentrating ability is observed sooner than renal azotemia (Price, 2002). However, measured USG in this study, at both T0 and T1, was not significantly different from that in control dogs. AT T0 and T1 a significant increase in UPC as oppose to controls was observed, however hemoglobinuria was present in approximately half of the dogs at T0, whereas at T1 severe hemoglobinuria was present in almost all dogs. Similarly,

hematuria was detected at both occasions, and it was severe in approximately half of the dogs at T1. In view of these results and the known pathogenesis of snake envenomation, the observed proteinuria in these dogs could have been either prerenal or renal. In less than half of the dogs, cellular casts, granular casts and RTE cells, indicating a tubular injury, were also reported at both occasions. The presence of cellular and granular casts indicates acute tubular damage but does not give information on the extent and possible reversibility of tubular injury (Villiers et al., 2005). Overall, based on the results of routine serum and urine markers, evaluation of the magnitude of renal dysfunction at an early phase was not possible. Correlation of our findings with results of histopathological examination would be interesting, however no renal biopsies were taken due to ethical reasons and possible complications arising from this procedure. Regardless, measurement of urinary biomarkers provided additional evidence of glomerular and tubular dysfunction induced by the snake venom.

Observed increases in uAlb and uIgG are usually a reflection of increased glomerular permeability (Haraldsson et al., 2008). Microalbuminuria has been described in various kidney diseases in dogs (Smets et al., 2010), but even though uAlb was significantly increased, the simultaneous measurement of uIgG was performed as it has been reported to allow more precise evaluation of the extent of glomerular damage (Bazzi et al., 2001). The HMW protein IgG is usually excreted when permselectivity of the glomerular capillary wall is severely disrupted (Bazzi et al., 2000, 2001; D’Amico and Bazzi, 2003). An added value of uIgG in selectivity of proteinuria was also described in dogs with different types of nephropaties (Maddens et al., 2010, 2011; Vinge et al., 2010; Smets et al., 2012; Nabity et al., 2012). Elevation of both these markers of glomerular injury in this study also correlates well with described venom-induced histopathological lesions in kidneys of humans, dogs and rats

(Rehan et al., 1986; Boer-Lima et al., 2002; Kanjanabuch and Sitprija, 2008; Mello et al., 2010).

A third HMW marker uCRP was detected only at T1. Comparable increase was also observed in the measured sCRP concentration, which was also significantly elevated only at T1. The presence of uCRP in urine is typically a result of a combination of increased concentration of sCRP and leakage of systemic CRP through the glomerular capillary wall. However, detection of CRP in urine is usually a sign of substantial damage to the glomerular barrier, regardless of its circulating concentration (D'Amico and Bazzi, 2003). Similar changes in sCRP concentration after envenomation were previously reported in human patients envenomed by snakes in which sCRP peak levels were also observed one to two days after hospital admission rather than at the time of admission (Barraviera et al., 1995). The latter is an acute phase protein produced in hepatocytes in response to IL-6 and TNF-α and serves as a sensitive marker of acute inflammation or infection (Baumann and Gauldie, 1994). In several studies, mice injected with snake venom demonstrated an elevation of IL-6 as well as TNF-α (Lomonte et al., 1993; Chaves et al., 2005). Increase in IL-6 was also described in human snake victims suggesting that envenomation shares a lot of similarities with acute inflammation (Barraviera et al., 1995). Nonetheless, the presence of uCRP in snake-envenomed dogs in this study is most likely a combination of sCRP elevation due to acute trauma and severe glomerular damage allowing the leakage of HMW proteins.

The LMW marker uRBP was also significantly elevated at T0 but returned to normal at T1. Trace levels of RBP are physiologically present in urine but higher concentrations of RBP are usually associated with tubular proteinuria (Bernard et al., 1987). Tubular necrosis is suspected to be a frequent cause of acute renal failure in human victims of snake

envenomation. Aside of this, several other tubular pathologies were also described after evenomation, namely infiltration of tubuli with different inflammatory cells, tubular interstitial lesions and oedema (Kanjanabuch and Sitprija, 2008). Furthermore, the combined elevation of two urinary markers of different molecular weight, namely the HMW IgG and the LMW RBP, as observed in this study, was also reported to be an excellent prognostic marker for ongoing renal injury in humans (Bazzi et al., 2001).

Three measured renal markers, uAlb, uIgG and uRBP were noted to be high at T0, but remained unaltered at T1, suggesting a transient character of venom induced proteinuria. Similar findings were reported in human snake victims in which the incidence of proteinuria usually resolved after clinical improvement of patients (Sitprija, 2006; Kanjanabuch and Sitprija, 2008). Rats injected with viperid venom also demonstrated a significant decline in proteinuria 48 h post-injection (Boer-Lima et al., 2002). Despite these observations, further *in vivo* studies assessing the course of events ultimately leading to proteinuria and renal failure after snake envenomation are needed. Moreover, the design of future studies should additionally address follow up and correlation with clinical outcome.

Conclusion

The results of this prospective clinical study demonstrated glomerular and tubular dysfunction in snake-envenomed dogs. Early renal damage was assessed using the urinary biomarkers, uAlb, uIgG, uCRP and uRBP. In contrast, other routine serum and urinary markers failed to demonstrate the magnitude of early nephrotoxicity induced by snake venom. The detected renal injury and its transient character as observed in this study seem to correlate well with current published histological and kinetic studies of renal pathology inflicted by

snake venom in dogs, rats and humans. To our knowledge this is the first study in veterinary medicine assessing the localisation of early nephrotoxicity of snake venom *in vivo.*

Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Table 1

Summary of hematology results and routine serum and urinary parameters for control, snake-envenomed dogs upon presentation (T0) and snake-envenomed dogs post-envenomation (T1). Values are expressed as median and range.

	Controls	Snake-envenomed (T0)	Snake-envenomed (T1)
Number of dogs	10	19	18
Age (years)	4.3 (1.3 – 8)	4.0 (1 – 12)	4.7 (1.4 – 12)
Body weight (kg)	17.6 (6 – 38)	12.6 (5.8 – 39)	16.8 (5.8 – 33.6)
Sex	3m, 1 mn, 4f, 2fn	7m, 2mn, 7f, 3fn	7m, 1 mn, 6f, 1fn
PCV (%)	49.5 (43 – 58)	53.0 (34 – 58)	43.5 (20 – 56)[b]
Platelets (10^9/L)	328 (218 – 455)	234 (0.5 – 501)[a]	195 (45–414)[b]
WBC (10^9/L)	8.8 (6.0 – 11.1)	14.1 (4.7 – 38)	14.0 (1.1 – 41.6)[b]
Neutrophils (10^9/L)	5.7 (2.3 – 8.1)	9.2 (1.8 – 29.6)[a]	10.4 (0.25 – 36.6)[b]
Lymphocytes (10^9/L)	2.5 (0.8 – 3.7)	2.0 (0.5 – 6.8)	1.7 (0.3 – 7.4)
Monocytes (10^9/L)	0.4 (0.0 – 0.72)	0.5 (0.2 – 2.5)	0.5 (0.2 – 1.8)
BUN (mmol/L)	6.1 (4.1– 11.8)	6.4 (2.9 – 12.1)	5.2 (2.1 – 11.5)
sCr (μmol/L)	68.7 (44 – 93)	82 (45 – 130)	56.2 (BDL – 111)
	Controls	Snake-envenomed (T0)	Snake-envenomed (T1)
Number of dogs	10	19	15
USG (g/L)	1.031 (1.010 – 1.050)	1.036 (1.010 – 1.050)	1.033 (1.004 – 1.050)
UPC	0.10 (0.05 – 0.21)	0.27 (0.08 – 24.1)[b]	0.34 (0.08 – 0.98)[b]

[a]Significant difference ($P < 0.05$); [b]Significant difference ($P < 0.01$); m, male intact; f, female intact; mn, male neutered; fn, female neutered; PCV, packed cell volume; WBC, white blood cells; BUN, blood urea nitrogen; sCr, serum creatinine; BDL, below detection limit; USG, urine specific gravity; UPC, urine protein - creatinine ratio.

Figure legends

Figure 1. Box and whisker plots of renal biomarkers uAlb/Cr, uIgG/Cr and uRBP/Cr in urine of control dogs (n=10), snake-envenomed dogs at T0 (n=19) and snake-envenomed dogs at T1 (n=15). Each box-and-whisker plot illustrates the median, quartiles, average (+) and outliers (•) values; significant difference at the level of *$P < 0.05$ and **$P < 0.01$ compared to control.

Figure 1