Kidney International, Vol. 67 (2005), pp. 659–667

Acute renal failure after *Crotalus durissus* snakebite: A prospective survey on 100 patients

FÁBIA M.O. PINHO, DIRCE M.T. ZANETTA, and EMMANUEL A. BURDMANN

Division of Nephrology, University of São Paulo Medical School, São Paulo, and Hospital de Doenças Tropicais (Tropical Diseases Hospital) of Goiânia, Brazil; Department of Epidemiology and Public Health; and Division of Nephrology, São José do Rio Preto Medical School, Brazil

Acute renal failure after *Crotalus durissus* snakebite: A prospective survey on 100 patients.

Background. Acute renal failure (ARF) is the main cause of death after the South American crotalid snakebite. The aim of this study was to assess the prevalence, risk factors, and characteristics of *Crotalus durissus* venom-induced ARF.

Methods. One hundred cases of *Crotalus durissus* bite were studied from hospitalization to discharge or death. Creatinine clearance (GFR) <60 mL/min/1.73m^2 in the first 72 hours after snakebite was defined as ARF. Data are expressed as median (range of variation) or %, and were analyzed by univariate analysis and logistic regression.

Results. Twenty-nine patients developed ARF. Of those, 24% required dialysis and 10% died. ARF patients had smaller body surface [1.55 (0.6–2.3) vs. 1.7 (0.6–2.1) m^2, $P = 0.0097$], received antivenom (AV) later [12 (2–48) vs. 2 (1–14) hours, $P < 0.0001$], received more AV [190 (90–536) vs. 158 (75–500) mg/m^2, $P < 0.0001$], presented lower diuresis at admission [62 (0–182) mL/hr vs. 100 (25–325) mL/hr, $P = 0.0004$], and showed a striking creatine kinase (CK) increase [50,250 (69–424,120) vs. 1108 (88–133,170) U/L, $P < 0.0001$]. Age <12 years (OR 5.6, $P = 0.026$), time for AV >2 hours (OR 11.1, $P = 0.032$), CK at admission >2000 U/L (OR 12.7, $P = 0.0009$) were identified as independent risk factors for ARF, whereas diuresis at admission >90 mL/hr (OR 0.20, $P = 0.014$) was an independent protector factor.

Conclusion. C. durissus venom-induced ARF had high prevalence (29%). Delay for AV treatment, CK at admission >2000 U/L, and age <12 years were independent risk factors for ARF development. Diuresis at admission >90 mL/hr was a protective factor.

Poisonous snakebites are a serious health challenge in tropical regions due to their incidence, morbidity, and mortality [1, 2]. The World Health Organization (WHO) estimates there are approximately 125,000 deaths out of 2,500,000 poisonous snakebites a year worldwide [2].

In Brazil, there are 20,000 poisonous snakebites a year, a mean incidence of 13.5 bites/100,000 inhabitants and a mortality rate of about 0.45% [3]. The *Crotalus* gender, of the *Viperidae* family and *Crotalinae* subfamily, is represented in Brazil by a single species, *Crotalus durissus*, or the South American rattlesnake. It is responsible for 7.7% of the notified cases, and for a mortality rate of 1.9%, the greatest among all Brazilian poisonous snakes [3].

The crotalic venom is a complex mixture of enzymes, toxins, and peptides. The main identified toxins are crotoxin, crotamine, giroxin, convulxin, and an enzyme similar to thrombin [4, 5]. Crotoxin is responsible for the high toxicity of the venom [5] and has neurotoxic [6], myotoxic [7–10], and nephrotoxic [11–13] activity.

The clinical picture of *C. durissus* snakebite is characterized by mild local injury and systemic manifestations, which are frequently severe. Eyelid ptosis, blurred vision, and/or double vision, ophthalmoplegia, and paralysis of facial muscles are examples of the venom neurotoxic activity. Its myotoxic action induces rhabdomyolysis, characterized by generalized myalgia and myoglobinuria. The coagulant activity triggered by the thrombin-like enzyme may lead to afibrinogenemia and blood incoagulability in 40% to 50% of the cases [1, 3].

Acute renal failure (ARF) is the major complication in patients surviving the initial effects of *C. durissus* envenomation, and is considered the major death cause in these accidents [14, 15]. The crotalid accident is approximately 10 times less frequent than the bothropic, but the absolute number of ARF cases reported in literature with both snake genders is similar [16]. Recent experimental studies suggest the pathogenesis of crotalid-induced ARF is related to rhabdomyolysis, renal vasoconstriction, and a direct nephrotoxic effect of the venom [11–13, 17, 18].

Although the reported prevalence of ARF after *C. durissus* snakebite is high [1, 19, 20], all available studies are retrospective and used parameters with poor

Key words: snakebite, acute renal failure, Crotalus, rattlesnake, rhabdomyolysis.

Received for publication June 12, 2004
and in revised form August 3, 2004
Accepted for publication August 18, 2004

sensitivity, such as serum creatinine [15, 19, 20], or that were not very specific, such as diuresis [1, 14, 21, 22], to evaluate renal function.

The objective of this study was to assess the prevalence, risk factors, and characteristics of ARF after envenomation by the South American crotalid.

METHODS

Victims of *C. durissus* snakebite admitted at the Hospital de Doenças Tropicais (HDT), Goiânia, Goiás (GO), were prospectively studied. HDT is a reference center for the treatment of snake envenomation in the State of Goiás, Brazil.

The diagnosis was based on the snake identification and/or by a clinical picture consistent with *C. durissus* envenomation within the first 24 hours after hospitalization. A typical clinical picture was defined as the presence of neurotoxic face (lid ptosis, flaccid paralysis of facial muscles, ophthalmoplegia), blurred and/or double vision, myalgia, and mild injury at the bite site.

Acute renal failure (ARF) was defined as endogenous creatinine clearance (GFR) <60 mL/min/1.73m^2 in the first 72 hours after envenomation with subsequent recovery of GFR. Patients who did not improve their GFR were carefully screened, and if there was clinical and/or laboratory evidence of chronic kidney disease they were excluded from the analysis. Chronic kidney disease was defined as baseline serum creatinine (previous to the accident) over 2 mg/dL, renal ultrasound with decreased kidneys or loss of the corticomedullary distinction, and/or history of active or past renal disease.

The study was approved by the Ethics Committees of the Tropical Diseases Hospital of Goiânia and Hospital das Clínicas, University of São Paulo Medical School. Patients were only included in the study after signing the informed consent. If the patient was younger than 18 years of age, informed consent was signed by the legal guardian.

All of the patients received specific crotalid antivenom (Instituto Butantan, São Paulo, Brazil) at HDT admission if adequate treatment had not been carried out previously. The crotalid antivenom (CAV) was administered intravenously, following the dosages recommended by the Health Ministry (HM), according to the classification of the accident (Table 1) [3]. Patients with changes in coagulation time 12 hours after antivenom administration received an additional CAV dose of 150 mg. Tetanus prophylaxis was used when required.

At admission, all patients were hydrated with an alkalinizing solution (500 mL of glucose 5%, 25 mL of mannitol 20%, 10 mL of sodium chloride 20%, and 20 mL of sodium bicarbonate 8.4%), 3 to 6 L/day, according to clinical picture. This solution was used to prevent renal injury by rhabdomyolysis. If the urinary flow remained satisfactory (1–2 mL/kg/hr in children and 30–40 mL/hr in adults), the solution was maintained for 48 to 72 hours. If the patient remained oliguric (diuresis <400 mL/day), the solution was discontinued and furosemide was administered (240 to 480 mg/day). When necessary (uremia and/or hypervolemia), intermittent peritoneal dialysis (IPD) was used.

Table 1. Classification of crotalid snakebite severity and recommended antivenom dosage

	Classification of the accident (initial evaluation)		
	Mild	Moderate	Severe
Myasthenic face and blurred vision	None or late	Mild or evident	Evident
Myalgia	None or mild	Mild	Intense
Dark urine	None	None or little evident	Present
Oliguria or anuria	None	None	None or present
Antivenom *mg/no. of vials*	75 mg	150 mg	300 mg
CAV-BCAV[a]	(5 vials)	(10 vials)	(20 vials)

Modified from National Health Foundation (FUNASA), 2001.
[a]CAV, crotalid antivenom; BCAV, bothropic-crotalid antivenom.

Clinical parameters

The bite site, severity of envenomation (Table 1), the type and the amount of antivenom given per body surface, the time elapsing between the snakebite, and the administration of specific antivenom were evaluated.

Age, gender, body surface, history of chronic diseases (heart failure, hypertension, or diabetes mellitus), use of concomitant drugs, hospitalization time, dialysis treatment, and mortality were recorded. The presence of myalgia and urinary abnormalities (volume and color), as assessed by the patient, and the occurrence of lid ptosis and bleeding were also screened.

Weight (kg), systolic and diastolic blood pressure (mm Hg), intravenous hydration volume (mL/kg/hr), and diuresis (mL/hr) were evaluated daily.

Laboratory evaluation

A blood specimen was collected at admission and daily until 72 hours after the snakebite. When the patient developed ARF, blood specimens were collected every three days until discharge or death. Blood samples were used to measure sodium, potassium, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), and lactic dehydrogenase (LDH) (Autoanalyzer Mega Merck 1.0; Merck, Darmstadt, Germany), to determine blood coagulation time (CT) (Lee White Method), hematocrit, hemoglobin, and platelets (Cell-Dyn 3.000–5.4, San Francisco, CA, USA).

On admission day, urine was collected for at least six hours to a maximum of 24 hours, depending on the time of hospitalization and the patient's urinary volume. It was then collected and analyzed daily in all patients (12-hour urinary volume) until 72 hours after the snakebite.

After that, it was collected every three days until hospital discharge or death in patients developing ARF. Urinary volumes at admission and 12-hour urinary volume were used to dose proteinuria (Bradford modified) and urinary creatinine (Merck).

A urine sample was collected at admission, daily in the first 72 hours after the snakebite, and every three days until discharge or death for patients developing ARF. This sample was used for measurement of sodium, potassium, and creatinine (Merck), pH (Multistix-Bayer Reagent, Rosario, Argentina), and for analysis of urinary sediment.

Fractional excretion of sodium (FeNa), fractional excretion of potassium (FeK), and endogenous creatinine clearance (GFR) were calculated according to the usual formulas.

Statistical analysis

Data were expressed as median (variation range) or %. Patients were analyzed as a group and were later allocated into two groups according to the presence of ARF (ARF group, with GFR <60 mL/min/1.73m^2 in the first 72 hours after envenomation, and non-ARF group with GFR ≥60 mL/min/1.73m^2 in the first 72 hours after envenomation). Differences between the two groups were compared using Fisher test or the Mann-Whitney test, as indicated.

Dispersion diagrams were shown for time to the administration of specific antivenom therapy and CK at admission.

Differences between GFR at admission, lowest GFR value, and GFR value at discharge for both groups were analyzed by analysis of variance (ANOVA), followed by Tukey's post-test or by Kruskal-Wallis test, as indicated. Spearman's correlation coefficients were calculated for GFR and time to receive specific antivenom therapy, for GFR and CK at admission, and for GFR and diuresis at admission.

Risk factors for ARF were determined by multivariate analysis with logistic regression. The initial model included significant variables at the univariate analysis and clinically significant variables. Two initial models were developed. Model 1 included age (up to 12 years of age and above 50 years, reference 13 to 50 years), time to antivenom (>2 hours), abnormal CT at admission (>10 minutes), CK at admission (>2000 U/L), and diuresis at admission (>90 mL/hr). In model 2, age was replaced by body surface (BS >1.5 m^2). Significant variables were evaluated by Wald's test.

$P < 0.05$ was considered statistically significant.

RESULTS

Analyzed sample

Of the 104 patients hospitalized at the HDT after *C. durissus* snakebite from July 1998 to May 2000 and from August 2001 to March 2003 (43 months), one was excluded due to previous chronic kidney disease (renal lithiasis with bilateral hydronephrosis and baseline creatinine >2 mg/dL), and three patients were not followed-up by the study (percentage of lost cases of 2.9%). The remaining 100 patients represent 17.3% of all *C. durissus* snakebites (578 cases) reported in the Health System of the State of Goiás in the same period (Toxicological Information Center, GO).

Time from snakebite to specific antivenom administration was three (1–48) hours. Nine patients received inadequate antivenom in the first medical care, outside of HDT. Seven were administered bothropic antivenom (BAV), and two received CAV doses lower than that recommended by the HM.

Mean age was 30 (3–61) years, 85% were male, and 19% were children ≤12 years. Three patients had a history of hypertension, and one had a history of diabetes mellitus. None of them had used nephrotoxic drugs. Mild inflammation observed at the bite site disappeared within 72 hours of the accident. None of the cases developed infection at the bite site. None of the patients had hypotension or shock within the first 72 hours after the accident. Mean hospitalization time was five (2–38) days. There were three deaths (3%).

Comparison of the ARF and non-ARF groups

Of the 100 patients studied, 29 had GFR <60 mL/min/1.73m^2 in the first 72 hours after the snakebite. Of these, seven required dialysis (24.1%) and three (10.3%) died.

All patients who developed ARF were hospitalized within 72 hours after the snakebite. Of these, 22 (76%) arrived within 24 hours of the bite, and all of them developed ARF in this period. The seven remaining patients who arrived at the hospital 24 to 72 hours after the bite were already admitted with ARF. Six of the nine (67%) patients receiving inadequate antivenom developed ARF (five received BAV and one received insufficient CAV).

Data regarding the accident and antivenom administration

The time interval between the accident and the administration of specific antivenom was significantly higher in the patients who developed ARF than in those who did not develop it (Fig. 1). There was a higher prevalence of cases classified as severe in the ARF group and, therefore, this group received more antivenom per body surface than the non-ARF group. There was no difference between both groups regarding the bite site (Table 2).

Patient data

Although ARF patients were younger, this difference was not statistically significant [24 (3–59) years vs. 32

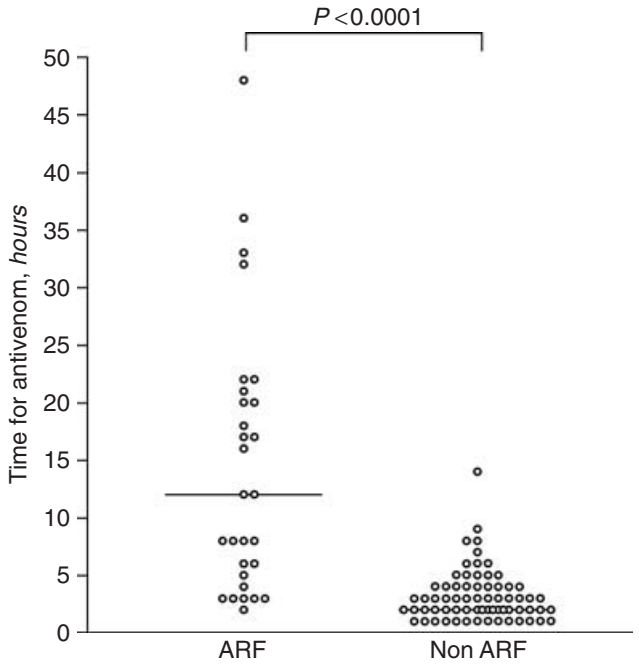

Fig. 1. Time interval from snakebite to administration of the specific antivenom in ARF and non-ARF groups. The horizontal bars indicate median values.

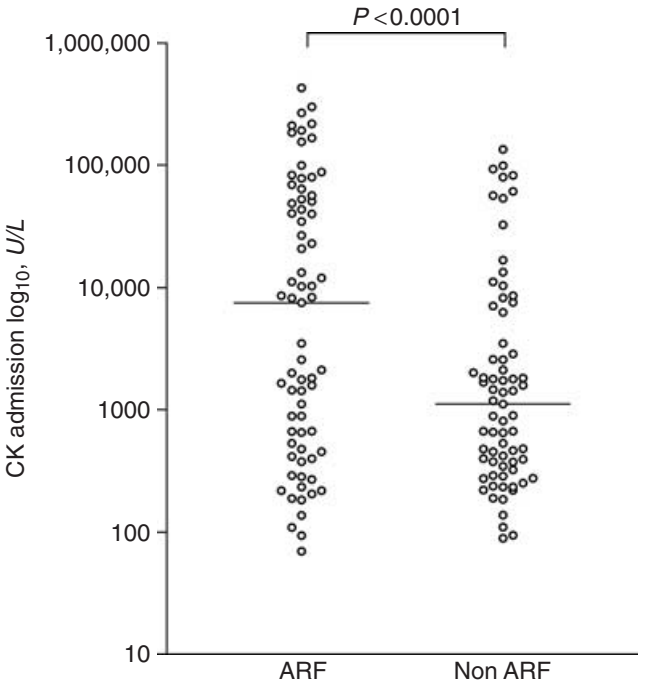

Fig. 2. Creatine kinase (CK) values at admission in the ARF and non-ARF groups. The horizontal bars indicate median values.

Table 2. Comparison between the two groups for data regarding the accident and the patients

	ARF ($N = 29$)	Non-ARF ($N = 71$)	P value
Time to AV *hours*	12 (2–48)	2 (1–14)	<0.0001
Amount of AV/BS *mg/m²*	190 (90–536)	158 (75–500)	<0.0001
Cases classified as severe%	90	54	0.0005
Bite of lower extremities%	83	78	0.7872
Age *years*	24 (3–59)	32 (3–61)	0.0882
Weight *kg*	56.3 (14.5–105.0)	64.0 (15.0–95.2)	0.0067
BS *m²*	1.55 (0.6–2.3)	1.70 (0.6–2.1)	0.0097
Males%	83	86	0.7599
Lid ptosis%	100	70	0.0003
Myalgia%	100	94	0.32

Abbreviations are: AV, antivenom; BS, body surface.
Values expressed as median (variation range) or %.

(3–61) years, $P = 0.088$]. Of the 19 children (≤12 years) included in the study, 11 developed ARF (58%), and of the 81 adults, 18 developed ARF (22%, $P = 0.004$ adults vs. children). Weight and body surface (BS) of the patients with ARF were significantly lower than the patients who did not develop ARF. Both ARF and non-ARF groups included more males.

The ARF group had significantly more patient complaints of urinary volume decrease (ARF, 86% vs. non-ARF, 31%, $P < 0.0001$) and dark urine (ARF, 83% vs. non-ARF, 49%, $P = 0.0032$). Lid ptosis and generalized myalgia were observed in all patients who developed ARF.

Both groups had similar systolic [ARF, 120 (90–180) mm Hg and non-ARF, 120 (100–180) mm Hg, $P = 0.8510$] and diastolic [ARF, 80 (60–110) mm Hg and non-ARF, 80 (60–120) mm Hg, $P = 0.4312$] blood pressure at admission.

Both groups received similar hydration at admission [ARF, 3.0 (0.6–10.2) and non-ARF, 3.0 (1.2–15.6) mL/kg/hr, $P = 0.4747$] and within the first 72 hours [ARF, 2.6 (0.7–9.6) and non-ARF, 2.2 (1.1–10.5) mL/kg/hr, $P = 0.60$].

Hospitalization time was longer in the ARF group [5 (2–38) days] when compared with the non-ARF group [5 (3–7) days, $P = 0.0016$].

Patient data are summarized in Table 2.

Laboratory evaluation of rhabdomyolysis

Both groups showed a significant increase of CK serum levels at admission. However, CK values observed in the ARF group were significantly higher than those in the non-ARF, reaching levels 260 times higher than normal (Fig. 2). Other markers of rhabdomyolysis, such as AST, ALT, and LDH were also significantly higher at admission in the ARF group when compared to the non-ARF group (Table 3).

Table 3. Comparison of both groups for rhabdomyolysis markers at admission

	ARF ($N = 29$)	Non-ARF ($N = 71$)	P value
CK *U/L*	50,250 (69–424,120)	1,108 (88–133,170)	<0.0001
AST *UI/L*	1544 (12–16,170)	60 (13–4170)	<0.0001
ALT *UI/L*	325 (10–3880)	25 (10–854)	<0.0001
LDH *UI/L*	2833 (154–28,990)	384 (180–4673)	<0.0001

Abbreviations are: CK, creatine kinase; AST, aminotransferase aspartate; ALT, aminotransferase alanine; LDH, lactic dehydrogenase; ARF, acute renal failure. Values expressed as median (variation range).

Table 4. Comparison of renal function data

	ARF ($N = 29$)	Non-ARF ($N = 71$)	P value
Diuresis at admission *mL/hr*	62 (0–182)	100 (25–325)	0.0004
Diuresis in the first 3 days *mL/hr*	90 (0–177)	116 (52–204)	0.0006
GFR at admission *mL/min/1.73m²*	38.6 (0–54.4)	91.6 (61–162.2)	<0.0001
GFR nadir *mL/min/1.73m²*	38.6 (0–54.4)	85.4 (61.1–110.2)	<0.0001
GFR at discharge *mL/min/1.73m²*	87.5 (21.9–104.7)	102.1 (90.7–145)	<0.0001
FeNa at admission%	2.4 (0.1–19.7)	1.0 (0.2–4.8)	<0.0001
Maximum FeNa%	4.1 (0.8–35)	1.9 (0.2–5.5)	<0.0001
FeK at admission%	28.3 (8.5–150)	9.8 (0.5–39.6)	<0.0001
Maximum FeK%	35.9 (8.5–164)	13.2 (2.4–55)	<0.0001
Proteinuria at admission *mg/m²/hr*	34.0 (3.6–160)	9.7 (1.5–95.4)	<0.0001

Abbreviations are: GFR, glomerular filtration rate; FeNa, fractional excretion of sodium; FeK, fractional excretion of potassium; ARF, acute renal failure. Values expressed as median (variation range).

Hematology and coagulation time

Hematocrit, hemoglobin, and platelet levels at admission were similar and normal in both groups (data not shown). Although the CT at admission was abnormal in 83% of the patients in the ARF group and in 51% of the non-ARF group ($P = 0.0033$), none of the patients had hemorrhage.

Renal function

Urinary volume at admission was 62 (0–182) mL/hr in the ARF group and 100 (25–325) mL/hr in the non-ARF group ($P = 0.0004$). When diuresis was compared in the first 72 hours of treatment, the statistically significant difference between groups remained. In the ARF group, only four patients were oliguric and required the use of diuretics.

GFR at admission, GFR nadir, and GFR at discharge are shown in Table 4. All of the patients in the ARF group had the lowest GFR values within the first 48 to 72 hours after the accident. Both the ARF and the non-ARF group presented a GFR at discharge significantly higher than the nadir of GFR (Fig. 3). Only five

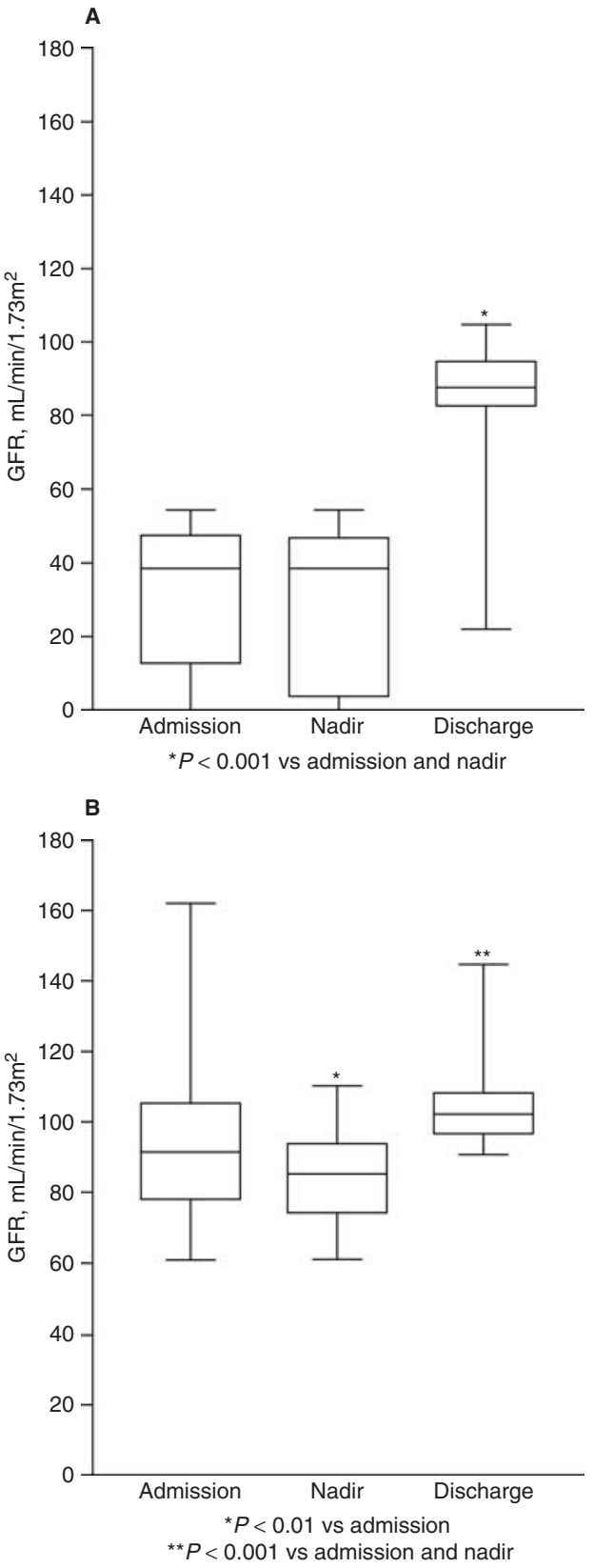

Fig. 3. GFR at admission, GFR nadir, and GFR at discharge in the ARF (*A*) and non-ARF (*B*) groups. Data are shown as box and whisker plots. The lines in the middle and those delimiting the boxes, respectively, indicate median and 25th and 75th percentile values.

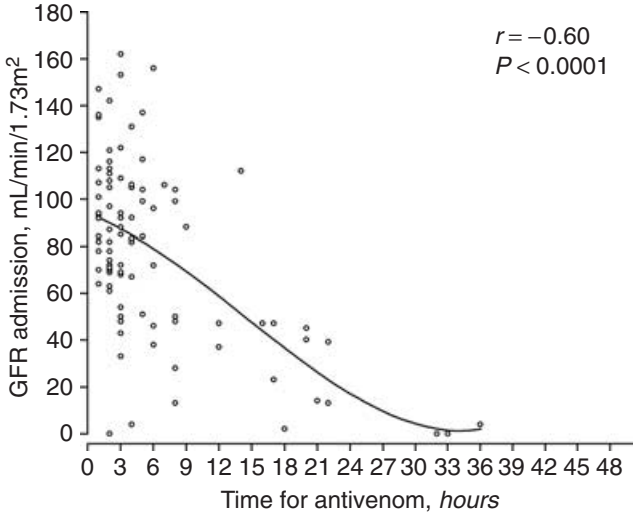

Fig. 4. Negative correlation between time to administration of the specific antivenom and GFR at admission.

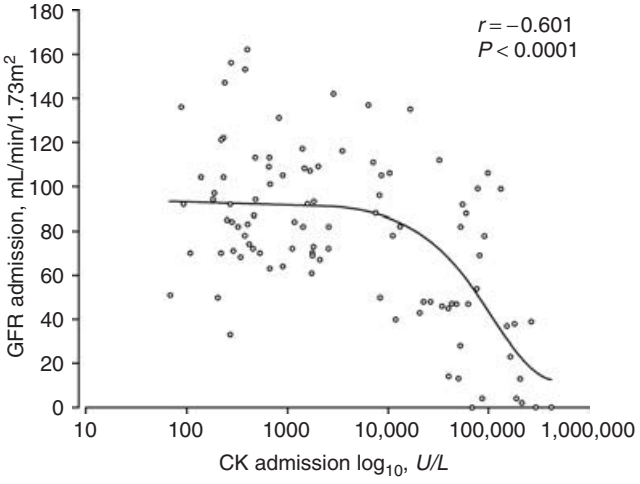

Fig. 5. Negative correlation between creatine kinase (CK) and GFR at admission.

of the 29 patients with ARF did not increase their GFR over 60 mL/min/1.73m^2 during hospitalization (actually, 23 were discharged with GFR over 80 mL/min/1.73m^2). Among these five patients, three died, and the two others were discharged with GFR of 21.9 mL/min/1.73m^2 (nadir 0 mL/min/1.73m^2) and 50.7 mL/min/1.73 m^2 (nadir 13.5 mL/min/1.73m^2), respectively. None of these five patients presented clinical or laboratory evidence of chronic kidney disease. All patients in the non-ARF group were discharged with GFR over 90 mL/min/mL/min/1.73m^2.

GFR at admission had a significant negative correlation with time to receive specific antivenom and with CK value at admission. A significant positive correlation was observed for GFR and diuresis at admission. Correlation curves are shown in Figures 4, 5, and 6.

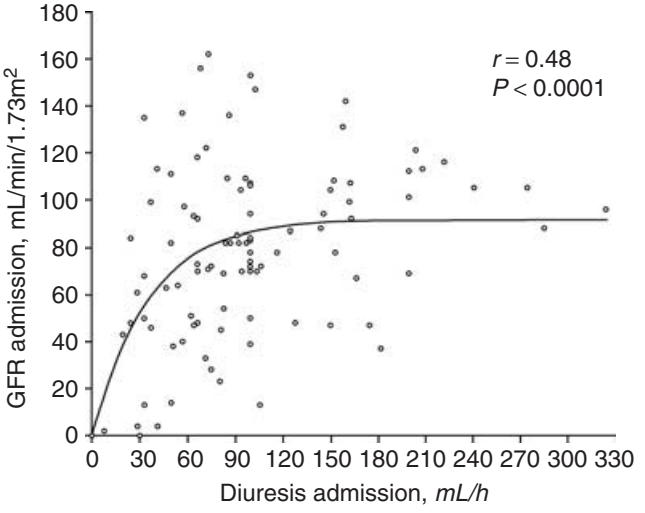

Fig. 6. Positive correlation between urinary volume and GFR at admission.

FeNa at admission was 2.4 (0.12–19.7%) in patients who developed ARF, reaching a peak value of 4.1 (0.8–35%). Both FeNa and FEK at admission and at peak were significantly higher in the ARF group compared to non-ARF group.

Urinary protein excretion at admission was 34.0 (3.6–160) mg/m^2/hr in the ARF group and 9.7 (1.5–95.4) mg/m^2/hr in the non-ARF group ($P < 0.0001$).

Urinary pH at admission was similar in both groups [6.5 (5.0–9.0) in ARF and 7.0 (5.0–8.5) in non-ARF, $P = 0.17$]. In the same way, both groups had similar maximum pH values [7.5 (5.0–8.5) in ARF and 8.0 (5.5–8.5) in non-ARF, $P = 0.25$]. ARF group had a significantly higher number of leukocytes and red blood cells in urine than the non-ARF group [10,000 (250–92,000) vs. 6000 (250–58,000) leukocytes, $P = 0.04$, and 10,000 (250–68,000) vs. 5000 (250–22,000) red blood cells, $P = 0.0077$].

Renal function data are summarized in Table 4.

Risk factors for ARF

Two logistic regression models were required to determine risk factors related to the development of ARF because age and body surface showed a great correlation and did not allow the analysis of both factors by the same model. The final results are shown in Table 5.

In both models, time to receive antivenom >2 hours and CK >2000 U/L at admission were identified as independent covariates for the development of ARF, and diuresis >90 mL/hr at admission was identified as a protection factor against the development of ARF. In the first model, age <12 years was a risk factor for the development of ARF.

Table 5. Logistic regression: Independent variables associated to ARF

	OR	95% CI	*P* value
Model 1			
Age <12 years	5.6	1.20–26.2	0.026
Time to receive antivenom >2 hours	11.1	1.23–101	0.032
CK at admission >2000 U/L	12.7	2.77–58	0.0009
Diuresis at admission >90 mL/hr	0.20	0.0548–0.738	0.014
Model 2			
Time to receive antivenom >2 hours	16.8	1.86–151	0.011
CK at admission >2000 U/L	12.3	2.93–51.9	0.0005
Diuresis at admission >90 mL/hr	0.20	0.0602–0.691	0.001

DISCUSSION

The results of this study, the largest prospective analysis of envenomation by South American rattlesnake reported in medical literature, clarify important aspects of renal impairment caused by crotalid envenomation.

Prevalence of ARF

The prevalence of ARF was extremely high (29%), although the patients were treated at a specialized hospital and prophylactic measures against myoglobinuria were used [23]. This prevalence is greater than that reported for several of the major nephrotoxic drugs, such as aminoglycoside, amphotericin B, or contrast media [24]. These data showed that most of the previous studies have underestimated the magnitude of the problem, reporting ARF prevalences of 12.9% to 18.4% after *C. durissus* snakebite [1, 19, 20].

It should be noted that the non-ARF group had GFR at discharge significantly higher than the lowest values observed during hospitalization, showing that some degree of renal injury might have taken place in almost all of the patients. The significant recovery of the GFR in almost all patients of the ARF group confirmed the acute nature of the renal insult.

Risk factors for the development of ARF

Antivenom. The heterologous crotalid antivenom (CAV) used was obtained from equine sensitization. One milliliter of CAV, which consists mainly of immunoglobulin fragments $F(ab')_2$, neutralizes 1.5 mg of *C. durissus* venom [3]. Circulating venom is not observed one hour after its administration, and CAV titers remain high up to 24 hours after treatment [25]. The amount to be administered is determined according to accident classification (Table 1).

The finding that the delay to administer an adequate dose of the CAV increases more than 10 times the risk to develop ARF is not unexpected because the venom continues to act until it is neutralized. The fact that six of nine of the patients treated with either incorrect or insufficient antivenom develop ARF reinforces the extreme importance of an early and adequate treatment. Recent data have shown that the CAV prevented proximal tubular injury in vitro only when it was added simultaneously with the snake venom [18]. Previous studies have already suggested there is a correlation between renal injury and time interval between the snakebite and the administration of antivenom [9, 20, 26]. The major indication of this finding is that treatment must be decentralized to allow an early CAV treatment. Antivenom should be available in health centers and emergency services of small communities, rather than concentrated in reference hospitals or services.

A possible factor related to the development of renal injury might be the administration of less CAV in the ARF group. However, these patients received more CAV per body surface than the non-ARF group, suggesting that the current recommendation for CAV amount in severe envenomation cases might be inadequate.

Rhabdomyolysis. Azevedo-Marques et al first demonstrated that the envenomation by the South American crotalid induced rhabdomyolysis and myoglobinuria associated with ARF [7, 8]. Experimental studies showed that sublethal doses of the venom cause very early rhabdomyolysis associated with GFR decrease [13]. It was shown later that crotoxin causes systemic and selective injury of muscles or skeletal muscle groups composed of type I and IIa fibers, which are extremely vascularized and rich in myoglobin [10]. Recent studies have suggested that calcineurin and nitric oxide pathways are essential mediators of crotoxin-related myonecrosis [27, 28].

The clinical diagnosis of rhabdomyolysis is established when CK increases five or more times above normal levels, with a suggestive clinical picture, and without heart and/or cerebral injury [22, 29]. In this study, the myotoxicity of the venom was confirmed by intense and generalized myalgia (100% of the patients), by the complaint of dark urine (83% of the patients), and by an early and striking increase of muscle enzymes, especially CK, which is considered the most sensitive marker of muscle injury [29]. Although the CK increase was observed in almost all patients, it was much higher in the ARF group, with an extremely high peak of the enzyme occurring soon after the accident.

Rhabdomyolysis is a well-known cause of renal injury [7, 29] and was strongly related to ARF in this study. The most effective measure for the prevention of ARF induced by rhabdomyolysis is extracellular volume expansion with saline solution combined with sodium bicarbonate and mannitol [23, 29]. This solution must be started early and maintained until myoglobinuria is no longer present [23]. The volume to be administered is three to six L/day, when supervision is not assured, or higher than 10 L/day, when continuous supervision is available [29]. Indeed, Sakwiwaktul et al, working with sea snake venom, which has a strong myolytic activity,

demonstrated that pretreatment with sodium bicarbonate prevented venom-induced renal injury in dogs in the presence of marked myoglobinuria [30]. In the present study, volume expansion with alkalinizing solution associated with mannitol was not sufficient to prevent universally ARF, although a pH >6.5, considered ideal to prevent renal injury by myoglobin, was obtained [3, 23, 31]. The delay to start this maneuver might have contributed for the treatment failure of some patients who arrived at the hospital later. Another possibility is that this prophylactic measure was in fact effective, and the mechanisms leading to ARF are related to direct renal activity of the venom, causing tubular injury and renal vasoconstriction [13]. Finally, considering the severity of the muscle injury shown by CK serum levels, prevention might have to be more aggressive.

Age. Another independent factor related to ARF in the multivariate analysis was age <12 years. Indeed, ARF patients were younger, although the difference was not statistically significant. Likewise, it was observed that children have a prevalence of ARF almost three times higher than adults, although they received more CAV per body surface, had a higher hydration volume per kg of body weight, and did not have a longer time interval from the snakebite to the administration of antivenom or more intensive rhabdomyolysis than adults (data not shown). Although children and adults with snakebites receive equal amounts of CAV [3], children are thought to be more affected by the venom [32]. Comparatively, children have a lower blood volume and smaller body surface, which might be related to a more severe clinical picture due to an increased concentration of the venom [32].

This evidence suggests that the amount of antivenom administered, or the criteria established to calculate its dose, should be reviewed for children. Prospective and controlled studies are required for further clarification.

Diuresis. The venom of the South American crotalid is predominantly excreted by the kidney, and its toxic components have a direct and indirect activity on renal cells [13, 22]. A urinary flow of 30 to 40 mL/hr in adults and 1 to 3 mL/kg/hr in children is recommended to prevent ARF in cases of envenomation by the South American crotalid [3, 31]. In the present study, diuresis at admission >90 mL/hr proved to be an independent protective factor against ARF development. An intense urinary flow might result in less exposure of renal tubular cells to myoglobin and to the venom, attenuating the injury and preventing tubular lumen obstruction by myoglobin cylinders and cellular remains.

Other factors. The group that developed ARF had a higher prevalence of severe envenomation. Lid ptosis is the most important and well-known clinical sign of neurotoxic face [1, 3], and myalgia is one of the most frequent symptoms reported by victims of crotalid envenomation [1, 3, 22]. Lid ptosis and myalgia were observed in all ARF patients in this study, and therefore, it was not possible to evaluate these variables by logistic regression. Nishioka and Silveira have described the association of myalgia and lid ptosis with renal failure in individuals older than 40 years [20]. Dark urine, another severity criterion, was more frequent in ARF patients, but it was not included in the logistic regression analysis due to its subjectivity. Abnormal CT, one of the criteria used to classify the severity of the accident, was not significantly associated with ARF in the multivariate analysis.

Other factors that might be related to the genesis of ARF, such as shock, hypotension, hemolysis, sepsis, or the use of nephrotoxic drugs were not observed in these patients.

ARF characteristics

ARF occurred early, within the first 24 to 48 hours after the accident, suggesting the direct nephrotoxicity of the venom shown experimentally might also be clinically present. The majority of cases were nonoliguric.

FeNa values in ARF patients were significantly higher than in patients who did not have ARF, reaching a maximum value of 4%, suggesting there was proximal renal tubular cell injury. The use of loop diuretics does not seem to have increased FeNa because only 14% of the patients who developed ARF had used furosemide. The amount of proteinuria observed in the ARF group also suggests the presence of tubular injury, although the possibility of some degree of glomerular injury might not be ruled out [33]. These findings are consistent with the description of acute tubular necrosis in biopsies and autopsies of victims of the South America crotalid snakebite [7, 34].

Increased FeK in ARF patients probably indicates an increase in the distal tubular secretion of potassium caused by an increased distal delivery of sodium, due to the decreased proximal tubular reabsorption of sodium. These data suggest the distal areas of the nephron are preserved.

Dialysis treatment was indicated in 24% of the cases. Previous studies have reported a greater demand for dialysis, ranging from 68% to 77% of the patients. This discrepancy is probably related to the low specificity of ARF diagnostic criteria used in these studies, only identifying the more severe cases of renal injury [14, 20, 34].

Renal function was recovered in the individuals who survived ARF. Even though, GFR values at discharge of these patients were significantly lower than GFR values for the patients without ARF, indicating there might have been an incomplete recovery of the glomerular filtration rate in the studied period. The late follow-up of renal function in these individuals would be very important, but is extremely difficult to accomplish because most of the patients live in faraway rural areas.

ARF caused a longer hospitalization time, most likely resulting in higher costs and more use of hospital resources.

The reported mortality for ARF after envenomation by the tropical rattlesnake is expressive and ranges from 8% to 17%, depending on the studied series [14, 20, 34]. In this study, the mortality rate of 10% is quite significant because it involved young and previously healthy individuals.

CONCLUSION

ARF after envenomation by *C. durissus* has a high prevalence (29%) and is related to significant mortality in young and healthy patients. A delay in the administration of specific antivenom, presence of CK >2000 U/L, and age <12 years were independent risk factors for ARF. Diuresis >90 mL/hr at admission was a protective factor.

ACKNOWLEDGMENTS

Emmanuel A. Burdmann and Dirce M.T. Zanetta were partially supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). Fábia M.O. Pinho was a recipient of a grant from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). We thank Lívia A. Burdmann for the grammar review of the manuscript. Parts of this study were presented at the Annual Meeting of American Society Nephrology in 1999 and 2002.

Reprint requests to Emmanuel A. Burdmann, Av. Brigadeiro Faria Lima 5416, São José do Rio Preto, SP, 15090–000, Brazil.
E-mail: burdmann@famerp.br

REFERENCES

1. Jorge MT, Ribeiro LA: The epidemiology and clinical picture of an accidental bite by the South American rattlesnake (*Crotalus durissus*). *Rev Inst Med Trop São Paulo* 34:347–354, 1992
2. Chippaux JP: Snake-bites: Appraisal of the global situation. *Bull World Health Organ* 76:515–524, 1998
3. ministério da Saúde, Brasil: *Manual de Diagnóstico e Tratamento de Acidentes por Animais Peçonhentos*, Brasília, Fundação Nacional de Saúde, 2001
4. Vital Brazil O: Venenos ofídicos neurotóxicos. *Rev Assoc Med Bras* 26:212–218, 1980
5. Oshima-Franco Y, Hyslop S, Prado JF, *et al*: Neutralizing capacity of antisera raised in horses and rabbits against *Crotalus durissus terrificus* (South American rattlesnake) venom and its main toxin, crotoxin. *Toxicon* 37:1341–1357,1999
6. Vital Brazil O, Franceschi JP, Waishich E: Pharmacology of crystalline crotoxin. Toxicity. *Mem Inst Butantan* 33:973–980, 1966
7. Azevedo-Marques MM, Cupo P, Coimbra TM, *et al*: Myonecrosis, myoglobinuria and acute renal failure induced by South American rattlesnake (*Crotalus durissus terrificus*) envenomation in Brazil. *Toxicon* 23:631–636, 1985
8. Azevedo-Marques MM, Hering SE, Cupo P: Evidence that *Crotalus durissus terrificus* (South American rattlesnake) envenomation in humans causes myolysis rather than hemolysis. *Toxicon* 25:1163–1168, 1987
9. Cupo P, Azevedo-Marques MM, Hering SE: Clinical and laboratory features of South American rattlesnake (*Crotalus durissus terrificus*) envenomation in children. *Trans R Soc Trop Med Hyg* 82:924–929, 1988
10. Salvini TF, Amaral AC, Miyabara EH, *et al*: Systemic skeletal muscle necrosis induced by crotoxin. *Toxicon* 39:1141–1149, 2001
11. Monteiro HAS, Silva IMSC, Martins AMC, Fonteles MC: Actions of *Crotalus durissus terrificus* venom and crotoxin on the isolated rat kidney. *Braz J Med Biol Res* 34:1347–1352, 2001
12. Martins AMC, Toyama MH, Havt A, *et al*: Determination of *Crotalus durissus cascavella* venom components that induce renal toxicity in isolated rat kidney. *Toxicon* 40:1165–1171, 2002
13. Vidal EC, Yu L, Burdmann EA: Snake venom-induced nephrotoxicity—In vivo and in vitro studies. *J Am Soc Nephrol* 8:131A, 1997
14. Amaral CFS, Rezende NA, Silva OA, *et al*: Insuficiência renal aguda secundária a acidentes ofídicos botrópico e crotálico. Análise de 63 casos. *Rev Inst Med Trop São Paulo* 28:220–227, 1986
15. Ribeiro LA, Alburquerque MJ, Campos VAFP, *et al*: Deaths caused by venomous snakes in the State of São Paulo: Evaluation of 43 cases from 1988 to 1993. *Rev Assoc Med Bras* 44:312–318, 1998
16. Burdmann EA, Woronik V, Prado EBA, *et al*: Snakebite-induced acute renal failure: An experimental model. *Am J Trop Med Hyg* 48:82–88, 1993
17. Martins AMC, Monteiro HAS, Júnior EOG, *et al*: Effects of *Crotalus durissus cascavella* venom in the isolated rat kidney. *Toxicon* 36:1441–1450, 1998
18. Castro I: Estudo da toxicidade das peçonhas crotálica e botrópica em túbulos proximais isolados de rins de ratos. Thesis. Federal University of São Paulo, Brazil, 2003
19. Pinto RNL, Souza LCS, Silva AM, *et al*: Estudo clínico-epidemiológico de 774 casos de acidentes ofídicos. *Rev Soc Bras Med Trop* 20:S56, 1987
20. Silveira PVP, Nishioka AS: South American rattlesnake bite in a Brazilian teaching hospital. Clinical and epidemiological study of 87 cases, with analysis of factors predictive of renal failure. *Trans R Soc Trop Med Hyg* 86:562–564, 1992
21. Silva OA, Lopez M, Godoy P: Intensive care unit treatment of acute renal failure following snakebite. *Am J Trop Med Hyg* 28:401–407, 1979
22. Magalhães RA, Ribeiro MMF, Rezende NA, Amaral CFS: Rabdomiólise secundária a acidente ofídico crotálico (*Crotalus durissus terrificus*). *Rev Inst Med Trop São Paulo* 28:28–33, 1986
23. Better OS, Stein JH: Early management of shock and prophylaxis of acute renal failure in traumatic rhabdomyolysis. *N Engl J Med* 322:825–828, 1990
24. Cronin RE, Henrich WL: Toxic nephropathies, in *The Kidney*, edited byBrenner BM, Philadelphia, WB Saunders, 2000, pp 1563–1596
25. Amaral CFS, Campolina D, Dias MB, *et al*: Time factor in the detection of circulating whole venom and crotoxin and efficacy of antivenom therapy in patients envenomed by *Crotalus durissus*. *Toxicon* 35:699–704, 1997
26. Bucaretchi F, Herrera SRF, Hyslop S, *et al*: Snakebites by *Crotalus durissus ssp* in children in Campinas, São Paulo, Brazil. *Rev Inst Med Trop São Paulo* 44:133–138, 2002
27. Miyabara EH, Tostes RC, Selistre-Araujo HS, *et al*: Cyclosporin A attenuates skeletal muscle damage induced by crotoxin in rats. *Toxicon* 43:35–42, 2004
28. Miyabara EH, Tostes RC, Selistre-Araujo HS, *et al*: Role of nitric oxide in myotoxic activity induced by crotoxin in vivo. *Toxicon* 43:425–432, 2004
29. Vanholder R, Sever MS, Erek E, Lameire N: Rhabdomyolysis. *J Am Soc Nephrol* 11:1553–1561, 2000
30. Sakwiwatkul K, Chaiyabutr N, Sitprija V: Renal function following sea snake venom (*Lapemis hardwicki*) administration in dogs treated with sodium bicarbonate solution. *J Nat Toxins* 11:111–121, 2002
31. Cupo P, Azevedo-Marques MM, Hering SE: Crotalid bites in children: Clinical, laboratory, epidemiological aspects and treatment approach. *Rev Soc Bras Med Trop* 24:87–96, 1991
32. Weber RA, White RR: *Crotalidae* envenomation in children. *Ann Plast Surg* 31:141–145, 1993
33. MacLean PR, Robson JS: Unselective proteinuria in acute renal ischemic renal failure. *Clin Sci* 30:91–102, 1966
34. Vêncio D: Estudo do ofidismo em Goiás: Comprometimento da função renal. *Rev Goiana Med* 34:95–116, 1988