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Russell's viper envenomation induced Acute Kidney Injury: a study of plasma NGAL and serum Creatinine

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Abstract

Viper envenomation contributes to acute kidney injury (AKI), which is further influenced by alterations in various physiological factors and nephrotoxic pharmacotherapeutic agents. Despite early appropriate intervention with Injection antisnake venom (ASV), progression of AKI has been demonstrated earlier. So, an attempt has been made to determine plasma neutrophil gelatinase associated lipocalin NGAL and the serum creatinine (sCr)while these cases of viper envenomation are on treatment at hospital, correlate with selected demographic (age and gender) and clinical parameters (bite to needle time, lymphadenitis and local swelling), and suggest the possible mechanisms involved in the process. A total of 104 confirmed Russell's viper bite presented within 4 hours of bite with evidence of clinical envenomation and confirmation of the snakes who satisfied certain inclusion and exclusion criteria were considered for the study. Apart from routine clinical assessment, they were evaluated by laboratory means. Selected demographic and clinical parameters of these cases were documented and correlated with plasma NGAL and sCr estimated on admission ("0" hour) and at different intervals of post-bite admission. The results were analyzed statistically. Plasma NGALand sCrrevealed a sustained elevation following viper envenomation at different intervals even after administration of ASV. These changes were independent of the demographic and clinical parameters. The mechanisms involved for the development of AKI in viper envenomation are attributable to the by nephrotoxins, cytokines and endothelin present in the venom, and the oxidative stress and thrombo inflammation induced by envenomation. So, an understanding of possible mechanisms involved in AKI will likely pave ways for early introduction of adjuvants for snake bite cases in order to avert or minimize renal damage.

Key words: *Viper envenomation - AKI - NGAL - creatinine –mechanisms - prevention*

Introduction

Snake envenoming is one of the major medical emergencies in the rural tropics (Rajendiranet al., 2014). It is observed more among farming communities. Young males become the victims mostly. In India, the majority of snake envenoming is due to "Big 4" which includes Cobra, Krait, Russell's viper and *Echiscarinata*. The clinical syndromes vary with snake envenoming (Simpson and Norris, 2007).

Among the viper species, Russell's viper (*Daboiarusselii*) is an important one in Asia. Russell's viper envenomation often leads to coagulopathy and in some it causes neurotoxicity. One another devastating complication observed in these cases is acute kidney injury (AKI). Even though the exact mechanisms for AKI in viper envenomation is not known, various risk factors for the development of AKI are longer duration between bite and administration of antisnake venom, hypotension, pigment nephropathy following microangiopathic haemolytic anaemia and /or rhabdomyolysis, venom-induced consumption coagulopathy and possibly the quality and quantity of nephrotoxins present in the venom (Vikrant *et al.,* 2017).

Acute kidney injury (AKI) results in significant morbidity and mortality. Currently available methods to diagnose AKI are clinical signs such as oliguria or anuria and elevation of serum creatinine (sCr) and blood urea nitrogen (BUN) which happen after significant injury to the kidney (Makris and Spanou, 2016). Moreover, sCr dependents upon various physiological factors i.e., age, gender, race, hydration status, muscle mass and nutritional status, which isfurther, aggravated by inadvertent use of nephrotoxic pharmacotherapeutic agents (Baxmann*et al.,*2008). So, timely diagnosis and appropriate intervention of AKI are likely to minimise or prevent the morbidity and mortality.

Early diagnosis of AKI remains a challenging one, and hence, there is a need to find out a renal biomarker to diagnose AKI much before elevation of sCr. Among the various renal biomarkers, plasma Neutrophil gelatinase associatedlipocalin (NGAL) has been studied extensively (Devarajan, 2010). Earlier the progression of NGAL and sCr was demonstrated in cases received ASV, which cause anxiety among the patients, care givers and providers of Health care. Hence the objectives of the present study were to determine the plasma NGAL and sCrat different intervals while on treatment at hospital, correlate with selected demographic and clinical parameters, and suggest the possible mechanisms involved in the process.

Materials and Methods

This prospective study was carried out in an emergency department (ED) of a teaching hospital from January 2019 to December 2020 with 104 confirmed viper bites who presented within 4 hours of bite with evidence of clinical envenomation and confirmation of the snakes as Russell's viper. The cases considered for the study purposes had snakebite either in the upper or lower limb only. Patients with no signs of envenomation after a period of 24 hours observation, definite bite by snakes other than Russell's viper, sCr level below 1.4 mg/dL on admission, preexistent renal diseases, long-standing diabetes or hypertension, overt congestive heart failure (New York Heart Association III–IV), hemodynamic instability of any cause, sepsis or systemic infectious diseases, injuries, contracted kidneys on ultrasound and history of exposure to nephrotoxic drugs/chemicals were excluded from the study. An informed written consent was obtained from every participant before enrollment. The study was performed in accordance with the Declaration of Helsinki and after the approval of the Institutional Ethics Committee.

These cases of Russell's viper bite were assessed clinically on admission and during hospital stay at periodic intervals or as and when required. Ten ml of venous blood was collected from all these patients for laboratory evaluation such as complete blood count including 20 minutes whole blood clotting time (20WBCT) as well as blood chemistry including plasma NGAL and sCr at the time of admission. The first sample is considered as "0" hour sample. Subsequently, another 5 ml of blood was collected at an interval of 12, 24 and 48hours of post-bite admission for sCr, and plasma NGAL at an interval of 12 and 24 hours. Plasma NGAL was estimated using the standardized Triage ® NGAL tests (Biosite Incorporated, San Diego, CA, USA). Serum creatinine (sCr) was determined by Jaffe's method.All these cases received anti-snake venom as per protocol and supportive measures (MoHFW, 2016). None of them developed adverse events following

administration of ASV. During the hospital course, all the patients were monitored for hourly urine output, and none received any nephrotoxic agents, vasopressors or steroids. Good laboratory and clinical practice were adhered all through the study period.

The demographic data included were age of the patient in years and gender. Also, for the study purposes, the patients were grouped into those below 25, 25 to 44, and 45 and above. The clinical parameters considered were bite to needle time, lymph node involvement and swelling at the site of snakebite. For the study purposes, bite to needle time was defined as the interval from the time of bite to the time at which ASV was initiated. Lymphadenitis was graded as "0" if there were no lymph node involvement and grade I, if patients had tenderness over lymphnodes with and without adenitis.Swelling at the site of snakebite was classified as Grade 0, 1, 2 and 3, as per the reference classification (Saravu*et al.,* 2012).The data were entered in excel spread sheet and analyzed statistically. The mean and SD were calculated for age group, gender and other clinical parameters individually for plasma NGAL and sCrat different intervals of post bite. Correlation coefficient was calculated for each parameter for plasma NGAL and sCr.

Results

Among the 104 cases taken for the study, there were 72 males and 32 females. Their ages ranged from 22 to 60 years. The mean and SD for plasma NGAL and sCr for each parameter in relation to demographic and clinical parameters and with regard to time series are depicted in Table 1 and 2 respectively. Among the study subjects, a progressive elevation of plasma NGAL and sCrwas observed at different intervals consistently and positively correlated. Significant elevation was noticed at 24 hours when compared with "0" hour for NGAL, and 24 and 48 hours for sCr to each of the parameter. As plasma NGAL levels were elevated very much at 24 hours in all the cases, evaluation of these biomarkers at 48 hours was not attempted. Significance was more as the age of the patient advances, time interval increases and clinical manifestations progresses. Of the 104, 13 cases (12.5%) required haemodialysis. All the 104 recovered and got discharged from the hospital.

Discussion

The occurrence of AKI in viper bite victims despite appropriate treatment causes concern among clinicians and creates displeasure among patients and care givers, and induces strained doctor patient relationship at times. The viper venom directly affects the renal tissues (Athappanet al., 2008). Apart from that the venom may possibly disturb the internal circulation within the kidney and may cause ischemic perfusion injury (IRI). In addition, better understanding of the other possible mechanisms for renal injury is essential in order to suggest adjuvants so as to avert or minimize the progression of renal damage.

The present study has demonstrated clearly that plasma NGAL and sCr increases in all the patients received ASV, when samples were analyzed at different intervals. The possible mechanisms for the elevation of plasma NGAL and sCr are the release of cytokines (Boda*et al.,* 2018), mediators of oxidative stress (Santhosh*et al.,* 2013; Sunitha*et al.,* 2015)andthrombo inflammation (Teixeira *et al.,* 2019), endothelin (Takasaki *et al.,* 1988) and other unknown nephrotoxins present in the viper venom which might have contributed to nephrotoxcity and led to decline in renal function. The sCr and urine output act as functional biomarkers, but its effectiveness is limited in snake bite victims. Urine output may be affected by many factors such as use of diuretics and altered ADH response or rarely due to Diabetes insipidus.

The rise in serum creatinine and the drop in urine output are not enough to diagnose AKI, as their sensitivity and specificity are too low for predicting AKI early. Despite its prognostic value, a rise in sCr characteristically occurs 24 to 48 hours after the snake bite owing to the concept of renal reserve. NGAL is a small siderophore protein, intensely up-regulated and excreted in acute tubular damage. It is also, readily filtered in the glomerulus and reabsorbed in the proximal tubular segments. It can be detected in plasma and urine in the early phases of AKI following viper envenomation (Senthilkumaran*et al.,*2017). Hence, it is one of the biomarker of AKI in patients with systemic inflammation, when multi-organ damage is less pronounced (Senthilkumaran*et al.,*2019; Ratnayake*et al.,*2019). In addition, the bioavailable dose of nephrotoxin following

snake envenomation is presumed to be highly variable but remains unknown, and the extent of exposure is further modified by varying elimination kinetics including those determined by baseline renal function.

During envenomation, various cytokinesare released which contribute to vasculopathy, coagulopathy, cytotoxicity and necrosis. In experimental models, the cytotoxic potency of viper venom was found to be higher than krait and cobra venoms. Venom induces hemorrhage and necrosis, and thereby causes local damage following the release of "damage associated molecular pattern (DAMPs) molecules". These DAMP molecules contribute to sustained oxidative stress and inflammation (Jamunaa*et al.,*2012). The authors have stated the usefulness of antioxidant treatment in addition to antivenom during the early stage of viper bite, which may reduce the occurrence complications following envenomation.

It is worth to recall the kinetics involved in the effects of venom and inflammatory response leading to osmotic stress in the renal cells and regulation of cell survivalwhich may lead to cell injury and apoptosis (Thiemicke and Neuert, 2021). These may explain why the plasma NGAL gets elevated in viper envenomation. These processes are further aggravated by nephrotoxins present in the venom. All these are potential areas for future research with regard to prevention of renal injury by way of early administration of adjuvants like antioxidants and steroids in order to protect, preserve and prevent the progression of cell death leading to AKI.

As the plasma NGAL and sCr got elevated in patients with viper envenomation despite early intervention, the patients and care givers shall be informed about the ongoing events related to the occurrence of AKI, and the need for dialysis at the time of admission and during the clinical course. They shall be counseled accordingly so as to avoid Doctor-patient conflicts. Moreover the treating physicians, practitioners and health science students shall be taught on the limitations of ASV therapy. They shall also, be motivated to monitor the overt and covert symptoms and signs of AKI by clinical and laboratory means, and asked to continue supportive care so as to minimize the progression of AKI.

Conclusion

AKI is observed in Russell's viper bite cases despite early intervention. Analysis of plasma NGAL and sCr revealed a progressive elevation of both at different intervals even after administration of anti-snake venom irrespective of the age, gender, bite to needle time, lymphadenitis and local swelling. The possible mechanisms for the occurrence of AKI in viper envenomation are attributable to the constituents of venom such as nephrotoxins, cytokines and endothelin, and the envenomation induced oxidative stress and thrombo inflammation. Better understanding of possible mechanisms involved in AKI will likely pave ways for early introduction of adjuvants like antioxidants and steroids for snake bite cases so as to avert or minimize renal damage.

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Table1**: A time series of plasma NGAL with Demographic and clinical parameters**

Parameters for 10 cases (N=104)	**Plasma NGAL (ng/mL) (Mean ±SD)**			**Coefficient of variance (CV)**
	0 hour	**12 hours**	**24 hours**	
Age group in years				
Below 25 (15)	232.67±32.3	349.33±119.9	344.00±127.7	0.273
25 – 44 (64)	231.87±21.7	327.05±131.9	330.55±155.5	0.243
45 and above (25)	236.40±38.2	351.80±137.1	358.60±153.9	0.294
Gender				
Male (73)	231.71±35.06	333.38±137.8	327.80±144.5	0.242
Female (31)	235.32±30.74	366.16±147.4	366.13±164.8	0.307
Bite to needle				
Less than 1 hour (22)	221.59±30.7	314.49±107.67	277.50±118.5	0.158
1 &<2 hours (39)	227.05±30.5	315.64±92.85	303.72±97.22	0.204
2 &<3 hours (33)	244.85±36.6	408.50±174.70	408.48±186.89	0.354
3 &<4 hours (10)	241.00±28.7	373.00±140.07	381.11±167.82	0.318
Lymphadenitis				
Grade 0 (50)	237.80±33.08	333.50±122.1	338.40±127.4	0.247
Grade 1 (54)	228.70±34.50	344.00±160.5	340.00±171.5	0.277
Swelling				
Grade 0 (26)	230.96±30.6	237.88±37.90	280.20±107.9	0.136
Grade 1 (65)	227.23±33.4	322.26±118.3	315.20±129.8	0.229
Grade 2 & 3(13)	263.44±21.7	514.33±120.0	547.00±119.4	0.496

[Figure in parenthesis indicate actual number of cases]

Table2**: A time series of serum creatinine with demographic and clinical parameters**

Parameters for 104 cases	**Serum creatinine (in mg%) (Mean ±SD)**				**CV**
	0 hour	**12 hours**	**24 hours**	**48 hours**	
Age group in years					
Below 25 (15)	1.12±0.13	1.16±0.10	1.41±0.31	2.27±1.11	0.162
25 – 44 (64)	1.10±0.11	1.17±0.10	1.40±0.36	2.05±1.08	0.169
45 and above (25)	1.09±0.12	1.14±0.11	1.42±0.32	2.22±1.24	0.185
Gender					
Male (73)	1.11±0.11	1.18±0.10	1.37±0.34	2.30±1.12	0.145
Female (31)	1.11±0.12	1.17±0.08	1.51±0.31	2.30±1.08	0.216
Bite to needle interval					
Less than 1 hour (22)	1.09±0.10	1.18±0.12	1.23±0.30	1.59±0.86	0.085
1 &<2 hour (39)	1.12±0.12	1.17±0.09	1.38±0.35	2.08±1.21	0.147
2 &<3 hour (33)	1.11±0.12	1.16±0.08	1.48±0.33	2.51±1.32	0.202
3 &<4 hour (10)	1.08±0.12	1.19±0.08	1.45±0.37	2.40±1.10	0.206
Lymphadenitis					
Grade 0 (50)	1.12±0.11	1.18±0.09	1.41±0.30	2.19±1.03	0.162
Grade 1 (54)	1.11±0.12	1.17±0.09	1.38±0.35	2.08±1.21	0.153
Swelling					
Grade 0 (26)	1.16±0.13	1.21±0.09	1.78±1.95	1.75±0.90	0.293
Grade 1 (65)	1.11±0.10	1.17±0.10	1.39±0.34	2.04±1.09	0.158
Grade 2 and 3 (13)	1.10±0.13	1.18±0.07	1.63±0.29	2.08±1.09	0309

[Figure in parenthesis indicate actual number of cases]