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Neutrophil Gelatinase-Associated Lipocalin as an Early Marker of Acute Kidney Injury in Snake Bites Victims

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ABSTRACT: The effects of viper envenomation in renal tissues leading to acute kidney injury (AKI) are well known. However, the usefulness of Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker to detect AKI in viper bite cases was not attempted much. Hence the present study was undertaken to find out the plasma NGAL levels among the hospitalized viperidae group snake bite victims, compare it with serum creatinine and assess the usefulness of NGAL. The plasma NGAL level was elevated within 6 to 8 hours of all the 131 cases of viper bites much before the elevation of serum creatinine levels, irrespective of the age and gender of the patient, and bite to hospital time. The sensitivity, specificity, positive predictability and negative predictability were 96.5, 70.5, 95.7 and 75% respectively which was significant statistically ($p<0.001$). Elevated plasma NGAL levels in viper bite cases acts as biomarker to detect AKI early, and also helps to plan for an appropriate intervention. So, it is suggested to include estimation of plasma NGAL in the point of care testing especially in emergency settings handling snake bite cases. However more studies are recommended to find out its serial levels in snake bites following different kind of snakes with different manifestations as observed in different age groups belonging to different gender in different population so as to arrive embarking conclusions / recommendations.

Keywords: Neutrophil gelatinase-associated lipocalin - NGAL – serum creatinine – biomarker – Acute kidney injury – viper bite – diagnostic test

I. INTRODUCTION

Acute Kidney Injury (AKI) remains a significant cause of morbidity and mortality among victims of viperidae group snake and sea snake bites [1]. In India, Russell's viper and Echiscarinatus are the important viper species that often cause acute tubular necrosis, at times acute cortical necrosis and rarely a combination of both [2]. The AKI which occurs after snake bite is usually reversible with supportive management and injection anti-snake venom, but if acute cortical necrosis develops, it may lead to an incomplete recovery.

The gold standard for the diagnosis of impaired renal function is the rising serum creatinine levels from the basal level. Currently accepted definition of AKI is absolute increase in serum creatinine by ≥ 0.3 mg/dl [3]. As elevation of serum creatinine is delayed by day(s), it becomes a less reliable biomarker in the scenario of viper envenomation. Therefore, there is a need to find out better biomarkers to recognize AKI at the earliest and institute appropriate measures so as to reduce the morbidity and mortality associated with it.

Neutrophil gelatinase-associated lipocalin (NGAL) was originally identified as a 25-kDa protein covalently bound to gelatinase of neutrophils. Although NGAL is expressed only at very low levels in several human tissues, it is markedly elevated in injured epithelial cells [4]. This is likely to be elevated whenever renal tubular or cortical cells are injured either by drugs or toxins. Moreover, this biomarker plays a central role in the control and regulation of cell survival, and proliferation. The expression of NGAL in proliferating and regenerating tubular epithelial cells indicates its role in the process of repair. NGAL, in the context of AKI, plays a role in the preservation of function and attenuation of apoptosis as well as reflects the proliferative response. This protective effect is dependent on the chelation of toxic iron from extracellular environment and the regulated delivery of siderophore and iron to intracellular sites [5].

II. MATERIALS AND METHODS

This prospective study was carried out in an emergency department of a tertiary care hospital from June 2012 to December 2016 with 131 cases of viper snake bite presented within eight hours of bite. Patients with AKI serum creatinine >1.2 mg/dL), pre-existent renal diseases, long standing diabetes or hypertension, overt congestive heart failure (NYHA III–IV), hemodynamic instability of any cause, sepsis or systemic infectious diseases, exposure to nephrotoxic drugs/chemicals or other biological toxins and injuries were excluded. Informed written consent was obtained from every participant before enrollment. The study was performed in accordance with the Declaration of Helsinki and with the approval of the institutional ethics committee.

Cases of viper bites were assessed clinically and then, subjected to laboratory evaluation such as complete blood count including clotting time and blood chemistry including plasma NGAL, and serum creatinine and urine for sediments and protein at the time of their arrival to Emergency Department. Plasma NGAL was estimated using the standardized Triage® NGAL test (Biosite Incorporated, San Diego, CA, USA). Serum creatinine was determined by Jaffe`s method at 0, 12, 24 and 48 hours. All the patients included in this study were monitored for hourly urine output and serial serum creatinine till the patients got discharged from the hospital. All the cases received anti-snake venom and supportive measures. Thorough out the study good laboratory and clinical practice were adhered too. During the hospital course, these patients were not given any nephrotoxic agents. The data were analysed statistically.

III. RESULTS

There were 90 (68.7%) males and 41 (31.3%) females, and their age ranged from 16 to 80 with a mean and median of 45.12 and 52 years respectively. Mean body weight of males and females included for the study were 66 and 59kgs respectively. All of them had local symptoms and signs of envenomation without any systemic manifestations. Their haematology and blood chemistry were within acceptable limits, but for prolonged clotting time. Their plasma NGAL and serum creatinine ranged from 210 to 615ngm/dl and 0.8 to 1.1mgs/dl with a mean and SD of 278.3 $\pm$ 62.5ngm/dl and 0.9 $\pm$ 03mgs/dl respectively at the time of admission. The mean plasma NGAL level was elevated in almost all the snake bite victims even at the time of arrival significantly ($p<0.001$) when compared with healthy control, even though their serum creatinine was well within normal levels at that time. The serum creatinine was estimated at serial intervals varied from 1.3 to 2.1mg/dl. During hospital stay their urine output was satisfactory and urinalysis was not contributory.

Based on the 24 hours post admission serum creatinine levels, the cases were considered to have developed non-oliguric AKI and hence, an attempt was made to compare these levels with initial NGAL levels. As NGAL was elevated in all these cases, a receiver operator characteristic (ROC) curve was attempted and the details are furnished in Table 1. The ROC for plasma NGAL yielded a sensitivity of 96.5% and specificity of 70.5% for a cut-off value of 245ngm/dl. As the Positive Predictive Value to detect AKI was 95.7% among the study population, it is likely that viper bite cases if have plasma NGAL level of 245ngm/dl or more, they will be considered to have developed AKI requiring appropriate care and intervention. During their hospital stay none developed any other complications including death.

Table 1: Sensitivity and Specificity for NGAL at cut-off value 245 ngm%.

NGAL values in ngm/dl	Serum Creatinine in mgms%	
	>1.4	< 1.4
>245	110	5
<245	4	12
Total	114	17

Sensitivity = a/(a+c) = 96.5%
Specificity = d/(b+d) = 70.5%
Positive Predictive Value = a/(a+b) = 95.7%
Negative Predictive Value = d/(c+d) = 75%

IV. DISCUSSION

Snake bite induced AKI is an important cause of mortality in rural areas. Estimation of NGAL in the detection of AKI and the mechanisms involved for the elevation of it has been highlighted earlier [5]. Our study also revealed that plasma NGAL was significantly ($p<0.001$) elevated in viper bite victims when compared with non-venomous snake bite cases and before the elevation of serum creatinine [6]. As elevation of NGAL is much before the elevation of serum creatinine with high sensitivity, plasma NGAL may be considered as a useful bio-marker to detect AKI at the bedside among snake bite victims. Preclinical transcriptome profiling studies identified NGAL as one of the most up regulated genes in the kidney very early after acute injury based on animal model [7]. Plasma NGAL rises after the renal insult by the venom even before renal cell necrosis develops. This indicates that viper venom is toxic to renal cells. In our study the Positive and Negative Predictive Values were 96.5 and 70.5% respectively.

Hence, we believe that elevated plasma NGAL is an early marker of AKI following viper and aids to diagnose AKI 48 hours prior to the diagnosis based on RIFLE criteria [8]. During the study period, serum creatinine did not accurately reflect renal insult by viper venom until a steady state was reached by 24 or 48 hours in different cases. At this juncture, one has to remember that the renal cellular response to viper venom may vary in different individuals because of the genomic nature and the chemokines involved in the production and release of NGAL vary from one another. The other contributory factors for cellular injury related to snake are the habits and age of the snake, amount of venom injected and the constituents of venom in addition to season, time of bite, bite to hospital time and time of investigation [9].

Thamarai and Sivakumar correlated plasma NGAL levels of their viper bite cases with RIFLE criteria [10]. They observed higher levels of plasma NGAL (mean 517 ngm/ml) in patients who fell into the category of "failure" (RIFLE) whereas patients who fell into the category of injury had mean plasma NGAL of 298.47ngm/ml. In our study we did not observe any correlation between the plasma NGAL and serum creatinine at the time of admission. However, there was significant correlation between plasma NGAL and serum creatinine value at 48 hours after admission. In our study the area for plasma NGAL under the ROC (AuC-ROC) was 0.93 (95% CI 0.88–0.97), thus suggesting that plasma NGAL is a better predictor over serum Creatinine, in diagnosing AKI.

In conclusion, the present study demonstrated an elevation of plasma NGAL level at 6 to 8 hours after viper bite measured and thereby indicating NGAL level is a useful biomarker to detect AKI much before serum creatinine and aid for an appropriate intervention. It is also suggested that estimation of plasma NGAL may be included in the point of care testing especially in an emergency settings handling snake bite cases.

Limitations of the study were small number of samples and single center study as well as non-estimation of plasma NGAL serially due to technical constraints. The strengths of the study are confirmation of viper bite by identifying the snake and adoption of good laboratory and clinical practice.

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