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Full Length Research Paper

The clinical and biochemical profile of snakebite patients- A hospital based comparative study of envenomed and nonenvenomed victims

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Envenomation and death resulting from snakebite is a particularly important public health problem in the rural tropics. Knowledge about the early clinical and biochemical manifestations of envenomation is imperative to plan effective management strategy. This study aimed to identify potential markers of envenomation. This is a prospective cohort study, wherein consecutive, hospitalized snakebite victims [envenomed and non-envenomed] were enrolled as Cohorts I and II. The complete clinical profile and biochemical parameters [Blood glucose, urea, serum creatinine, Alanine transaminase, Aspartate transaminase, Creatine Kinase and Lactate Dehydrogenase] were assessed. The findings were computed and analyzed. There were 61 patients in each Cohort. In Cohort I, 61.1% were hemotoxic, 24.2% neurotoxic, 9.8% both and 4.9% local envenomation. The predominant clinical manifestations included edema 72.5%, coagulopathy 70.1%, neuroparalysis 34 % and oliguria 33.1%. Biochemical evaluation revealed statistically significant increase in LDH and CK in Cohort I on all 3 days. Snake bite is seasonal. Clinically hemotoxic envenomation is more common than neurotoxic. Biochemically, an early rise in LDH and CK could be used as a reliable marker of systemic envenomation.

Key words: Snake bite, envenomation, lactate dehydrogenase, creatine kinase.

INTRODUCTION

Snakes are ubiquitous, being found throughout most of the world, except in few frozen environments and high altitudes (Anuradhani Kasturiratne, et al., 2008). According to a recent analysis, the estimated number of snakebites worldwide could be as high as 5,500,000 per year, resulting in 1,841,000 envenomation and 94000 deaths, Ariaratnam CA, (2009). Nowhere is the burden so felt as in the developing tropical countries. India has the highest number of deaths due to snake bite in the world with 35,000–50,000 people dying per year according to World Health Organization (WHO) direst estimates Bergmeyer HW (1965). Most appropriately, snakebite is classified by the WHO as a neglected tropical disease.

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The spectrum of snake bite envenomation can range from an insignificant local edema to fulminant toxemia and death. Warrel et al. (1995) showed that envenomation by South Asian viperid snakes results in local pain and tissue damage as well as coagulopathy and Acute Renal Failure Bhagwat K, Amar L (2013). In addition, Russell's viper can cause neurotoxicity, as has been shown in several studies conducted in South India and Sri Lanka Bomb BS, Roy S, Kumawat DC, Bharjatya M (1996). Burtis CA, Ashwood ER (1994) Progressive descending paralysis is the hallmark of systemic envenoming by elapid snakes in South Asia Chippaux JP (1998), Hussain I (1997) Faiz MA, (1995). The Indian scenario is dominated by the "big four poisonous snakes" namely, common cobra (Najanaja), common krait (Bungarus caerulus) and viperidae including Russell's viper and saw scaled viper. However, not all snakebites result in systemic envenomation. In Kerala, Suchithra and

Papachan [2008] showed that only 219 out of 635 patients (34%) with proven snake bite developed signs of systemic envenomation. Likewise, in Bangladesh the proportion of nonenvenomed bites reported in hospital-based studies varied between 60% and 80%. Hansdak SG, (1998) Du NT (2009). Despite the abundance of literature on the subject, few researchers have focused on identifying clinical markers of systemic envenomation. Most physicians in South Asia have to rely on the bite circumstances and clinical features as a guide to specific management.

The need for sensitive and specific markers of systemic envenomation is of utmost importance for those involved in this field. Kale Bhagwat et al. (2013) proved that an increase in serum creatine kinase activity can best be utilized to assess extent of rhabdomyolysis whereas serum lactate dehydrogenase activity serves as a marker of rhabdomyolysis as well as hemolysis, Kularatne SA (2002). In our country, given the existing constraints, timely clinical identification of venomous bites and reliable cost effective biochemical markers could go a long way in reducing mortality. This study aimed to achieve just that.

BIOCHEMICAL CHANGES

Snake venom is considered to be one of the most highly developed and complicated of all toxins produced by plants and animals Kularatne SA (2003). It is a complex mixture containing polypeptides, enzymes, glycoprotein and other lipid components, capable of producing severe tissue damage (WHO, 2001). The neurotoxic elements of elapid venom are polypeptides that bind post synaptically and reversibly block acetylcholine receptors, Mittal BV (1994). Phospholipases A2 are the most extensively studied of all the venom enzymes. They have been proved to cause mitochondrial damage, hemolysis, thrombocytopenia, skeletal muscle and vascular endothelial membrane lysis. Metalloproteinase causes local and systemic hemorrhage, myonecrosis, blistering and edema, Dhingra N, (2011) Kini RM (2007).The resultant tissue damage triggers the release of muscle enzymes like creatine kinase and lactate dehydrogenase, both of which may be utilized as markers of systemic envenomation.

MATERIALS AND METHODS

Study Design: Ours was a prospective, observational study conducted from May 2012 to April 2013, so as to span all the seasons.

Study Centre: This study was conducted at Govt Villupuram Medical College and Hospital, Villupuram, India. This is a 500 bedded, tertiary care level teaching institute located in a rural district of Southern India. The study was done by the Departments of Biochemistry and Internal Medicine in unison.

Subject Selection: A total of 134 patients were enrolled into the study, of whom 12 were lost to follow up and hence excluded. Patient enrollment was done based on inclusion and exclusion criteria outlined below:

- **Inclusion Criteria**: Hospitalized, adult patients with snakebite, who presented within 6 hrs of the bite.
- **Exclusion Criteria**:
  - Adult patients who presented later than 6 hrs.
  - Patients in whom the biting organism could not be identified.
  - Patients with severe co morbid illnesses like Chronic Kidney Disease, Heart Failure, and Diabetes.

The sample included 61 consecutive adult patients who showed evidence of systemic or local envenomation from snake bite. These were grouped as Cohort I. Likewise 61 consecutive patients who suffered snakebite, but failed to show any objective evidence of envenomation were grouped as Cohort II. The diagnoses of systemic envenomation, local envenomation and non envenomation were assigned based upon predefined criteria.

METHODOLOGY

Upon enrollment, a detailed history was taken and comprehensive clinical examination done for all the patients by the researchers. The findings were documented on the clinical case proforma, designed for the study. Subsequently, the patient underwent basic investigations like Blood glucose, Blood urea, Serum creatinine, Urine analysis and Electrocardiogram.

Targeted Laboratory workup

5ml venous sample was collected from study participants. The blood samples were centrifuged at 3000rpm for 10min to separate serum and cells. Specific investigations like, Whole Blood Clotting Test (WBCT), Serum Creatinine, Lactate Dehydrogenase (LDH), Creatine Kinase (CK) Schumann (2002). Dissanayake S (2002) and Liver Function Tests were performed by researchers from the Department of Biochemistry at 6, 12, 24, 48 and 72 hour intervals. The biochemical parameters were analyzed by fully auto analyzer (Beckman Coulter). Physical examination paralleled blood investigations till the end of 72 hours and thereafter based on the clinical scenario. The findings were computed periodically. The treatment was entirely at the discretion of the treating physician.

HEMOTOXIC ENVENOMATION **Whole Blood Clotting Test [WBCT] > 20 minutes on one or more occasions after 6 hours of bite and/or bleeding manifestations [gum bleeds, venipuncture site ooze].**
NEUROTOXIC ENVENOMATION **Presence of neuromuscular paralysis [ptosis, swallowing difficulty, respiratory muscle weakness] or signs of neuropathy [increased salivation, postural hypotension, diaphoresis]**
LOCAL ENVENOMATION **Presence of edema and inflammatory markers [warmth, tenderness] at bite site only and without the features enlisted above for neuro and hemotoxic envenomation and absence of features of Systemic Inflammatory Response Syndrome [SIRS].**

Figure 1. Diagnostic Criteria for snakebite envenomation.

Determination of Creatine Kinase Assay

The creatine kinase activity was assayed by immunoinhibition method.Soe (2005).1ml of reconstituted reagent was mixed with 20 µl of serum in which the creatinine kinase present releases the phosphate group from creatine phosphate and this phosphate combines with glucose to form glucose 6-phosphate by enzyme Hexokinase. G-6-P is oxidized to produce NADPH. Increasing absorbance of NADPH is monitored at 340nm and is directly proportional to CK activity. Increasing optical density was measured 4 times at one minute interval. The average absorbance difference per minute (ÄAbs/min) was calculated. The ÄAbs/min was multiplied by the factor 8095 to get CK activity in IU/L

Determination of Lactate Dehydrogenase Assay

The Lactate dehydrogenase activity is estimated by kinetic method. 1 ml of reconstituted reagent was mixed with 20µl of serum. In this reagent the contained pyruvate is catalyzed by LDH to form lactate and NAD.Decrease in absorbance due to oxidation of NADH is monitored at 340nm and is directly proportional to LDH activity. Decreasing optical density was measured 4 times at one minute interval. The average absorbance difference per minute (ÄAbs/min) was calculated. The ÄAbs/min was multiplied by the factor 8199 to get Lactate dehydrogenase activity in IU/L

ETHICAL ISSUES

This study was approved by the Institutional Ethics Committee, Government Villupuram Medical College and Hospital. All participants gave written, informed consent.

RESULTS

A total of 61 envenomed patients were categorized as Cohort I and an equal number of nonenvenomed patients as Cohort II. Most of the bites occurred between 1800 hrs to 2200hr. Most of the bites occurred in lower limbs (64.5%).There was a significant seasonal predominance with approximately 52% of bites occurring during the period from August to November. This coincides well with the harvesting season of the peasants, wherein the exposure rate is enhanced.

The overall male to female ratio was 3:2 and there was no significant difference between the 2 Cohorts. The mean age of the victims was 34.3yrs. There were no deaths during the study period.

The most common symptoms were pain (74.1%), swelling (72.5%) and giddiness (50%). Others included, vomit (29%), dyspnoea (14.5%) and headache (12.9%). Clinical signs assessed included pulse rate, blood pressure, temperature, urine output, hemotoxic manifestations like bleeding, petechiae, edema, etc and neurotoxic features like ptosis, lower cranial nerve palsies and respiratory weakness. The majority suffered hemotoxic envenomation (61.1%). 24.2% were affected by neurotoxic bite and 4.9% experienced only local envenomation. The commonest clinical findings included tenderness (74.1%), edema (72.2%), bleeding from venipuncture site (33.5%) and ptosis (22.5%). The bleeding manifestations correlated well with WBCT values. Respiratory failure occurred in 4 patients [6.5%], of whom 2 required ventilator support. A significant decrease in urine output occurred in Cohort I (33.1%) on day 2, but thereafter normalized. Acute kidney injury (Serum creatinine >1.2mg %) occurred in 4 patients, of whom 3 were hemotoxic envenomation. There was a non-significant increase in heart rate among patients in Cohort I [p=0.27] on day 2. Apart from a subjective sense-

Table 1. The clinical features of snake bite envenomed patients [Cohort 1]

Clinical profile of Cohort I	Percentage[n]
Pain and tenderness	74.1%
Edema	72.5%
Bleeding manifestation	70.1%
Giddiness	50%
Neuroparalysis	34%
Oliguria	33.1%
Vomit	29%
Dysponea	14.5%
Headache	12.9%
Fever	8.7%

Table 2. Comparison of LDH and CK values between two cohorts.

Parameter	Day 1 Mean± SD	P Value	Day2 Mean± SD	P Value	Day3 Mean± SD	P value
LDH						
COHORT 1	425 ± 306		391.26 ± 281.23		384.41±280.86	
COHORT 2	347.25±72.05	<0.05*	370.69 ± 71.66	< 0.05*	346.20 ±89.53	<0.05*
CK						
COHORT 1	339.85 ± 304.59		322.98 ±270.12		257.21±257.30	
COHORT 2	60.97±7.80	<0.0001 *	185.62 ±52.95	0.000*	180.70 ±70.26	<0.03*

***** Significant difference observed (P<0.05)

sation of giddiness (54%) and anxiety (37.3%), no major clinical perturbations were evident in Cohort II.

The major biochemical alteration observed was however in the levels of Creatine Kinase (339.89+/-304.59IU /L[Mean+/-SD]) and Lactate Dehydrogenases (LDH 425.93+/-306.06 IU/L). The mean values of CK and LDH for the two Cohorts on all 3 days are summarized . Between the two Cohorts , there was a significant difference in CK and LDH levels on all 3 days , showing a phenomenal rise in Cohort I(p<0.05). Other alterations revealed were, a nonsignificant rise in serum creatinine on day 3 in Cohort I (p=0.08). Likewise, Cohort I patients showed a non significant rise in serum Aspartate Transaminase (AST Mean 55.26-+/- 44.26) on day 1[p =0.04]. The remaining tests like blood glucose, serum bilirubin, serum electrolytes showed no significant difference between the two Cohorts.

DISCUSSION

Snakebites are a major problem in India especially in rural areas, among the farming community. The million death study estimated that approximately 50,000 people die of snakebite every year in India, indicating a major public health problem Sujathan P (2008) . The incidence, morbidity and mortality vary widely in different parts of the country. It is therefore obvious that the information from one region cannot be extrapolated onto another. That said, the clinical picture of envenomation however does not change much for the given 4 major snake species in the nation.

There are 5 syndromes that are identified with snake bite in South East Asia (See WHO guidelines for management of snake bite in South East Asian region) Warrell DA (1995). However, one of the major limitations of this approach is syndromic overlap for many snake species, eg. Russel's viper bite can cause neuroparalytic features as well as hemotoxic and Acute Kidney Injury Warrell DA (1996). In a previous research work, Ariaratnam et.al concluded that the syndromic approach lacked sensitivity Swe TN (1987). In this study, the diagnostic criteria for envenomation
(hemotoxic, neurotoxic and local) were based on clinical and laboratory features outlined in this study.

Many previous research works identified clinical features, but not markers Bharjatya M (1996). Previous research works report ptosis in 70–93% of patients in most series, and extra ocular muscle weakness in 68–82%. Respiratory muscle weakness has been reported in 27–87% of cases. Das JC (1995).

Case fatality rates with neurotoxic envenoming again show wide variation, usually ranging between 4–11%. Chippaux JP (1998) This study focused on identifying potential clinical markers of envenomation. Most bites were unprovoked and timed during nocturnal hours (1800hrs to 2200 hrs).

Local swelling and pain dominated the clinical picture, not withstanding bleeding venipuncture sites, ptosis and salivation. A noteworthy observation was that a rapidly progressive swelling (edema which exceeds the proximal joint within 6 hrs) was consistently associated with systemic envenomation ($p< 0.05$).

As in previous studies, hemotoxic envenomation (61.1%) constituted the major bulk of cases, thereby predicting viperine species predominance in the region WHO (2005 &2007). Neuroparalytic features were present in 24.2% of envenoming. In this study, about 9.7%% of cases had features of both heamo and neurotoxic manifestations. Another important observation was the decrease in urine output on day3. The reason for the same could not be ascertained.

It is hypothesized that an insult to the kidneys either by venom per se or antisnake venom sera could be the potentiating cause.

The search for newer and sensitive biochemical markers for systemic envenomation is a field of active ongoing research. Previous research work in this area has revealed the potential utility of serum LDH and serum Creatine Kinase activity in the diagnosis and prognosis of snake bite cases. Also, blood hemoglobin level has been used to assess the extent of hemolysis, renal failure and rhabdomyolysis Kularatne SA (2002) A prospective study from Brazil showed that among children with moderate to severe snake bite envenomation as early rise in serum LDH levels up to 48 hours which correlated well with degree of haemotoxicity of snake bite Kularatne SA (2002).

In this study, we found that early rise in CK and LDH levels were significantly associated with systemic envenomation both neuro and hemotoxic.

The cause for this rise was attributed to local and vascular endothelial damage as well as hematological toxicity.

The incidence of Acute Kidney injury was very low in this study, probably related to early intervention and adequate management. There was a non-significant rise of Serum Creatinine on day 3 which was preceded clinically by a reduction in urine output during the same time frame. Likewise Serum Aspartate Transaminase showed an early rise in envenomed patients, though it lacked specificity.

LIMITATIONS

Our study was not powered enough to unveil better and novel biochemical markers due to financial and logistic constraints.

CONCLUSION

This study was instrumental in revealing a few facts about snake bite envenomation. Firstly, the need for a comprehensive physical examination cannot be overemphasized. From the results, it is inferred that a rapidly progressive local edema could serve as a potential marker for inevitable systemic envenomation. Secondly, the clinical profile of snakebite patients both envenomed and non envenomed was defined for this region. Thirdly, this study uncovered the importance of biochemical markers like CK and LDH. Significantly elevated CK and LDH levels on day 1 and 2 in these patients could reliably predict systemic envenomation. Further targeted research is the need of the hour and will hopefully allow better and more specific diagnostic markers to be unveiled. This study paves the path for more extensive and multi-centric research focused on the subject to reduce the mortality and morbidity for this ever-expanding social malady.

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Abbreviation: Lactate dehydrogenase (LDH), Creatine kinase (CK), Aspartate transaminase (AST), Alanine transaminase (ALT).

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