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ASSESSING SERUM C-REACTIVE PROTEIN AND LACTATE DEHYDROGENASE AS HEMOTOXICITY INDICATORS IN PATIENTS WITH VASCULOTOXIC SNAKEBITES

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ABSTRACT

Background : Snakebites are a significant public health issue, particularly in tropical regions, with a focus on South Asia. It emphasizes the high incidence of snakebites among rural populations and the challenges of underreporting and underestimation of the problem. The study highlights the diversity of venomous snakes, particularly in India, and the various effects of snake envenomation on different body systems, emphasizing the importance of swift diagnosis and treatment. This study aims to investigate the potential of serum C-reactive protein (CRP) and lactate dehydrogenase (LDH) as markers of hemotoxicity in snakebite cases. **Methods:** This was a prospective observational study conducted at MGM Medical College, Navi Mumbai, India, from March 2021 to December 2022 and included 70 snakebite cases. using convenient sampling. **Results:** Majority of the subjects were female (81.4%) within the 21-40 age group, with lower limb snakebites being common, and most patients seeking medical attention within 6 hours. Envenomation severity ranged from none to severe, and 37.1% of subjects exhibited bleeding manifestations. Variations in CRP and LDH levels were associated to envenomation severity. Patients with bleeding manifestations displayed significant increases in CRP and LDH levels over 24 hours, indicating inflammation and cellular damage associated with bleeding. Receiver operating characteristic (ROC) curve analysis identified CRP and LDH at 24 hours as moderate predictive biomarkers for bleeding manifestations, with optimal cut- off values of CRP > 5 mg/L and LDH > 300 U/L. **Conclusion:** Serum CRP and LDH levels demonstrated significant variations across envenomation grades, particularly elevated in severe cases, indicating their potential as hemotoxicity markers in snakebite patients. These markers also aid in distinguishing severe from mild envenomation, facilitating early assessment and management.

KEYWORDS :

INTRODUCTION:

Snakebites represent a significant public health issue in tropical and subtropical countries, particularly among rural populations, including farmers, plantation workers, herders, and laborers. However, this problem often goes unnoticed and underreported. Accidental snakebites frequently occur when people inadvertently step on snakes or while sleeping on the ground in open-style dwellings. South-East Asia is disproportionately affected due to its dense population and extensive agricultural practices, with India reporting the highest snakebite-related mortality. Despite these alarming statistics, the true global burden of snakebite remains unknown, as many victims initially seek traditional healers rather than medical facilities. (1)

The world hosts over 3,000 snake species, with approximately 600 being venomous. In Southeast Asia, the Elapidae family (cobras and kraits) and Viperidae family (vipers and pit vipers) contain important venomous snakes. (2) India's "Big 4" venomous snakes are Russel's viper, Cobra, Common Krait, and Saw-scaled viper, while other venomous species like the king cobra and monocle cobra also pose threats in specific regions. (2,3) Snake envenomation affects various body systems and can be hemotoxic, neurotoxic, myotoxic, or a combination, emphasizing the need for swift identification and treatment. Snake venom is believed to act as an acute-phase reactant, leading to the production of inflammatory mediators like Interleukin-

6 (IL-6) and acute-phase reactants such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). Serum lactate dehydrogenase (LDH) levels are associated with hemotoxicity and envenomation severity, prompting this study to explore their potential as markers of hemotoxicity in snakebite cases. (3–5)

India houses approximately 270 snake species, with six being notably venomous, including the "big four" responsible for most snakebite cases: King Cobra, Indian Krait, Russell's Viper, and Saw Scaled Viper. Other venomous species like the Indian Cobra, Malabar Pit Viper, Bamboo Pit Viper, and Hump-Nosed Pit Viper are also regionally significant. Additionally, the Banded Sea Krait and Indian Rock Python add to India's diverse snake population. Snake venom, a complex mixture of various molecules, primarily proteins, varies in composition among species, locations, and individual snakes. Venom types, categorized as neurotoxic and hemotoxic, result in diverse snakebite manifestations, influenced by venom components such as phospholipase A2, hyaluronidase, acetylcholinesterase, and neurotoxins. The intricate composition of snake venom underlies the wide range of snakebite effects. (6,7)

Assessing the global burden of snakebite envenoming is challenging, especially in South Asia where reliable data is limited. India, with an estimated 35,000 to 50,000 annual snakebite- related deaths, faces the highest impact worldwide. Pakistan, Nepal, and Sri Lanka also report significant cases and fatalities. Snakebites disproportionately affect outdoor workers during the rainy season and extreme weather events, making it a substantial health concern in the region. Improved monitoring and healthcare interventions are urgently needed to address this issue. (4)

Serum CRP as a Marker of Hemotoxicity in Snake Bite:

Venomous snake bites can exhibit characteristics of an acute-phase reaction, including leukocytosis with neutrophilia, lymphopenia, and eosinophilia, along with changes in various blood parameters such as mucoproteins, clotting time, and C-reactive protein. These observations suggest that

snake bites share similarities with acute trauma. Snake venom components may interact with various target cells, leading to the release of inflammatory mediators. Studies by Bielory et al (8), B. Barraviera et al (9), Ranjini M et al. (10) have shown that C-reactive protein (CRP) levels can serve as markers for hemotoxicity in snakebite victims and may aid in predicting prognosis. Additionally, Bhagwat K et al. (11) found significant elevations in serum CRP levels in severe envenomation cases compared to mild envenomation cases.

Lactate Dehydrogenase: Lactate dehydrogenase (LDH) is an enzyme with four subunits that plays a key role in converting pyruvate to lactate and NADH to NAD+. There are four LDH genes: LDHA, LDHB, LDHC, and LDHD. LDHA is mainly found in skeletal muscle, while LDHB is primarily present in the heart. LDHA and LDHB can form homo- or heterotetramers, producing five different LDH isoenzymes. Each isoenzyme has distinct kinetic properties due to differences in charged surface residues around the active site, influencing their preference for converting pyruvate to lactate or lactate to pyruvate. (12)

Serum LDH as a Marker of Hemotoxicity in Snake Bite

Researchers are actively seeking more sensitive biochemical markers to identify systemic envenomation in snakebite cases. Previous studies have indicated that serum lactate dehydrogenase (LDH) activity could be a valuable tool for diagnosing and predicting the prognosis of snakebite victims. For example, research in Brazil found that children with moderate to severe snakebite envenomation exhibited an early increase in serum LDH levels within 48 hours, correlating with the degree of hemotoxicity. Studies by Ranjini M et al (10) and Bhagwat K (11) also noted significant differences in LDH levels across various envenomation grades, suggesting LDH as a potential marker for hemotoxicity and prognosis. Additional research by Kandasamy S. and Patil SL (13, 14) supported these findings, underscoring the importance of monitoring LDH levels to assess snakebite severity. Moreover, Kulkarni S et al.'s study highlighted the link between acute inflammatory markers and LDH levels, aiding in the differentiation of severe envenomation and offering early prognosis indicators for snakebite patients (15)

MATERIAL AND METHODS:

The study was conducted at the Department of Medicine, Mahatma Gandhi Mission Medical College in Navi Mumbai, Maharashtra, India. The study population consisted of individuals with either a confirmed history of snakebite or those with a history of an unknown bite but clinically suspected to be a snakebite. This prospective observational study employed a non- probability sampling method, specifically convenient sampling, and involved a sample size of 70 participants. The study spanned from March 2021 to December 2022. Inclusion criteria encompassed patients who reported a snakebite or those with strong clinical suspicion of a snakebite and who arrived at the hospital within 12 hours of the incident. Patients with bleeding disorders or a history of vasculitis were excluded from the study.

The study commenced after obtaining approval from the Institutional Ethical Committee and included a total of 70 patients selected through purposive sampling based on specific inclusion and exclusion criteria, with informed consent obtained. Upon admission, vital signs and the site of the snakebite were recorded. Patients with a history suggestive of snakebite underwent a battery of lab tests, including a complete blood picture, ESR (erythrocyte sedimentation rate), PCV (packed cell volume), urine routine and microanalysis, serum CRP and LDH, PT-INR (prothrombin time with international normalized ratio), and APTT (activated partial thromboplastin time), all conducted at admission and repeated 24 hours later. Additionally, clotting time, bleeding time, and the 20-minute whole blood clotting test were monitored every 6 hours for the first 24 hours of hospital admission. Patients were categorized into groups based on the severity of envenomation, ranging from no envenomation to mild, moderate, and severe envenomation, following a predetermined scale. The study analyzed the relationship between these severity groups and the levels of serum LDH and CRP.

RESULTS:

The study subjects were characterized based on several key variables. Table 1 indicates that the age distribution revealed that a majority of participants fell within the 21-40 age group (51.4%), with smaller proportions in the 41-60 age group (35.7%), and those over 60 (11.4%). Gender- wise, the study population consisted primarily of females (81.4%). In terms of the site of the snakebite, the lower limbs (LLL and RLL) were the most common locations, while the time interval since the bite was predominantly within 6 hours for the majority of cases (92.9%). Envenomation grades ranged from none (8.6%) to severe (11.4%), with mild and moderate cases making up significant proportions. Bleeding manifestations were present in a substantial portion of subjects (37.1%), highlighting the importance of assessing this factor in snakebite cases.

Table 1 Distribution of study subjects according to study variables

Parameter		Frequency	Percentage
Age	≤ 20	02	2.9%
	21-40	36	51.4%
	41-60	25	35.7%
	>60	08	11.4%
	Mean ± SD = 42.17 ± 4.16		
Gender	Male	13	18.6%
	Female	57	81.4%
Site of Bite	LLL	22	31.4%
	LUL	11	15.7%
	RLL	25	35.7%
	RUl	12	17.1%
Time Interval (in hrs)	≤ 6 hours	65	92.9%
	6 – 12 hours	03	4.3%
	12 – 24 hours	02	2.9%
Grade of envenomatio n	None	06	8.6%
	Mild	33	47.1%
	Moderate	23	32.9%
	Severe	08	11.4%
Bleeding manifestation	Yes	26	37.1%
	None	44	62.9%

Table 2 table illustrates the comparison of mean CRP (C-reactive protein) and LDH (lactate dehydrogenase) levels at both baseline and 24 hours across different grades of envenomation. Notably, the findings indicate significant variations in these biomarkers based on the severity of envenomation.

For patients with "None" envenomation, the mean CRP levels remained relatively stable, with a slight decrease from 4.8 mg/L at baseline to 4.5 mg/L at 24 hours (p-value = 0.75). Similarly, LDH levels also showed no significant change, with values of 211.6 U/L at baseline and 265.5 U/L at 24 hours (p-value = 0.17). In contrast, patients with severe envenomation demonstrated substantial alterations in both CRP and LDH levels. Specifically, patients with "Mild" envenomation exhibited a significant increase in mean CRP levels from 5.7 mg/L at baseline to 8.9 mg/L at 24 hours (p-value = 0.16), along with a corresponding increase in LDH levels from 267.2 U/L to 305.1 U/L (**p-value = 0.024***). Patients with "Moderate" envenomation experienced a remarkable increase in mean CRP levels from 5.6 mg/L at baseline to 15.7 mg/L at 24 hours (**p-value < 0.01**), and a substantial elevation in LDH levels from 277.6 U/L to 479.0 U/L (**p-value < 0.01****). The most

pronounced changes were observed in patients with "Severe" envenomation, as they exhibited a substantial increase in mean CRP levels, from 10.3 mg/L at baseline to 48.5 mg/L at 24 hours (p-value < 0.01). Moreover, LDH levels in this group increased significantly from 585.1 U/L at baseline to 828.2 U/L at 24 hours (**p-value < 0.01****).

Mean CRP levels from 4.5 mg/L at baseline to 6.9 mg/L at 24 hours (p-value = 0.114). Similarly,

LDH levels showed no statistically significant difference, with values of 252.1 U/L at baseline and 296.8 U/L at 24 hours (p-value = 0.42).

Conversely, individuals with bleeding manifestations ("Yes") displayed substantial and statistically significant increases in both CRP and LDH levels over the 24-hour period. Specifically, mean CRP levels increased from 7.1 mg/L at baseline to 20.2 mg/L at 24 hours (p-value < 0.01), indicating a significant inflammatory response. Additionally, LDH levels increased significantly from 351.8 U/L at baseline to 594.3 U/L at 24 hours (p-value < 0.01), reflecting cellular damage associated with bleeding manifestations.

Table 2 Comparison of mean CRP and LDH levels at baseline and at 24 hours with grade of envenomation and bleeding manifestations

		CRP (mg/L)			LDH (U/L)		
		At baseline	At 24 hours	p-value	At baseline	At 24 hours	p-value
		Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)	
Grade of envenomation	None	4.8 (1.2)	4.5 (1.9)	0.75	211.6 (111.4)	265.5 (168.7)	0.17
	Mild	5.7 (8.7)	8.9 (9.6)	0.16	267.2 (78.6)	305.1 (116.5)	0.024*
	Moderate	5.6 (4.0)	15.7 (9.7)	<.01**	277.6 (77.9)	479.0 (271.2)	<.01**
	Severe	10.3 (5.8)	48.5 (35.8)	<.01**	585.1 (123.4)	828.2 (173.4)	<.01**
	p-value	< .01	< .01		< .01	< .01	
Bleeding manifestations	No	4.5 (1.3)	6.9 (5.7)	0.114	252.1 (81.2)	296.8 (133.2)	0.42
	Yes	7.1 (8.4)	20.2 (23.8)	<.01	351.8 (135.4)	594.3 (176.5)	<.01
	p-value	< .01	< .01		< .01	< .01	

Table 3 and figure 1 indicates the outcome of receiver operating characteristic (ROC) curve analysis that was conducted to assess the utility of CRP (C-reactive protein) and LDH (lactate dehydrogenase) levels at 24 hours as predictive biomarkers for bleeding manifestations in snakebite cases. The results revealed that both CRP and LDH exhibited statistically significant discriminative power, as indicated by their respective area under the curve (AUC) values of 0.706 and 0.712. These AUC values, which fall between 0.5 (no discriminative power) and 1.0 (perfect discrimination), signify that both biomarkers have moderate predictive ability.

Furthermore, the ROC analysis identified optimal cut-off values for CRP (> 5 mg/L) and LDH (> 300 U/L) at 24 hours. For CRP, a cut-off value of > 5 mg/L yielded a sensitivity of 75.0% and specificity of 54.0%, meaning that it correctly identified 75.0% of patients with bleeding manifestations while correctly identifying 54.0% of those without. Similarly, LDH at 24 hours with a cut-off > 300 U/L exhibited a sensitivity of 77.3% and specificity of 50.0%. These results suggest that both CRP and LDH levels at 24 hours hold promise as predictive indicators for bleeding manifestations in snakebite cases.

Table 3. Receiver operative characteristic curve and optimal cut-off of CRP and LDH levels for prediction of bleeding manifestations

Area under the curve: Bleeding Manifestations					
				Asymptotic 95% CI	
Test Result Variable(s)	Area	SE	p-value	Lower Bound	Upper Bound
CRP at 24 hours	0.706	0.062	0.004	0.585	0.827
LDH at 24 hours	0.712	0.061	0.003	0.591	0.832
Ideal Cut-off	Sensitivity	Specificity			
CRP> 5 mg/l	75.0%	54.0%			
LDH > 300 U/L	77.3%	50.0%			

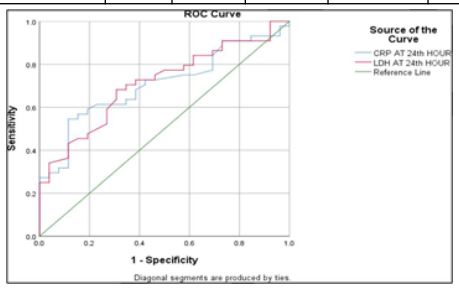

Figure 1 ROC Curve

DISCUSSION:

Snake bites are a highly dangerous form of bio-weaponry found in nature, capable of causing local and systemic complications such as neurotoxicity or hematotoxicity. This medical emergency is particularly prevalent in tropical countries like India, presenting a significant occupational hazard. Envenomation primarily affects the nervous system, kidneys, heart, lungs, liver, blood coagulation system, vascular endothelium, and can have local effects at the site of the bite (3). Snake bites, whether hemotoxic, neurotoxic, myotoxic, or a combination of these, are considered grave medical emergencies necessitating immediate identification and treatment. In this region, the majority of snakebite patients exhibit hematotoxic symptoms. Detecting the degree of envenomation upon presentation poses a challenge, and the identification of novel markers for this purpose could guide treatment and prognosis. The objective of this study was to investigate the relationship between serum CRP and LDH levels and hematotoxicity, and to determine their potential utility as markers of hematotoxicity in snakebite victims.

The study involved 70 cases with a reported history of snakebite. Serum CRP and LDH levels were assessed upon admission and again 24 hours later. Following investigations and examinations, cases were categorized into groups based on the absence or presence of envenomation (no, mild, moderate, and severe) and the presence or absence of bleeding manifestations.

Baseline Data: The average age of the study subjects was 42.17 years, with half falling in the active age group of 21-40 years. Males predominated in this study, comprising 81.4% of the cases, while females constituted 18.6%. The most common site of snakebite was the right lower limb (35.7%), followed by the left lower limb (31.4%). Comparable findings regarding age and gender distribution have been reported in previous studies, demonstrating consistency with our study. (13,14)

Envenomation and Bleeding Manifestations: The study revealed mild to moderate envenomation in 47.1% and 32.9% of cases, respectively, while severe envenomation was observed in 11.4%. Bleeding manifestations were reported in 62.9% of cases. These findings are in line with observations from other studies (10,13,14), which also noted a predominance of hemotoxic envenomation and bleeding manifestations in snakebite victims.

Role of CRP: Serum CRP levels were significantly higher in cases with bleeding manifestations both at baseline (7.1 vs. 4.5 mg/l; p<0.01) and at 24 hours (20.2 vs. 6.9 mg/l; p<0.01). Moreover, CRP levels were significantly elevated in cases with severe envenomation compared to mild or moderate envenomation (10.3 vs. 5.56 mg/L; p<0.01). The increase in CRP levels correlated with the severity of envenomation, with the highest rise observed in cases with severe envenomation (10.3 to 48.5 mg/L; p<0.001). Raised CRP levels were found in 100% of cases with severe envenomation compared to 87% and 63.6% in cases with moderate to mild envenomation, and 33.33% of cases without signs of envenomation (p<0.01). Elevated CRP was seen in 86.4% of cases with bleeding manifestations compared to 50% of cases without bleeding manifestations (p<0.01). The ROC curve analysis showed that CRP (>5 mg/l) was a significant predictor of bleeding manifestations after snakebite, with an AUC of 0.706 (95% CI – 0.585-0.827; p<0.01), and sensitivity and specificity of 75% and 54%, respectively. Other studies (9,10,11) have also demonstrated the potential of CRP as a marker for envenomation severity and prognosis in snakebite cases.

Role of LDH: Mean LDH levels were significantly higher in cases with severe envenomation compared to mild or moderate envenomation (585.1 vs. 271.3 U/L; p<0.01). By 24 hours, LDH levels had increased in all cases, with the most significant rise observed in cases with moderate (277.6 to 479.0 U/L; p<0.001) and severe envenomation (585.1 vs. 828.2 U/L; p<0.001). Elevated LDH levels were seen in 100% of cases with severe envenomation compared to 95.7% and 66.7% in cases with moderate to mild envenomation, and 50% of cases without signs of envenomation (p<0.01). Furthermore, raised LDH levels were observed in 90.9% of cases with bleeding manifestations compared to 61.5% of cases without bleeding manifestations (p<0.01). According to the ROC curve, LDH (>300 U/L) was a significant predictor of bleeding manifestations after snakebite, with an AUC of 0.712 (95% CI - 0.591 – 0.832; p<0.01), and sensitivity and specificity of LDH was 77.3% and 50% respectively. Other studies (13,14,15) have also demonstrated the importance of LDH levels as a marker for envenomation severity and bleeding manifestations in the patients with snakebite.

CONCLUSION:

From our study, it can be concluded that serum CRP and serum LDH levels have substantially difference in the snakebite patients at the time of admission and 24 hours after the admission in various grades of envenomation. It was also found that these two were good predictors of bleeding manifestations after snakebite.

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