Study Of C-Reactive Protein Levels In Vasculotoxic Snake Bite Patients

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KEYWORDS	ABSTRACT
hemolysis, viperoid species, vasculotoxic snake bite, c –reactive protein (crp), envenomation .	**Introduction-** Snakebites pose frequent medical emergencies and represent a significant occupational hazard in India, particularly in rural and suburban areas where agriculture is a major source of employment. The World Health Organization (WHO) estimates a global annual incidence of 5.4 million snakebites, with approximately 2.7 million being venomous and 5% resulting in mortalities. Snakebites, whether vasculotoxic, myotoxic, neurotoxic, cytotoxic, or a combination, are considered medical emergencies with consequences ranging from local tissue injury to multi-organ dysfunction. The occurrence of hematotoxic snake envenomation is primarily attributed to two Viperoid species: Russell's viper and the Saw-scaled viper (Echis carinatus). Vasculotoxic snake envenomation lead to necrotoxic, neurotoxic, and hematotoxic harm to the body, the mechanisms through which vasculotoxic snakebites contribute to illness. **Aim-**To find out the relation between C-reactive protein (CRP) predicting the severity in snake bite (envenomation) patients **Objectives-** To study the role of C-reactive protein (CRP) plasma levels in cases of snake envenomation. To study grades of envenomation based of serum CRP levels. Predicting the severity of snake bite (envenomation) victims using serum CRP levels. To study serum CRP as prognostic indicator. **MATERIAL AND METHODOLOGY-** This will be a single center hospital-based prospective observational study conducted in patients admitted to the Krishna hospital under medicine wards and ICU over a period of 18 months from 2 nd September 2022 to 29 th February 2024. **RESULT-**The grade of envenomation in relation to CRP (C-reactive protein) levels shows distinct patterns across different severity categories. In cases with no envenomation, CRP was positive in 4.55% of cases compared to 20.59% where it was negative, with a significant.For mild envenomation, 25.76% had elevated CRP levels versus 38.24% without positive CRP. In moderate cases, 42.42% exhibited elevated CRP levels compared to 35.29% without elevation. Severe envenomation showed positive CRP in 27.27% of cases, contrasting with only 5.88% where CRP was negative. Outcomes based on CRP (C-reactive protein) positivity were analysed, revealing a significant association with mortality in snakebite envenomation cases.Cases where CRP was positive, 22.73% resulted in death, compared to only 2.94% in cases where CRP was negative. **CONCLUSION-**Analysis of C-reactive protein levels revealed that 84% of study group survived, while 16% did not. Among cases where CRP levels were positive, the mortality rate was markedly higher as compared to cases with normal CRP levels. Positive CRP levels were significantly associated with severe envenomation. The CRP **Categories:** Forensic Medicine, Internal Medicine .

Introduction

Snakebites pose frequent medical emergencies and represent a significant occupational hazard in India, particularly in rural and suburban areas where agriculture [1] is a major source of employment. The World Health Organization (WHO) estimates a global annual incidence of 5.4 million snakebites, with approximately 2.7 million being venomous and 5% resulting in mortalities. [2]

Snakebites, whether vasculotoxic, myotoxic, neurotoxic, cytotoxic, or a combination, are considered medical emergencies with consequences ranging from local tissue injury to multi-organ dysfunction. The occurrence of vasculotoxic snake bites is primarily attributed to two Viperoid species [3]: Russell's viper and the Saw- scaled viper (Echis carinatus).Vasculotoxic snakebites lead to necrotoxic, neurotoxic, and hematotoxic harm to the body, the mechanisms through which vasculotoxic snakebites contribute to illness remain unknown [4].

Complications arising from snake bites can be broadly classified into two primary categories depending on the location of manifestation: local and systemic.Some local complications linked to vasculotoxic snake bites includes necrosis and cellulitis [4]. Meanwhile, systemic complications involve coagulopathy, acute renal failure (ARF), and hemolysis [5,6]. Early manifestations include local pain, swelling, bleeding from the bite site, local necrosis, and cellulitis [7].

Identifying the severity of envenomation is challenging, and the utilization of novel markers proves to be a valuable [8]. There is a suspicion that snake venom acts as an acute-phase reactant, triggering the release of inflammatory mediators such as Interleukin-6 (IL-6) and IL-8. This cascade may lead to increased production of acute-phase reactants, including C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), serum amyloid, and haptoglobin, while reducing negative acute-phase proteins like albumin [9].

C-reactive protein (CRP) is a plasma protein that exhibits strong phylogenetic conservation and high resistance to proteolysis. It is primarily synthesized in the liver in response to proinflammatory cytokines, notably IL-6, IL-1β, and TNF. Elevated CRP levels can occur due to diverse inflammatory processes, including infection, collagen diseases, inflammatory conditions, as well as necrosis following myocardial infarction, acute pancreatitis, trauma, and specific types of cancers [10]. Also, elevated serum CRP levels are observed in cases of mild-to-severe envenomation, indicating its potential as a biomarker for both the diagnosis and prognosis of snakebite cases. [11]

Serum C-Reactive Protein (CRP) is widely utilized in both basic and clinical research, as well as in clinical practice. Biomarkers, like CRP, play a crucial role in enhancing our understanding of complex molecular mechanisms. [12] CRP has the ability to improve diagnostic accuracy, predict clinical outcomes, aid in patient selection for clinical trials, monitor disease progression, and identify new treatment targets. Nevertheless, the bleeding tendency induced by viperine envenomation can only be effectively counteracted through the administration of snake antivenom. [13]

Despite the widespread availability of anti-snake venom, a significant number of snakebite victims succumb to complications. Swift administration of Anti-Snake Venom (ASV) is essential to neutralize the venom and restore normal coagulation, along with addressing other signs of envenomation. [14] Therefore, the study aims to investigate the correlation between C-reactive protein levels and the prediction of severity in vasculotoxic snake bite patients.

Materials And Methods

This will be a single center hospital-based prospective observational study conducted in IPD patients and patients admitted to the Krishna hospital under medicine wards and ICU over a period of 18 months from 2nd September 2022 to 29th February 2024.

Study Design: This was a prospective observational study.

Study Period: The study was conducted from 2nd September 2022 to 29th February 2024.

Study Setting: A tertiary care hospital and a teaching institute in Western Maharashtra. Patients admitted in the wards and ICU in the Department of General Medicine at Krishna Institute of Medical Sciences, a tertiary care teaching institute and research center, Karad, Maharashtra.
Sample size: 100

 Inclusion criteria: All patients aged over 18 years presenting with features suggestive of snakebite (envenomation) or having a confirmed snakebite (envenomation) were considered in the study.

Exclusion criteria:

·   Patients other than snake bite.

·   Patients administered ASV prior to hospital admission.

·   Patients with Co-morbidities.

. Patients with malignancy Methodology:

Patients with a history of snakebite underwent the laboratory tests. These included a haemoglobin, prothrombin time-INR, activated partial thromboplastin time (APTT), bleeding time(BT),clotting time (CT),serum creatinine, random blood sugar level, Liver function test (LFT), whole blood clotting test (WBCT),serum CRP.

All tests were conducted at admission and wbct repeated after 24 hours.

Clotting time, bleeding time, and a 20-minute whole blood clotting test were assessed at initial 24 hours of hospital admission and whole blood clotting test after 24 hours later.

Dry bites were characterized as instances where patients reported a history of snakebite but exhibited no signs or symptoms of local or systemic envenomation, nor did they display any laboratory abnormalities even after 24 hours of observation in the hospital. Subsequently, patients were categorized into groups of none, mild, moderate, and severe envenomation based on a predetermined scale.

Results:

TABLE 1: Gender distribution in the study groups

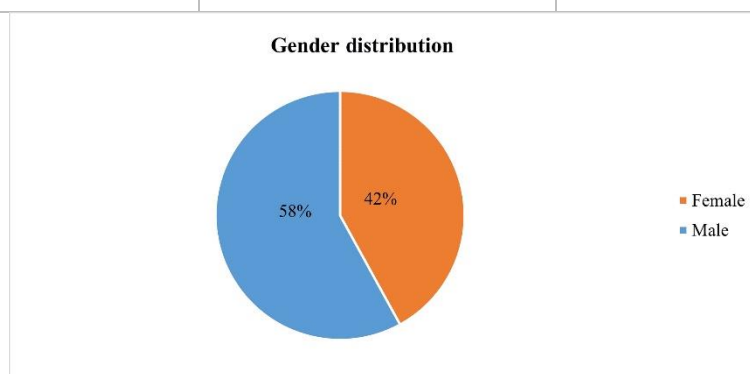| Gender	Cases	Percent
Female	42	42%
Male	58	58%
Total	100	100%

FIGURE 1: Graphical representation of gender distribution in the study groups

In examining the gender distribution within the study groups investigating C-reactive protein (CRP) levels and severity prediction in snake bite patients, it was found that out of a total of 100 participants, 58 were male (58%) and 42 were female (42%).

Age group	No of cases	Percent
18-40	36	36%
41-50	20	20%
51-60	13	13%
>60	31	31%
Total	100	100%
Age (Mean ± SD)	51.18 ± 19.89	

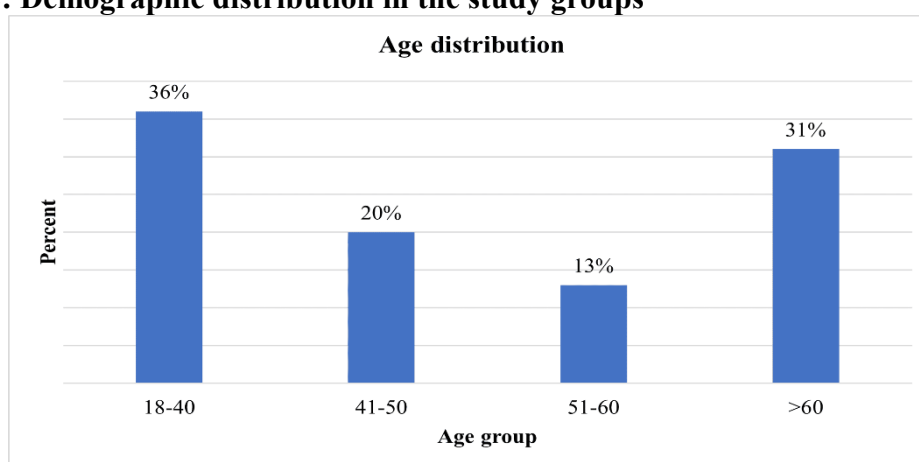**TABLE 2: Demographic distribution in the study groups**

FIGURE 2: Graphical representation of demographic distribution in the study groups

In a study investigating the relationship between C-reactive protein (CRP) levels and predicting severity in snake bite patients, the mean age of participants was 51.18 years with a standard deviation of 19.89 years.

Haematological parameters	No of cases	Percentage	P-value
HB<10	40	40%	0.004
Prothrombin (>15 sec)	69	69%	<0.0001
INR (>1.5)	60	60%	0.004
APTT (>30 sec)	70	70%	<0.0001
BT	35	35%	<0.0001
CT	41	41%	0.01
Creatinine	59	59%	0.01
R. BSL	36	36%	0.0001
S.T Bilirubin	39	39%	0.001
Direct bilirubin	58	58%	0.02

Indirect bilirubin	33	33%	<0.0001
SGOT	43	43%	0.04
SGPT	59	59%	0.01
ALP	63	63%	0.0002
Serum Albumin	69	69%	<0.0001
Globulin	30	30%	<0.0001
Total protein	42	42%	0.02
Platelet (<100000)	20	20%	<0.0001

TABLE 3: Haematological parameters in envenomed patients in the study group
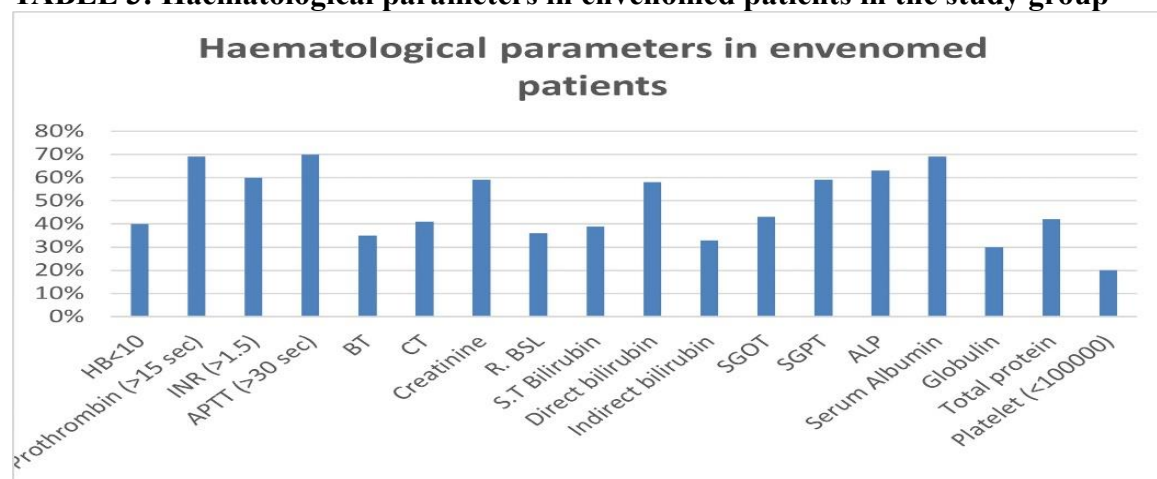

FIGURE 3: Graphical representation of haematological parameters inenvenomed patients

Significant findings included high values of severe haematological abnormalities such as low haemoglobin (<10 g/dl) in 40% of cases, prolonged clotting times (PT and APTT) in over 60% of patients, and elevated liver enzymes and bilirubin levels. Elevated creatinine levels indicated renal involvement, while decreased albumin, globulin, and total protein levels suggested disrupted protein metabolism. Platelet counts below 100,000/mm³ in 20% of cases indicated coagulation issues.
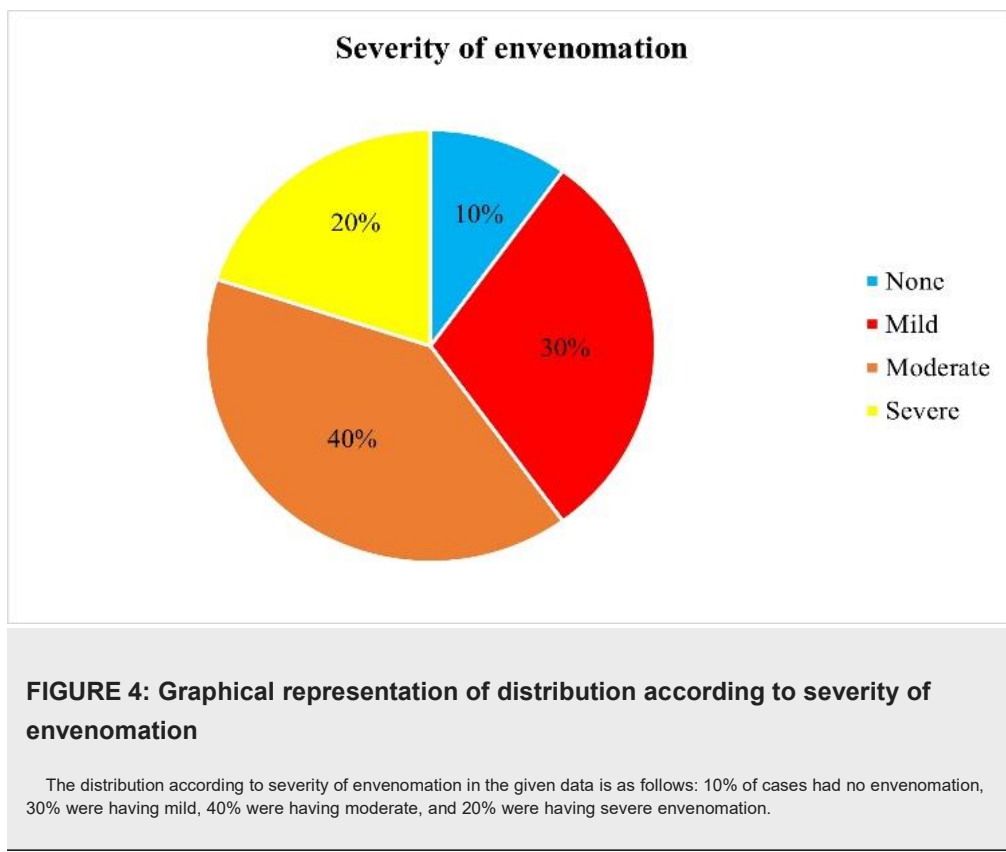
FIGURE 4: Graphical representation of distribution according to severity of envenomation

The distribution according to severity of envenomation in the given data is as follows: 10% of cases had no envenomation, 30% were having mild, 40% were having moderate, and 20% were having severe envenomation.

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Severity of envenomation	CRP POSITIVE		CRP NEGATIVE		Chi- Square test	P-value
	No of cases	Percent	No of cases	Percent		
None	3	4.55%	7	20.59%		
Mild	17	25.76%	13	38.24%		
Moderate	28	42.42%	12	35.29%	12.35	
						0.0004
Severe	18	27.27%	2	5.88%		
Total	66	66.00%	34	34.00%		

TABLE 4: Relation of CRP levels and Severity of envenomation
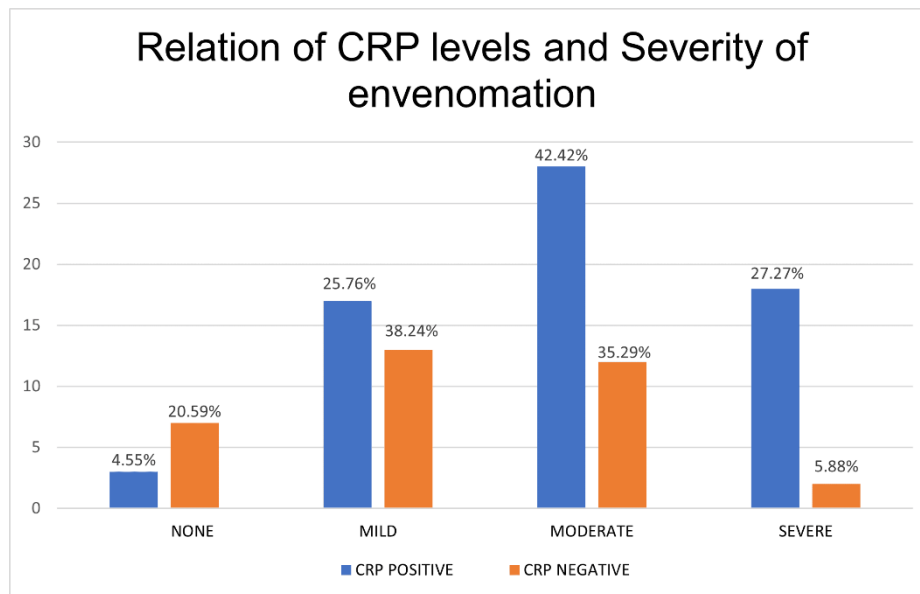The grade of envenomation in relation to CRP (C-reactive protein) levels shows distinct

FIGURE 5: Graphical representation of relation of CRP levels and Severity of envenomation

patterns across different severity categories. In cases with no envenomation, CRP was elevated in 4.55% of cases compared to 20.59% where it was not elevated, with a statistically significant P-value of 0.0004. For mild envenomation, 25.76% had elevated CRP levels versus 38.24% without elevated CRP. In moderate cases, 42.42% exhibited elevated CRP levels compared to 35.29% without elevation. Severe envenomation showed elevated CRP in 27.27% of cases, contrasting with only 5.88% where CRP was not elevated.

Severity of envenomation	WBCT at admission	WBCT after 24 hour
	Mean ± SD	Mean ± SD
None	9.2 ± 7.74	23 ± 2.58
Mild	15.3 ± 9.07	23 ± 2.58
Moderate	24.87 ± 2.79	23.45 ± 2.87
Severe	23.4 ± 10.03	25.12 ± 2.91

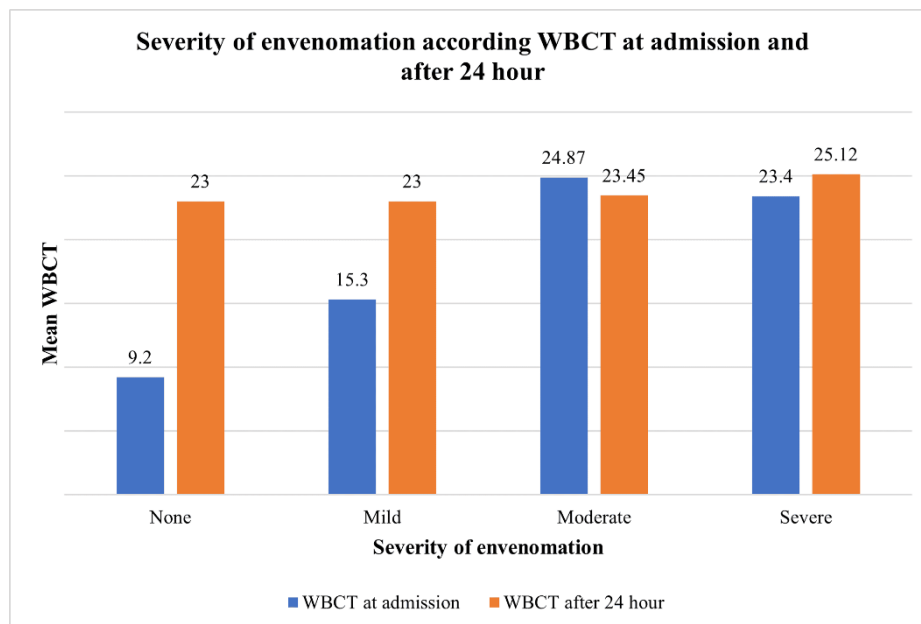**TABLE 5: Severity of envenomation according WBCT at admission and after 24 hours**

FIGURE 6: Graphical representation of severity of envenomation according WBCT at admission and after 24 hour

Outcome	No of cases	Percentage
Survived	84	84.00%
Not survived	16	16.00%

TABLE 6: Distribution according to outcome in the study groups
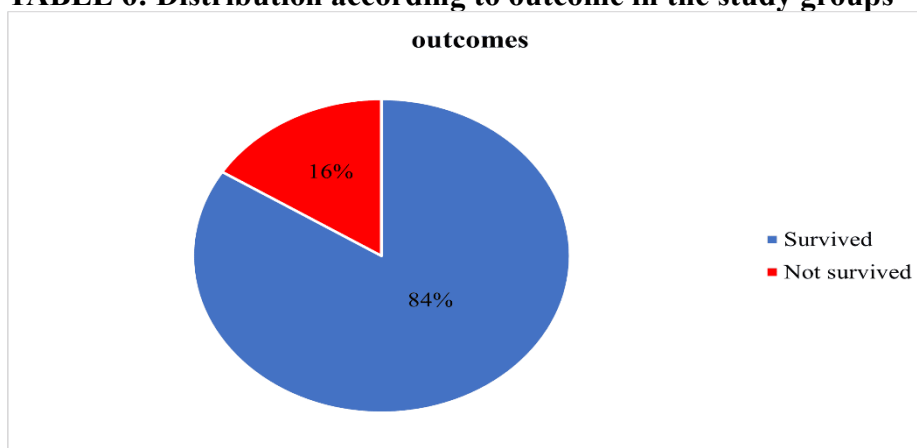

FIGURE 7: Graphical representation of distribution according to outcomes in the study groups

In the study focusing on snake bite severity and C-reactive protein (CRP) levels, the outcomes of the participants were analysed to understand the overall impact of the condition. Out of a total of 100 cases studied, 84 patients survived, representing 84.00% of the group. While 16 patients did not survive, constituting 16.00% of the total cases.

Outcome	CRP positive		CRP Negative		Chi-square	P-value
	No of cases	Percent	No of cases	Percent		
Not Survived	15	22.73%	1	2.94%		
Survived	51	77.27%	33	97.06%	6.54	0.01
Total	66	66%	34	34%		

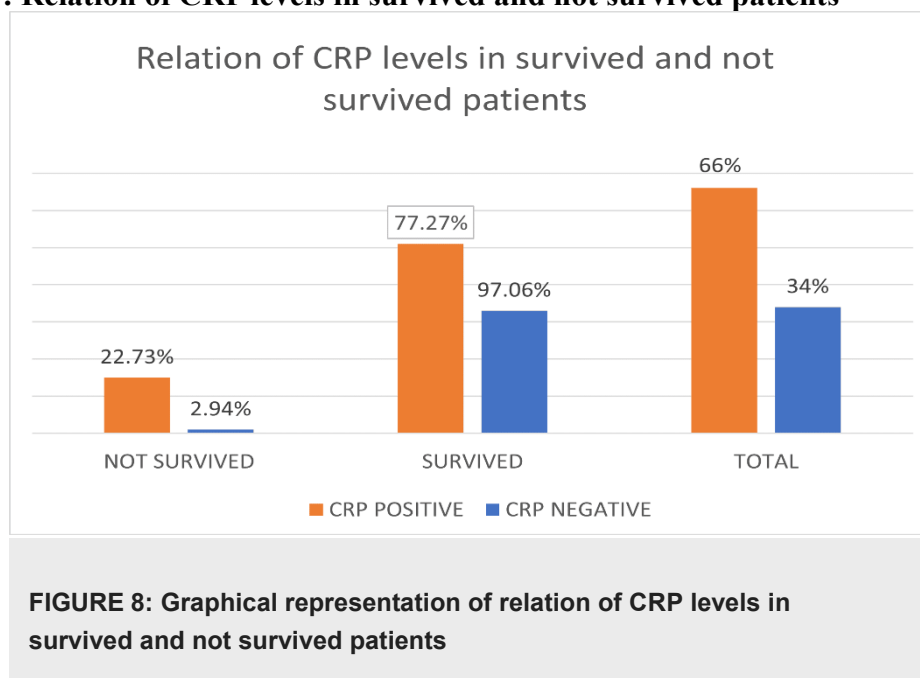**TABLE 7: Relation of CRP levels in survived and not survived patients**

FIGURE 8: Graphical representation of relation of CRP levels in survived and not survived patients

Outcomes based on CRP (C-reactive protein) positivity were analysed, revealing a significant association with mortality in snakebite envenomation cases. Among cases where CRP was positive, 22.73% resulted in death, compared to only 2.94% in cases where CRP was negative.

Discussion

Snake bites present clinically dynamic challenges, where apparently minor bites can escalate into severe, complex situations. Many components found in snake venom, such as PLA (phospholipase A), serine proteases, metalloproteinases, lectins, l-amino-acid oxidases, bradykinin potentiating factors, natriuretic factors, and integrin, are known to induce various pharmacological actions. [15] These actions include neurotoxicity (affecting the nervous system), myotoxicity (damaging muscle tissue), cytotoxicity (causing cell damage), hemotoxicity (affecting blood cells), and antimicrobial activity. These diverse effects contribute to the complex clinical manifestations observed in snakebite envenomation.

Studies reported that, serum LDH (lactate dehydrogenase) and CRP (C-reactive protein) serve as markers of hemolysis in cases of venomous snakebites, which trigger acute phase reactions characterized by pathophysiological changes. These changes include moderate leukocytosis with neutrophilia, lymphopenia, and eosinophilia, alongside elevated LDH and C-reactive protein levels, and decreased total proteins, erythrocyte sedimentation rate, and albumin. Findings from an in silico study propose that serum LDH and CRP-1 activities could potentially serve as useful biomarkers for diagnosing and prognosticating snakebite cases. Elevated LDH levels following snakebites are indicative of hemolysis, which can lead to acute renal failure. Additionally, snake venom induces inflammatory responses in vascular smooth muscles and

alters CRP levels, contributing to elevated CRP levels observed in hematological analyses of snakebite patients.

The current study included participants across various age groups, 36% of the cases were aged 18-40 years, 20% were aged 41-50 years, 13% were aged 51-60 years, and 31% were over 60 years old. Similar findings were observed in another study conducted by Gore BP, 28.3% of the participants were aged 18-40 years, 31.7% were aged 41-50 years, 11.7% were aged 51-60 years, and 28.3% were above 60 years old. This distribution shows that the highest proportion of participants fell within the 41-50 age group, while the 18- 40 and above 60 age groups were equally represented. The smallest proportion of participants was in the 51- 60 age group.

In current investigation, the participants had a mean age of 51.18 years. This correlated with other studies done by Suchithra N et al. [11], Monteiro FN et al., and Ferhat Icme et al. where the mean age was 40, 40.7 and 37 years respectively. In contrast Mahmood K et al. the mean age of presentation was 33.3 years.

The results of the present study were in parallel with the study conducted by Kulkarni S and Sreedevi, T. A.[16] who found 50% of the snake bite victims with decreased serum albumin level showed severe grade of envenomation with p value less than 0.001 which is statistically significant. Bhasker S et al. found reduction in platelets count.

The gender distribution in the present study revealed that, out of 100 participants, 58% were male and 42%

were female. Similarly, a study by Mahmood K et al., found that 80% of the patients were male, reflecting a 4:1 male-to-female ratio. Another study by Chube SP et al., reported that 56% of the patients were male and 44% were female. Also, Nisar [17] et al. out of 65 snakebite patients admitted over one year, 40 patients (61.5%) were male and 25 (38.5%) were female. These findings underscore that men are more frequently affected by snake bites than women, likely because men often work in fields during the day where snake encounters are more common.

In the present research, significant findings revealed a high prevalence of severe hematological abnormalities, including low hemoglobin levels (<10 g/dl) in 40% of cases and prolonged clotting times (PT and APTT) in over 60% of patients. Additionally, elevated liver enzymes and bilirubin levels were observed. Renal involvement was indicated by elevated creatinine levels (59%), while decreased albumin (69%), globulin (30%), and total protein levels (42%) suggested disrupted protein metabolism. Coagulation issues were evidenced by platelet counts below 100,000/mm³ in 20% of cases.

Envenomation leads to prolonged prothrombin time due to decreased levels of fibrinogen and factors V, VIII, and X. This results in a prolonged activated partial thromboplastin time (APTT) and an increased international normalized ratio (INR). [18]

The grade of envenomation in relation to CRP (C-reactive protein) levels shows distinct patterns across different severity categories. In cases with no envenomation, CRP was elevated in 4.55% of cases compared to 20.59% where it was not elevated, with a statistically significant P-value of 0.0004. For mild envenomation, 25.76% had elevated CRP levels versus 38.24% without elevated CRP. In moderate cases, 42.42% exhibited elevated CRP levels compared to 35.29% without elevation. Severe envenomation showed elevated CRP in 27.27% of cases, contrasting with only 5.88% where CRP was not elevated.

Wbct and severe envenomation both at admission and 24 hours later (p value 0.0001).

Overall, 84% of the patients survived, while 16% did not. A significant association between CRP positivity and mortality was observed, with 22.73% of CRP-positive cases resulting in death compared to 2.94% of CRP-negative cases. This study highlights the importance of CRP as a prognostic indicator in snakebite envenomation cases.

While CRP was investigated as a marker for severity and outcomes, the study did not explore other potential biomarkers or their interactions. Future studies could benefit from examining a broader panel of biomarkers to better understand their combined predictive value.

The study primarily focused on mortality as an outcome measure. Future studies could consider additional clinical endpoints such as long-term sequelae, quality of life measures post-discharge, or functional recovery.

Conclusions

Based on the study findings, the majority of participants were in the 41-50 age group. Significant observations included a high prevalence of severe hematological abnormalities, such as low hemoglobin levels, prolonged clotting times (PT and APTT) and low platelet count in patients. Elevated liver enzymes and bilirubin levels were also noted. Analysis of C-reactive protein (CRP) levels revealed that 84% of the study group survived, while 16% did not. Among cases where CRP levels were positive, the mortality rate was notably higher as compared to cases with normal CRP levels. Positive CRP levels were significantly associated with severe envenomation. The CRP levels were identified as potential markers for hemotoxicity in snakebite victims, suggesting their utility in predicting prognosis and guiding treatment decisions.

Additional Information

Disclosures

Human subjects: Consent for treatment and open access publication was obtained or waived by all participants in this study. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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