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Original article

Snake bite: Biochemical changes in blood after envenomation by viper and cobra

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Abstract

Snake bite poisoning is a significant cause of mortality and morbidity in tropical and sub-tropical countries like India. The present study was taken up to evaluate the biochemical changes in snake bite cases in different time periods. The clotting time (C.T) was 55.83±38.5 in viper bite cases, much higher than in controls, 5.07±1.33, which was normalized after anti venom administration; however no significant changes were observed in cobra bite cases. Thus evaluation of C.T. may help to differentiate viper bites from cobra bites and to choose specific monovalent anti-venom treatment. The blood urea level in viper bite cases increased significantly after the sixth hour: 58.19±27.6 mg% in cases; 25.80±4.9mg% in controls. Since anti-venom does not decrease the blood urea to normal, dialysis is required for normalization of urea level. Blood creatinine level in the majority of viper bite cases was found to be increased (1.60–7.4 mg%) after the sixth hour, where as in cobra bite cases it was found only in 9% (1.5-2mg%); this increased creatinine level in viper bite cases caused the renal failure. Sodium and potassium levels were not increased in both cobra and viper bite cases, up to the fourth day. However, in 50% of viper bite cases, significant elevation in sodium level was observed on 5th and 6th day, due to the secondary effect of renal failure. 50% of the viper and cobra bite cases showed rise in potassium level on the sixth day which ranged between 5.1– 4.14 mEq/litre. No significant difference was observed in serum calcium level between viper and cobra bite cases. In the present study, clotting time increases immediately after viper bite, detection of which within six hours is a good indicator of envenomation by viper bite. The other biochemical parameters would be helpful to assess the severity of renal failure predominant after six hours of envenomation.

Key words: snake bite, serum calcium, serum creatinine, sodium level, potassium level

Snake bite poisoning is a significant cause of mortality and morbidity in tropical and sub-tropical countries[1]. Being a tropical region, India has a wide variety of poisonous and non-poisonous snakes[1,2]. Figures of mortality in India are 15,000 per annum; however these statistics are not accurate mainly because most snake bite incidents take place in villages and forest areas

and all the victims do not reach hospitals. In western countries, fewer snake bite deaths are reported. In England, there were only 14 deaths due to snake venom poisoning in the past hundred years. Snakes are poikilothermic vertebrates[3,4]. Poisonous snakes have two specialized teeth in the upper jaw called fangs which are larger than the rest. These have grooves or venom channels either on the inside or by the sides. Snakes belong to the class Reptilia under order Ophidia, in 5 Families - Elapidae, Viperidae, Hydrophidae, Crotalidae, Colubridae. It is estimated that out of 3000 species of snakes 375 are poisonous. Some of the common species like Russell's viper, saw scaled viper, cobra and krait are predominant in Kerala[5].

Snake venom is considered to be one of the most highly developed and complicated of all toxins produced by plants and animals[6]. It is a complex mixture containing peptides, polypeptides, enzymes, glycoproteins and other substances, capable of several pharmacological activities[7]. The more lethal venom fractions of snake venoms appear to be certain non-enzymatic proteins. In addition, snake venoms contain inorganic substances including metals like sodium, calcium, magnesium, zinc and small amounts of Iron[2]. Some snake venom, contains carbohydrates (glycoprotein)[7], lipids and biogenic amines while other venom contain free amino acids. Snake venom consists of at least 26 enzymes although no single venom contains all of these enzymes. At least 10 enzymes are found in most of the venoms, while the remaining are scattered throughout the venoms of the five families of poisonous snakes. Elapid venoms are rich in acetyl cholinesterase, while crotalid and viperoid venoms lack this enzyme but are rich in endopeptidase[4]. The important enzymes in snake venom includes proteolytic enzymes, thrombin like enzymes, argenine ester hydrolase, collagenase, hyaluronidase, phospholipase A2, phospholipase B, phospholipase C, lactate dehydrogenase, phosphomonoesterase, phosphodiesterase, acetylcholinesterase, RNase, DNase, 5'–nucleotidase[7].

Snake venom mainly affects the cardiovascular, nervous, renal and respiratory systems[8]. Generally snake venom is classified into two, hemotoxic and neurotoxic. Due to the high concentration of neurotoxins in the venom of elapid family, the usual clinical manifestations are neuromuscular paralysis, ptosis, opthalmoplegia and bulbar paralysis. On the other hand the viper venom produces shock, hemorrhage and disseminated intravascular coagulation[8-11].

One of the most effective treatments of snake venom poisoning is intravenous infusion of polyvalent anti-venom, with infusion of antihistamine prior to anti-venom infusion for avoiding possible anaphylactic reactions. Jacob suggested that in patients with minimal envenomation, 20 ml anti-venom is sufficient to reverse the venom toxicity, but in serious bites 40ml anti-venom should administered initially[12]. The further requirement of anti-venom in viper bites can be judged by clotting time, other laboratory parameters and clinical observations. In the case of elapid bites, it is based on clinical observation. So anti-venom should be continued till the neuroparalytic signs are cleared[12].

The biochemical changes after envenomation being of vital importance, our study aims to investigate the biochemical parameters such as blood urea, creatinine, sodium, potassium, calcium levels, and clotting time, in human blood, resulting from the bites of viper and cobra within six successive days.

Materials and methods

Blood biochemistry of 32 patients of snake bite was analyzed. Among these, 21 were viper bite and 11 were cobra bite cases, which were admitted in Calicut Medical College Hospital, Kerala.

Sample collection

The first blood sample from each case was taken as soon as possible after the bite (generally between 2-6hrs). Further samples were collected from each patient at different intervals such as 22-26hr, 46-50 hr, 70-74 hr, 94-98 hr and 118-122 hrs. 15 normal persons were taken as control.

Determination of blood clotting time

As we know, whole blood when removed from the vascular system and exposed to a foreign surface will form a solid clot. The time required for the formation of the solid clot is a measure of the clotting time[13,14]. Clotting time (C.T.) was determined by Lee and White's method.

Estimation of blood urea

The procedure was done according to Berthelot et al with modifications introduced by Fawcett and Scott[13,14]. This method uses urease to hydrolyse urea to produce ammonia and carbon dioxide. The ammonia generated reacts with alkaline hypochlorite and sodium salicylate in the presence of sodium nitroprusside to form a colored chromaphore. The intensity of the color produced is proportional to the amount of urea in the specimen.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + 2CO_2 \quad \text{.....(1)}$$

$$NH_4 + 5NaOCl + 2\ \text{Salicylate} \longrightarrow \text{Indophenol} + 5\ NaCl + 5\ H_2O \quad \text{……(2)}$$

Estimation of serum creatinine

Serum creatinine levels were estimated by the method of Jaffe reaction. Creatinine develops an orange red color when treated with picric acid in the presence of strong alkali. This color is due to the formation of creatinine picrate, which depends on the amount of creatinine present[13,14]. In brief, 0.5 ml serum was taken in a test tube and was placed in a boiling water bath, then cooled and 1.5 ml of distilled water was added slowly along the sides of the test tube. Samples were heated for eight minutes in a boiling water bath, cooled and 3 ml freshly prepared alkaline picrate was added. The mixture was allowed to stand for 15 min. at room temperature and was read at 490 nm.

Estimation of serum sodium and potassium by flame photometry

In this instrument the solution to be tested is passed carefully under controlled conditions as a very fine spray in the air supply to the burner. In the flame, the solution evaporates and the salt dissociates to give neutral atoms. Some of these, though only a very small proportion, move into a high energy state. When these electrons of the atom fall back to their original orbit, they release energy in the form of light, which is used in flame photometry. The most satisfactory dilution to use should be established experimentally. For sodium, this may be 1 in 100, for potassium it is usually lower, often 1 in 50, but it is possible by varying the sensitivity to use the same dilution for both the salts. Sodium and potassium can interfere with each other. Therefore in standard solutions, both the elements are added[14,15].

Estimation of serum calcium

Serum calcium level was estimated using modified OCPC method. At alkaline pH, serum calcium forms a colored complex O-cresophthalene. 8-hydroxy quinoline is included in the reagent as a chelating agent of magnesium ions which can otherwise interfere in the reaction[13,14].

Statistical analysis

Statistical evaluation was done with the multiple range test (Duncan test with significance level of 0.05) for each of the parameters studied.

Results and discussion

Clotting time

There was a marked increase in C.T in the case of viper bites. This increase was observed in all the viper bite patients immediately (2-6 hr.) after the bite. In the present study the C.T is 55.83 ± 38.5 minutes in viper bite cases, where as in the control subjects, it is only 5.07 ± 1.33 minutes. In three cases out of 21 cases studied, the clotting time were prolonged beyond three hours. After 22-26 hours, and thereafter, there was significant reduction of C.T. which came to normal on the fifth day (Table I and Fig 1). This reduction in clotting time may be due to the anti venom administration. In cobra bite cases, a significant rise in C.T was observed only in four cases (36%) 11.55 ± 7.22 minutes which came to normal in the second day after the administration of anti venom (mean C.T. in the 2-6 hours sample is 11.55 ± 7.22), whereas in control the mean value of C.T, is 5.07±1.33. Our results show that one of the major signs in the envenomation of viper venom is the prolongation of the clotting time. Agarwal reported in 2005 & 2006 that there is marked coagulation disorder in viper bite cases which can be controlled by the administration of anti venom[4,16]. The prolongation of clotting time is very high in viper bite cases in comparison to cobra bite cases. So the detection of C.T. may help to differentiate viper bites from cobra bites[1,3,6]. This will help in the treatment by choosing specific monovalent anti-venom, since it is more effective than the polyvalent anti-venom.

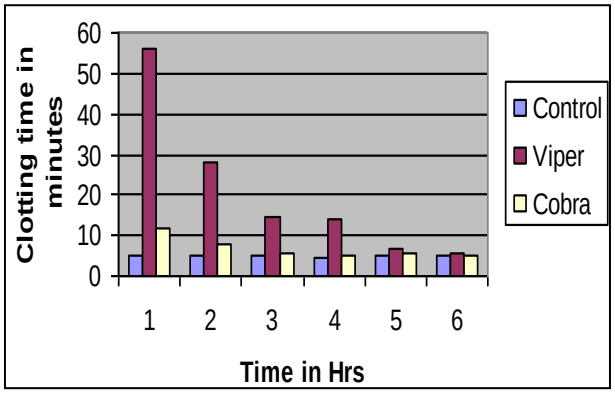

Fig 1. Blood clotting time in control versus snake bite cases

Blood urea and serum creatinine

The blood urea level in viper bite cases showed that the urea level increased significantly after the sixth hour. The increase was observed in 67% of cases which ranged between 50-242 mg% (58.19 ± 27.6) where as in control subjects it was 25.80 ± 4.9 mg% (Table II). In cobra bite cases only 28%

Table I. Blood clotting time (minutes) in snake bite cases

Time (hrs)	Controls	Viper bite cases	Cobra bite cases
1-6	5.00 ± 1.33	55.83 ± 11.20	11.55 ± 7.22
22-26	5.00 ± 1.36	28.21 ± 10.60	7.82 ± 3.76
46-50	5.00 ± 1.56	14.43 ±10.33	5.82 ± 1.33
70-74	4.60 ± 1.35	14.14 ±10.59	5.09 ± 1.14
94-98	4.80 ± 1.37	6.70 ±1.71	5.45 ± 1.30
118-122	5.00 ± 1.31	5.62 ±1.12	5.00 ± 1.60

Values represent mean ± standard deviation

Table II. Concentration of blood urea (mg%) in snake bite cases

Time (hrs)	Controls	Viper bite cases	Cobra bite cases
1-6	25.60 ± 4.54	30.76 ± 5.59	26.36 ±4.41
22-26	25.80 ± 4.90	58.19 ± 27.16	31.27 ±11.97
46-50	25.40 ± 5.40	80.52 ± 41.30	36.45 ± 20.50
70-74	25.53 ± 5.08	97.76 ± 56.20	38.27 ± 23.31
94-98	25.87 ± 4.90	98.76 ± 54.30	39.45 ± 25.80
118-122	25.53 ± 4.81	97.52 ± 52.28	37.36 ± 21.43

Values represent mean ± standard deviation

Table III. Concentration of serum creatinine (mg%) in snake bite cases

Time (hrs)	Controls	Viper bite cases	Cobra bite cases
1-6	0.65 ± 0.10	1.00±0.20	0.91±0.15
22-26	0.62 ± 0.12	1.93±1.32	1.04±0.29
46-50	0.63 ± 0.14	2.99±2.19	0.95±0.36
70-74	0.61 ± 0.10	3.42±2.35	0.88±0.24
94-98	0.61 ± 0.10	3.47±2.20	0.83±0.09
118-122	0.63 ± 0.12	3.47±2.25	0.85±0.12

Values represent mean ± standard deviation

showed increased urea concentration which ranged between 46-92 mg% (31.27 ± 11.97), where as in control, it ranged between 17-32 mg% (25.80 ± 4.90) (Table II). It was noted that the urea level in viper bite cases were not decreased even on the sixth day after the administration of anti-venom (97.52 ± 54.30) (Fig 2). Since anti-venom dose not decrease the blood urea to normal, dialysis is found to be more effective in attaining that. Table III shows the blood creatinine level in viper bite cases, which is found to be increased after the sixth hour that is observed in 67% of the cases. This increase in creatinine level ranged from 1.60 – 7.4 mg%, where as in cobra bite, the blood creatinine level increased only in 9% cases with the value ranging from 1.5- 2mg% (Fig 3).

This increased creatinine level in viper bite cases, suggests that renal failure is the main consequence of envenomation, where as in cobra bite cases it was found that there is no significant renal failure. This may be one of the reasons why dialysis is carried out in the most of the viper bite victims which is found to be effective in lowering the concentration of urea and creatinine.

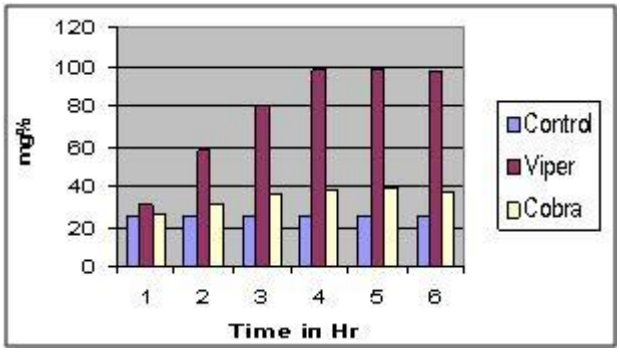

Fig 2. Serum urea level in control versus snake bite cases

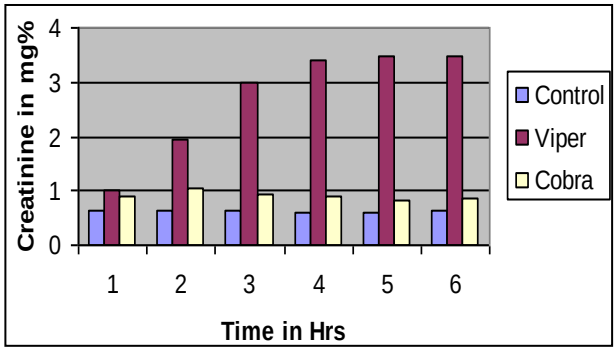

Fig 3. Serum creatinine level in control versus snake bite cases

Table IV. Concentration of serum sodium (mEq/litre) in snake bite cases

Time (hrs)	Controls	Viper bite cases	Cobra bite cases
1-6	137.47 ± 4.24	134.71 ± 6.39	139.09 ± 6.09
22-26	136.80 ± 4.20	138.48 ± 6.90	137.82 ± 6.05
46-50	136.87 ± 4.24	140.43 ± 7.16	137.91 ± 6.88
70-74	136.93 ± 4.22	141.81 ± 6.83	138.55 ± 9.88
94-98	137.27 ± 3.97	142.81 ± 6.97	137.55 ± 10.78
118-122	137.40 ± 4.01	143.90 ± 7.52	137.73 ± 10.42

Values represent mean ± standard deviation

Table V. Concentration of serum potassium (mEq/litre) in snake bite cases

Time (hrs)	Controls	Viper bite cases	Cobra bite cases
1-6	3.95 ± 0.29	4.01 ± 0.51	4.01 ± 0.44
22-26	3.95 ± 0.31	4.42 ± 0.57	4.01 ± 0.36
46-50	3.95 ± 0.28	4.66 ± 0.65	4.03 ± 0.47
70-74	3.98 ± 0.27	4.72 ± 0.79	4.07 ± 0.53
94-98	3.97 ± 0.28	4.87 ± 0.70	4.11 ± 0.52
118-122	3.99 ± 0.28	4.80 ±0.84	4.14 ± 0.58

Values represent mean ± standard deviation

Table VI. Concentration of serum calcium (mg%) in snake bite cases

Time (hrs)	Controls	Viper bite case	Cobra bite cases
1-6	9.43 ± 0.66	9.65 ± 1.98	9.91 ± 1.96
22-26	9.39 ± 0.65	9.89 ± 1.76	9.69 ± 1.79
46-50	9.38 ± 0.63	9.80 ± 1.87	9.66 ± 1.80
70-74	9.40 ± 0.65	9.87 ± 1.84	9.75 ± 1.92
94-98	9.39 ± 0.65	10 ± 1.97	9.65 ± 1.89
118-122	9.44 ± 0.66	9.86 ± 1.89	9.77 ± 1.83

Values represent mean ± standard deviation

Sodium, potassium and calcium levels

Results of sodium estimation showed that there was no significant increase in Na^+ level in both cobra and viper bite when compared to normal control subject up to the fourth day (70-74hrs) (Table IV). However, 50% cases of viper bite showed a significant elevation in sodium level on the 5^{th} and 6^{th} days, but in cobra bite cases there was no significant increase with respect to control (Table IV). This increase may be due to the secondary effect of renal failure.

Results of potassium estimation showed that there was no significant increase in both viper and cobra bite cases. However, 50% of the viper bite cases showed rise in potassium level on the sixth day which ranged between 5.1 – 4.14 mEq/liter which came within the normal range. Almost similar results were obtained in the cobra bite cases, where only 17% showed rise in the potassium level. But the mean of the samples were within the normal range (Table V). This slight possible increase may be due to the secondary effect of renal failure.

Serum calcium levels show that there is no significant difference between viper and cobra bite cases and this value is comparable with that of normal during various periods of envenomation (Table VI). So the prolongation of clotting time in viper bite cases may not be due to the lowering of serum calcium concentration and calcium is not a valuable indicator for accessing the severity of snake bite cases.

Among the six parameters which were analyzed, only clotting time increases immediately after bite. The other parameters showed an increase only after six hours of envenomation. In the present study we report that the detection of clotting time within six hours is a good indicator of envenomation by viper bite. The other parameters which have been analyzed may be helpful to assess the severity of renal failure which may ensue.

Conflict of interest: None

Acknowledgments: None

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