ENZYMATIC CHANGES IN SNAKE ENVENOMATION- AN OBSERVATIONAL STUDY

Sidharth Kapoor[1]

[1]*Associate Professor, Department of Medicine, Acharya Shri Chander College of Medical Sciences, Sidhra.*

ABSTRACT

BACKGROUND

Snakes are the most feared and the most worshipped living creatures on the earth. Snakes are called venomous when envenomation or human fatalities after their bite are known. Snakebite is an acute medical emergency faced by temperate and tropical regions with heavy rainfall and humid climate. The specific therapy for snakebite in India is still polyvalent ASV and clinical practice ASV is not recommended until the victim of snakebite presents either with the evidence of bite by a poisonous snake such as definite fang marks, swelling or pain at the bite site or with clinical or laboratory evidence of envenomation such as local and systemic bleeding. In some cases, institution of ASV may also be initiated on the identification of offending snake brought by the patient or attendants, but most of these are subjective matters and subject to fallacies. Also, that out of polyvalent and monovalent ASV available, since it is monovalent ASV, which is desirable due to its less side effects and more effectiveness, but its use warrants the identification of snake, which is practically not possible in every case and/or on the objective evidence of peripheral neurological signs and symptoms and haematological alterations, which may not be dependable in many cases.

MATERIALS AND METHODS

Snake envenomation is in fact a multifactorial stress phenomenon, which produces altered physiological states including death and one of the consequences of the stress phenomenon is generation of several lysosomal enzymes and formation of free radicals. Extensive data search on Medline has failed to show study of this type in any part of the world, so this study being taken up as a preliminary attempt to evaluate the pattern of enzymatic changes in snake envenomation.

RESULTS

The patients included in the study were be those coming to the Emergency Department of Government Medical College, Jammu, bitten by poisonous snakes during the period May 2003 to April 2004. The case were worked up according to proforma. Both neurotoxic and haemotoxic snakebite cases were included and samples were taken on day 0 and day 4. For comparison, blood samples from healthy persons were be tested for the enzymes like ACP, ALP, AST, ALT, malondialdehyde, superoxide dismutase, serum bilirubin (conjugated and unconjugated) to establish a control group.

Haemotoxic Cases- Amongst 32 haemotoxic cases in which enzymatic assays were studied on day 0 and day 4 following conclusions were drawn- AST level was elevated significantly on day 0 and it registered a further rise on day 4 as compared to age-matched healthy control. Mean AST levels in 32 haemotoxic cases was 57 IU/L on day 0, but on day 4, the mean AST levels was 94 IU/L as against the control means of 25 IU/L. ALT level was significantly high on day 0. On day 4, a further rise in its level was found as compared to age-matched healthy control. Mean ALT levels on day 0 was 73 IU/L, and on day 4, it was 83 IU/L against the control mean of 23 IU/L. Acid phosphatase showed a rise of about two-fold on day 0, while on day 4, a four-fold rise was there as compared to age-matched healthy controls. Mean acid phosphatase levels on day 0 was showing two-fold increase (5.8 KA units), but on day 4, the mean levels rose further to 8.3 KA units; the control mean being 2.4 KA units. Alkaline phosphatase was elevated on day 0, but on day 4, its levels showed a downward trend, although normalisation was not achieved. Mean alkaline phosphatase level on day 0 was 920 IU/L, and on day 4, it was 360 IU/L against the control mean of 179 IU/L. Significant superoxide dismutase activity suppression was found on day 0, but on day 4, this activity were restored to some extent, but normalisation was not achieved. About 38% suppression in mean SOD activity on day 0 (22.37 IU/L), but on day 4, the levels increased and mean SOD activity suppression was only 8% (33.47 IU/L); the control mean being 36.99 IU/L. Significantly, higher levels of malondialdehyde, an in vivo marker of lipid peroxidation was achieved on day 0, but on day 4, the levels showed a downward trend, although control value could not be achieved.

In Neurotoxic Cases- Amongst 12 neurotoxic cases in which enzymatic assay was studied on day 0 and day 4, following conclusions were drawn. On day 0, both AST and ALT registered a rise of approximately three-fold, but on day 4, the levels showed a downward trend, although a two-fold rise was still found (on day 4) as compared to age-matched healthy controls. The mean serum AST levels on day 0 and day 4 were 57 IU/L and 73 IU/L respectively against a control mean of 23.5 IU/L. Mean serum ALT levels on day 0 and day 4 was found to be 73 IU/L and 57 IU/L respectively against control mean of 24.3 IU/L. ACP levels were elevated on day 0, but on day 4, a further rise in levels was found. Acid phosphatase on day 0 and day 4 was 4.08 KA units and 5.3 KA units, respectively against control mean of 2.58 KA units. The ALP levels were elevated on day 0 and no significant fall on day 4 was found. Mean alkaline phosphatase levels on day 0 and day 4 was 387 IU/L and 365 IU/L respectively against a control of 200 IU/L. The SOD activity suppression on day 0 and day 4 was found to be nonsignificant. The MDA activity rise was significant on day 0, but on day 4, the levels were considerably reduced to achieve the value similar to those in the control levels. There was two-fold mean rise (6.53 mmol/L) in 32 haemotoxic cases on day 0, but on day 4, the mean levels reduced to 4.71 mmol/L, which was still higher than the control mean (3.27 mmol/L). In all 12 neurotoxicity cases were studied, the SOD activity suppression on day 0 and on day 4 was found to be statistically nonsignificant. The MDA levels

on day 0 showed a rise, which was less than two-fold (5.80 mmol/L), and on day 4, the mean levels were reduced to 3.27 mmol/L.

CONCLUSION

It was found that the mean rise in AST level was higher in neurotoxic cases than in haemotoxic cases on day 0, but on day 4, haemotoxic cases had still higher levels, but the value of AST had trend towards normalisation in neurotoxic cases. Since the mortality figures were too low to perform statistical analysis, the prognostic value of these changes could not be determined, hence, a larger study will be more informative. Since there is only one study available in literature in which effect of viperine venom enzymatic changes on male albino rats was studied, no definite conclusions could be drawn on the clinical studied, no definite conclusions could be drawn on the clinical implications of such changes in enzymatic assay after snake envenomation in human beings. Secondly, it is still not clear whether the oxidative stress after snake envenomation is because of effect on venom or is just on epiphenomenon, therefore, further work of similar nature is required before drawing definite conclusion.

KEYWORDS

Superoxide Dismutase, Malondialdehyde, Neurotoxic, Haemotoxic, Reactive Oxidant Species, Aspartate Aminotransferase, Alanine Aminotransferase.

HOW TO CITE THIS ARTICLE: Kapoor S. Enzymatic changes in snake envenomation- An observational study. J. Evid. Based Med. Healthc. 2017; 4(46), 2783-2789. DOI: 10.18410/jebmh/2017/552

BACKGROUND

Snakes are the most feared and the most worshipped living creatures on the earth. The snakes have been proved to be of great importance due to the presence of venom (Ahuja ML. Singh G).[1]

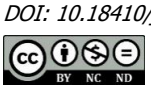
The venomous snakes of the world are grouped into families Viperidae, Elapidae), Hydrophiinae (the sea snakes), Atractaspididae (the burrowing asps) and Colubridae (a large group of which only a few species are dangerously toxic to humans) (Underwood, 1979).[2]

Reid (1968)[3] has described the bite of venomous snake into two types, the first being a business bites in which a large amount of venom is injected and the victim dies rapidly. The second type of bite is a matter of defence or warning and little or no venom is injected, the snake's object being to escape. When a venomous snake, bites human beings, it generally uses the second type of bite. Hence, it is important to realise that poisonous snakebite is not necessarily the same thing as the snakebite poisoning.

Jammu region, which lies in the sub-Himalayan region is enormously infested with snakes (Murthy et al, 1976,[4] Duda and Sahi, 1977).[5] Of all the main families of venomous snakes, saw scale viper (Echis Corinatus) is most abundant in Jammu region (Sharma, 1975; Sahi, 1979).[6]

The specific therapy for venomous snakebite in India is still polyvalent ASV and in clinical practice ASV is not recommended until the victim of snakebite presents either with the evidence of bite by a poisonous snake such as definite fang marks, swelling or pain at the bite site or with clinical or laboratory evidence of envenomation such as local and systemic bleeding (Reid et al, 1963; Reid, 1968).[7,3,8]

Financial or Other, Competing Interest: None.
Submission 25-05-2017, Peer Review 30-05-2017,
Acceptance 05-06-2017, Published 06-06-2017.
Corresponding Author:
Dr. Sidharth Kapoor,
No. 46, New Rehori, Opposite Park, Jammu.
E-mail: sidkap10@gmail.com
DOI: 10.18410/jebmh/2017/552



When there is either an increased production of reactive oxygen species or a decrease in the levels of antioxidant defenses or both, the toxic effects of such a scenario can be summed up as Oxidative Stress (OS) (Pugliese, 1998).[9]

An important, but almost wholly rejected facet of snakebite poisoning is the detection of enzymatic effects of venom for diagnostic purpose and for prognostic purposes.

Extensive data search on Medline and indexed journals has failed to show study of this type in any part of the world, so this study being taken up as a preliminary attempt to evaluate the pattern of enzymatic and free radical changes in snake envenomation.

MATERIALS AND METHODS

The patients included in the study were those coming to the Emergency Department of Government Medical College Hospital, Jammu, bitten by poisonous snakes during the period May 2003 to April 2004. Patients of both sexes irrespective of age formed the basis of study.

Both neurotoxic and haemotoxic snakebite cases were included and samples were taken on day 0 (before giving ASV) and day 4 (after ASV treatment), irrespective of whether the patient had recovered or was still under the impact of envenomation.

For comparison, blood samples from healthy persons were tested for the enzymes like aspartate aminotransaminase, alanine aminotransaminase, acid phosphatase, alkaline phosphatase, malondialdehyde, superoxide dismutase to establish a control group.

Inclusion Criterion

1. Presence of definite fang marks.
2. The identification of biting snake brought dead/alive by the patient.
3. Clinical local/systemic features of envenomation.
4. Laboratory features suggestive of envenomation.
5. No history suggestive of receipt of ASV prior to coming to emergency department.

Exclusion Criterion

1. All those patients who had received ASV prior to arrival.
2. All patients suffering from any comorbid condition (s).

Methods

The following laboratory investigations were carried out in each case-

1. a) Haemoglobin estimation (cyanmethemoglobin method described by Dacie and Lewis, 1975).
   b) Examination of total and differential leucocyte count - All by standard techniques advocated by Dacie and Lewis (1975).
2. a) Whole blood clotting time (Lee-White, 1913).
   b) Bleeding time (Ivy et al, 1940).
3. Platelet count.
4. Serum urea (diacetyl monoxime method), serum creatinine (Folin Wu alkaline picrate method), serum Na+ and serum K+ (flame photometry method) (Varley, 1967a; 1967b).
5. Routine examination of urine especially for proteinuria and haematuria followed by, if required, 24-hour total urinary proteins with Esbach's albuminometer.

Enzymes/Free Radical Estimation

1. Serum transaminases (AST/ALT)- After Reitman and Frankel (1957).[10]
2. Serum phosphatases (ACP and ALP) activity Measured after Mitchell et al (1970).
3. Thiobarbituric acid reactive products activity levels- It was ascertained by formation of Malondialdehyde (MDA) and measured by thiobarbituric acid method as described by Chatterjee and Aggarwal (1988).[11]
   Principle- The product of lipid peroxidation, i.e. MDA reacts with Thiobarbituric Acid (TBA) to give a red species, the absorption of which was read at 535 nm.
4. Superoxide dismutase activity- Superoxide dismutase activity was measured by the method of Beauchamp and Fridovich (1971).[12]

Principle- Hydroxylamine hydrochloride in alkaline solution produces O_2 anion, which reduces Nitroblue Tetrazolium (NBT) and the rate of this reaction can be monitored at 560 nm. Superoxide Dismutase Assay (SOD) activity is estimated by monitoring the rate of inhibition of NBT reduction by the enzyme.

Storage of Samples

The heparinised blood (5.0 mL) was collected aseptically from snakebite patients, stored in sterilised vials and subjected to tests without undue lapse of time. But, whenever the delay was unavoidable, these were stored in deep freezer at -80°C.

Statistical Analysis

Analysis was performed using computer software Microsoft Excel. Proportions were calculated for categorical variables and Chi-square test applied to see statistically significant difference. Mean ± SD was calculated after quantitative variables and 't' test was applied for testing statistical significance. P-value, if <0.05, was considered statistically significant.

The present study of enzymatic changes in snake envenomation was conducted in 44 victims of snakebite.

RESULTS

1. The present study was carried out to primarily evaluate the enzymatic changes in snake envenomation. Additional objective was to find out any alteration, if any, in haemotoxic and neurotoxic snakebites after ASV therapy and to see the clinical implications if any of these changes.
2. **Haemotoxic Cases-** Amongst 32 haemotoxic cases in which enzymatic assays were studied on day 0 and day 4 following conclusions were drawn-
   - AST level was elevated significantly on day 0 and it registered a further rise on day 4 as compared to age-matched healthy control. Mean AST levels in 32 haemotoxic cases was 57 IU/L on day 0, but on day 4, the mean AST level was 94 IU/L as against the control means of 25 IU/L.
   - ALT level was significantly high on day 0. On day 4, a further rise in its level was found as compared to age-matched healthy control. Mean ALT levels on day 0 was 73 IU/L, and on day 4, it was 83 IU/L against the control mean of 23 IU/L.
   - Acid phosphatase showed a rise of about two-fold on day 0, while on day 4, a four-fold rise was there as compared to age-matched healthy controls. Mean acid phosphatase levels on day 0 was showing two-fold increase (5.8 KA units), but on day 4, the mean levels rose further to 8.3 KA units; the control mean being 2.4 KA units.
   - Alkaline phosphatase was elevated on day 0, but on day 4, its levels showed a downward trend, although normalisation was not achieved. Mean alkaline phosphatase level on day 0 was 920 IU/L, and on day 4, it was 360 IU/L against the control mean of 179 IU/L.
   - Significant superoxide dismutase activity suppression was found on day 0, but on day 4, this activity were restored to some extent, but normalisation was not achieved. About 38% suppression in mean SOD activity on day 0 (22.37 IU/L), but on day 4, the levels increased and mean SOD activity suppression was only 8% (33.47 IU/L); the control mean being 36.99 IU/L.
   - Significantly higher levels of malondialdehyde, an in vivo marker of lipid peroxidation was achieved on day 0, but on day 4, the levels showed a downward trend, although control value could not be achieved.
3. **Neurotoxic Cases-** Amongst 12 neurotoxic cases in which enzymatic assay was studied on day 0 and day 4, following conclusions were drawn-
   - On day 0, both AST and ALT registered a rise of approximately three-fold, but on day 4, the levels showed a downward trend, although a two-fold rise

was still found (on day 4) as compared to age-matched healthy controls. The mean serum AST levels on day 0 and day 4 were 57 IU/L and 73 IU/L respectively against a control mean of 23.5 IU/L. Mean serum ALT levels on day 0 and day 4 was found to be 73 IU/L and 57 IU/L respectively against control mean of 24.3 IU/L.

- ACP levels were elevated on day 0, but on day 4, a further rise in levels was found. Acid phosphatase on day 0 and day 4 was 4.08 KA units and 5.3 KA units, respectively against control mean of 2.58 KA units.
- The ALP levels were elevated on day 0 and no significant fall on day 4 was found. Mean alkaline phosphatase levels on day 0 and day 4 was 387 IU/L and 365 IU/L respectively against a control of 200 IU/L.
- The SOD activity suppression on day 0 and day 4 was found to be nonsignificant.
- The MDA activity rise was significant on day 0, but on day 4, the levels were considerably reduced to achieve the value similar to those in the control levels. There was two-fold mean rise (6.53 mmol/L) in 32 haemotoxic cases on day 0, but on day 4, the mean levels reduced to 4.71 mmol/L, which was still higher than the control mean (3.27 mmol/L).

4. Since the mortality figures were too low to perform statistical analysis, the prognostic value of these changes could not be determined, hence a larger study will be more informative.
5. Since there is only one study available in literature in which effect of viperine venom enzymatic changes on male albino rats was studied, no definite conclusions could be drawn on the clinical implications of such changes in enzymatic assay after snake envenomation in human beings.

Secondly, it is still not clear whether the oxidative stress after snake envenomation is because of effect on venom or is just on epiphenomenon, therefore, further work of similar nature is required before drawing definite conclusion.

In all, 12 neurotoxicity cases were studied. The SOD activity suppression on day 0 and on day 4 was found to be statistically nonsignificant.

The MDA levels on day 0 showed a rise, which was less than two-fold (5.80 mmol/L), and on day 4, the mean levels were reduced to 3.27 mmol/L.

It was found that the mean rise in AST level was higher in neurotoxic cases than in haemotoxic cases on day 0, but on day 4, haemotoxic cases had still higher levels, but the value of AST had trend towards normalisation in neurotoxic cases.

On day 0, the rise in ALT was almost identical in haemotoxic and neurotoxic cases, but on day 4, the haemotoxic cases had higher levels than in neurotoxic cases, the normalisation being achieved in none.

There was increased acid phosphatase levels in haemotoxic cases on day 0 as compared to neurotoxic cases. On day 4, there was no decrease in ALP levels found in both haemotoxic and neurotoxic cases, nevertheless it was found that levels were higher than that found on day 0, the rise being more in haemotoxic cases.

Mean rise in alkaline phosphatase was more in haemotoxic cases on day 0, but on day 4, mean levels in both type of bites decreased the normalisation being achieved in none.

The suppression of SOD activity was more (38%) in haemotoxic bites on day 0 as compared to neurotoxic cases (13%). On day 4 of SOD activity in haemotoxic as well as neurotoxic bites increased, but 8% suppression of SOD activity was still there in haemotoxic cases, while in neurotoxic cases, this activity normalised.

The mean rise in MDA levels in haemotoxic bites was more (two-fold) as compared to neurotoxic bites (one and a half-fold), and on day 4, mean level in haemotoxic cases decreased (4.71 mmol/L), but remained high as compared to mean levels in healthy controls (3.27 mmol/L) while that in neurotoxic cases levels almost normalised.

Systemic Manifestation	Male	Female	Total
Haemorrhagic			
Bleeding gums	4	3	7
Haematuria	3	0	3
Ecchymosis	8	3	11
Haematemesis/Melaena	3	2	5
Ooze from bite site	5	2	7
Haemoptysis	0	1	1
Subcutaneous haematoma	2	2	4
Epistaxis	1	0	1
Subconjunctival haemorrhage	5	3	8
Neurological			
Ptosis/generalised weakness	8	4	12
Dyspnoea	6	3	9
Miscellaneous			
Oliguria/anuria	8	3	11
Death	3	0	3

Table 1. Showing Distribution of Various Systematic Manifestations

Haemotoxic Cases

Serum AST Levels (IU/L)			
	Number	**Range**	**Mean ± SD**
Controls	32	5-40	25.34 ± 8.26
Patients	32	27-107	57.62 ± 25.43

Table 2. Serum AST Levels in Healthy Controls and Snakebite Cases (Day 0)

Serum AST Levels (IU/L)			
	Number	**Range**	**Mean ± SD**
Controls	32	5-40	25.34 ± 8.26
Patients	32	59-183	94 ± 25.50

Table 2a. Serum AST Levels in Healthy Controls and Snakebite Cases (Day 4)

Serum ALT Levels (IU/L)			
	Number	**Range**	**Mean ± SD**
Controls	32	5-35	23.37 ± 7.77
Patients	32	39-93	73.96 ± 16.9

Table 3a. Serum ALT Levels in Healthy Controls and Snakebite Cases (Day 0)

Serum ALT Levels (IU/L)			
	Number	**Range**	**Mean ± SD**
Controls	32	5-35	23.37 ± 7.77
Patients	32	63-140	83 ± 14.7

Table 3b. Serum ALT Levels in Healthy Controls and Snakebite Cases (Day 4)

Serum Acid Phosphatase Levels (KA units)			
	Number	**Range**	**Mean ± SD**
Controls	32	1-4	2.43 ± 0.84
Patients	32	2.8-7.9	5.85 ± 1.67

Table 4a. Serum Acid Phosphatase in Healthy Controls and Snakebite Cases (Day 0)

Serum Acid Phosphatase Levels (KA units)			
	Number	**Range**	**Mean ± SD**
Controls	32	1-4	2.43 ± 0.84
Patients	32	5.6-10.3	8.37 ± 1.57

Table 4b. Serum Acid Phosphatase in Healthy Controls and Snakebite Cases (Day 4)

Serum Alkaline Phosphatase Levels (IU/L)			
	Number	**Range**	**Mean ± SD**
Controls	32	108-306	179.87 ± 50.35
Patients	32	256-567	420 ± 121.58

Table 5a. Serum Alkaline Phosphatase Levels in Healthy Controls and Snakebite Cases (Day 0)

Serum Alkaline Phosphatase Levels (IU/L)			
	Number	**Range**	**Mean ± SD**
Controls	32	108-306	179.87 ± 50.35
Patients	32	210-480	360 ± 102.16

Table 5b. Serum Alkaline Phosphatase Levels in Healthy Controls and Snakebite Cases (Day 4)

Serum SOD Levels (UI/mL)			
	Number	**Range**	**Mean ± SD**
Controls	32	30.8-57.8	39.37 ± 8.09
Patients	32	16.3-28.8	22.37 ± 3.79

Table 6a. Serum Superoxide Dismutase Activity in Healthy Controls and Snakebite Cases (Day 0)

Serum SOD Levels (UI/mL)			
	Number	**Range**	**Mean ± SD**
Controls	32	30.8-57.8	39.37 ± 8.09
Patients	32	26.5-39.54	33.47 ± 3.90

Table 6b. Serum Superoxide Dismutase Activity in Healthy Controls and Snakebite Cases (Day 4)

Serum MDA Levels (mmol/L)			
	Number	**Range**	**Mean ± SD**
Controls	32	2.24-4.44	3.27 ± 0.66
Patients	32	4.31-8.73	6.53 ± 1.28

Table 7a. Serum MDA Levels in Healthy Controls and Snakebite Cases (Day 0)

Serum MDA Levels (mmol/L)			
	Number	**Range**	**Mean ± SD**
Controls	32	2.24-4.44	3.27 ± 0.66
Patients	32	2.97-6.36	4.71 ± 1.08

Table 7b. Serum MDA Levels in Healthy Controls and Snakebite Cases (Day 4)

Neurotoxic Cases

Serum AST Levels (IU/L)			
	Number	**Range**	**Mean ± SD**
Controls	12	5-40	23.5 ± 7.77
Patients	32	27-148	75.8 ± 36.6

Table 8a. Serum AST Levels in Healthy Controls and Snakebite Cases (Day 0)

Serum AST Levels (IU/L)			
	Number	**Range**	**Mean ± SD**
Controls	12	5-40	23.5 ± 7.77
Patients	12	32-97	57 ± 20.48

Table 8b. Serum AST Levels in Healthy Controls and Snakebite Cases (Day 4)

Serum ALT Levels (IU/L)			
	Number	**Range**	**Mean ± SD**
Controls	12	5-35	24.33 ± 7.70
Patients	12	37-125	73 ± 30.01

Table 9a. Serum ALT Levels in Healthy Controls and Snakebite Cases (Day 0)

Serum ALT Levels (IU/L)			
	Number	**Range**	**Mean ± SD**
Controls	12	5-35	24.33 ± 7.70
Patients	12	33-187	57.83 ± 21.96

Table 9b. Serum ALT Levels in Healthy Controls and Snakebite Cases (Day 4)

Serum Acid Phosphatase Levels (KA units)			
	Number	**Range**	**Mean ± SD**
Controls	12	1-4	2.58 ± 0.84
Patients	12	2.3-5.9	4.08 ± 1.23

Table 10a. Serum Acid Phosphatase Levels in Healthy Controls and Snakebite Cases (Day 0)

Serum Acid Phosphatase Levels (KA units)			
	Number	**Range**	**Mean ± SD**
Controls	12	1-4	2.58 ± 0.84
Patients	12	2.9-8.3	5.3 ± 2.00

Table 10b. Serum Acid Phosphatase Levels in Healthy Controls and Snakebite Cases (Day 4)

Serum Alkaline Phosphatase Levels (IU/L)			
	Number	**Range**	**Mean ± SD**
Controls	12	108-306	200 ± 40.31
Patients	12	135-503	386.5 ± 102.34

Table 11a. Serum Alkaline Phosphatase Levels in Healthy Controls and Snakebite Cases (Day 0)

Serum Alkaline Phosphatase Levels (IU/L)			
	Number	**Range**	**Mean ± SD**
Controls	12	108-306	200 ± 40.31
Patients	12	128-489	364.16 ± 98.10

Table 11b. Serum Alkaline Phosphatase Levels in Healthy Controls and Snakebite Cases (Day 4)

Serum SOD Levels (UI/mL)			
	Number	**Range**	**Mean ± SD**
Controls	12	30.8-57.8	39.79 ± 8.27
Patients	12	21.7-44.8	34.88 ± 7.91

Table 12a. Serum Superoxide Dismutase Activity in Healthy Controls and Snakebite Cases (Day 0)

Serum SOD Levels (UI/mL)			
	Number	**Range**	**Mean ± SD**
Controls	12	30.8-57.8	39.79 ± 8.27
Patients	12	34.7-57.9	45.91 ± 7.29

Table 12b. Serum Superoxide Dismutase Activity in Healthy Controls and Snakebite Cases (Day 4)

Serum MDA Levels (mmol/L)			
	Number	**Range**	**Mean ± SD**
Controls	12	2.24-4.44	3.34 ± 0.69
Patients	12	3.27-7.18	5.84 ± 1.41

Table 13a. Serum MDA Levels in Healthy Controls and Snakebite Cases (Day 0)

Serum MDA Levels (mmol/L)			
	Number	**Range**	**Mean ± SD**
Controls	12	2.24-4.44	3.34 ± 0.69
Patients	12	2.29-4.70	3.27 ± 0.82

Table 13b. Serum MDA Levels in Healthy Controls and Snakebite Cases (Day 4)

DISCUSSION

In the present study, it was found that snake envenomation increased the levels of phosphatases and transaminases activity in the blood. Probable cause for these enzymatic changes is that there is lysis of lysosomal cells on different cellular organelles due to the presence of various enzymes, e.g. phospholipases present in snake venom (Alam et al, 1998).[13]

When there is either an increased production of reactive oxygen species or a decrease in the levels of antioxidant defenses or both the toxic effects of such a scenario can be summed up as oxidative stress (Pugliese, 1998).[9]

The malfunction of electron transport chain leads to electron leakage and hence increased generation of superoxide anions. The free radicals generated cause oxidative deterioration of polyunsaturated fatty acids, which are important constituents of biological membranes. Damage to biological membranes generates a number of secondary products both from fission and endo-cyclisation of oxygenated free radicals (Dexter et al, 1989).[14]

Although, oxidative stress has been implicated in a variety of disorders, however, it is still not clear whether oxidative stress has a primary role in these conditions or is secondary stage epiphenomenon (Ichiropoulos, 2003).[15]

Reactive oxygen species are generated during different processes of cellular aerobic metabolism. The reactive oxygen species like superoxide anion (O_2, hydrogen peroxide (H_2O_2)), hydroxyl radicals and peroxynitrite (ONOO) can potentially damage different macromolecules, such as proteins, nucleic acids and lipids and thereby leading to cellular degeneration. To counter this, the cells maintain a battery of detoxifying enzymes and small molecule antioxidants. These include glutathione, glutathione peroxidase, glutathione reductase and superoxide dismutase (Cohen et al, 1987).[16]

The prognostic value of these findings could not be ascertained as mortality data in the present study was too low (3 deaths in all; 2 haemotoxic and 1 neurotoxic) to perform statistical analysis. One died due to intracranial haemorrhage, second died due to upper gastrointestinal bleed while third death occurred due to pneumonia leading to septicaemia.

The exact cause of venom-induced changes in blood enzymes and free radical formation as estimated by thiobarbituric acid products formation (MDA levels) and SOD activity is still not clear.

Further work is needed to establish the findings supporting the effect of snake envenomation on enzymatic changes.

The present study was undertaken with the chief aim of evaluating the enzymatic changes in snake envenomation.

In Jammu region of J and K State, snakebites are very common during the summer and rainy seasons. About 800-1000 patients of snakebite are admitted in Government Medical College Hospital each year. Being a referral hospital of Jammu province, it caters for most of the cases from the surrounding areas.

The commonest poisonous snakebite in this region is due to Echis carinatus. In the study area, both neurotoxic and haemotoxic snakebites are seen. We did not encounter any case, which had combined features of haemotoxicity and neurotoxicity.

Majority (65%) of the patients were young adult males with the mean age of approximately 32 years. Most of them were outdoor workers who work and walk barefoot, hence distal parts like feet were bitten more frequently than proximal parts. Similar observations have been made by Bhat (1974), Warrel et al (1977).

72% of patients in the study by Bhat (1974)[17] and 85% of those seen by Lahori et al (1981) had reported within 24 hours.

In this study, out of 44 cases of snakebite poisoning, the majority (77%) of cases showed two fang marks, but one (2.2%) case revealed only solitary fang marks at the site of bite. On the other hand, 9 patients had no fang marks, but showed only scratches over site of bite.

Russell (1980)[18] observed fang marks in 100% and Lahori et al (1981)[19] in 94% cases. Bhat (1974) observed fang marks in 73% cases, since many (27%) of his patients had given local incisions.

Regarding the haemotoxic bites, the most frequent systemic clinical features were constituted by haemorrhagic manifestations. These were observed in 84.09% cases and consisted of ecchymosis, subconjunctival haemorrhage, bleeding gums, ooze from bite site, haematemesis or melaena, subcutaneous haematoma, haematuria, haemoptysis and epistaxis in that order of frequency.

Apart from clinical evidence of excessive haemorrhages, laboratory evidence of bleeding diathesis was observed in 29 (65%) out of 44 cases when considered on the basis of prolonged clotting time. The predominance of excessive bleeding tendency (either clinically manifest or with prolonged clotting time) in the present study is due to the fact that most common snake in our cases is Echis carinatus as also reported by Bhat (1974) and Verma et al (1982).

Amongst 11 cases who showed evidence of azotaemia, there was evidence of severe dehydration in 6, one had

septicaemia while in remaining 4 cases, since there was no other apparent cause identified and the possibility of direct nephrotoxic involvement was considered.

Though there are four medically important venomous land snakes in India, but only viper has been reported as a cause of renal failure in this country (Sitprija et al, 1974; Shastry et al, 1977).[20-21]

Out of 44 cases studied, clinical evidence of neurotoxicity in form of ptosis, generalised weakness, speech difficulty, breathing difficulty, etc. was observed only in 27% cases. One of the patients also had convulsions and excessive sweating with salivation.

CONCLUSION

In the present study, the most frequent clinical features were consistent with some other works on study of viperine snakebite poisoning (Reid et al, 1963; Bhat 1974; Warrell et al, 1977)[22] and cobra/krait poisoning (Reid, 1964; Warrell et al, 1983).[23]

Over the past one decade or so, much interest has been generated on the role of free radicals, especially oxy-free radicals in various diseases. Lipid peroxidation and oxidative stress are known to be involved in the pathogenesis of atherosclerosis and cancer (Hasan, 2003).[24] As far as the present study of evaluation of enzymatic/free radical changes in snake envenomation is concerned, no other studies are available in the literature for comparison with the results of present study except for the one animal study by Alam et al (1998).[13] In that study too, only viper venom-induced inflammation and inhibition of free radical formation in male albino rats was discussed. They proposed that snake envenomation leads to oxidative stress leading to increased formation of hydrogen peroxide and oxy-radicals.

REFERENCES

[1] Ahuja ML, Singh G. Snakebite in India. Ind J Med Res 1954;42(4):661-668.

[2] Underwood G. Classification and distribution of venomous snakes in the world. In: Lee CY, ed. Snake venoms. Berlin: Springer 1979:p. 15.

[3] Reid HA. Snakebite in tropics. Br Med J 1968;3(5614):359-362.

[4] Murthy TSN, Sharma BD. A contribution to the herpetology of J & K State. Brit J Hespit 1976;5:537-538.

[5] Duda PL, Sahi DN. A checklist to the herptiles of Jammu & Kashmir State. University Review 1977;6:87-94.

[6] Sahi DN. A contribution to the herpetology of Jammu & Kashmir State. Ph.D. Thesis, University of Jammu: 1979.

[7] Reid HA, Thean PC, Chan KE, et al. Clinical effects of bites by Malayan viper (Ancistrodon rhodostoma). Lancet 1963;1(7282):617-621.

[8] Reid HA. Symptomatology, pathology and treatment of land snakebite in India and & Southeast Asia. In: Bucherl W, Buckley EE, Deulofen V, eds. Venomous animals and their venoms. New York: Academic Press 1968;1:611-642.

[9] Pugliese PT. The skin's antioxidant systems. Dermatol Nurs 1998;10(6):401-416.

[10] Reitman S, Frankel S. A calorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology 1957;28(1):56-64.

[11] Chattergee SN, Agarwal S. Liposomes as membrane models for study of lipid peroxidation. Free Radical Biol Med 1988;4(1):51-72.

[12] Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 1971;44(1):276-287.

[13] Alam MI, Gomes A. Viper venom-induced inflammation and inhibition of free radicals formation of pure compound (2-hydroxy, 4-methoxy benzoic acid) isolated and purified from Anantamul (Hemidesmus indicus R. BR.) root extract. Toxicon 1998;36(1):207-215.

[14] Dexter DT, Carter CJ, Wells FR, et al. Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease. J Neurochem 1989;52(2):381-389.

[15] Ischiropoulos H, Beckman JS. Oxidative stress and nitration in neurodegeneration: cause, effect, or association? J Clin Invest 2003:111(2):163-169.

[16] Cohen GM, Arey DM. Free radical mediated cell toxicity by redox cycling chemicals. Br J Cancer 1987;8:46-52.

[17] Bhat RN. Viperine snakebite poisoning in Jammu. J Ind Med Assoc 1974;63(12):383-392.

[18] Russell FE. When a snake strikes. Emerg Med 1980;22(12):20-25, 33-34, 37-40, 43.

[19] Lahori UC, Sharma DB, Gupta KB, et al. Snakebite poisoning in children. Indian Pediatrics 1981;18:193-197.

[20] Shastrv JC, Date A, Carman RH, et al. Renal failure following snakebite. A clinicopathological study of nineteen patients. Am J Trop Med Hyg 1977;26(5 Pt 1):1032-1038.

[21] Sitprija V, Benyajati C, Boonpucknavig V. Further observations of renal insufficiency in snakebite. Nephron 1974;13(5):396-403.

[22] Warell DA, Davidson NMcD, Greenwood BM, et al. Poisoning by bites of saw-scaled or carpet viper (Echis carinatus) in Nigeria. Q J Med 1977;46(181):33-62.

[23] Warrell DA, Looareesuwan S, White NJ, et al. Severe neurotoxic envenoming by Malayan krait Bungarus candidus (Linnaeus): response to antivenom and anticholinesterases. Br Med J 1983;286(6366):678-680.

[24] Mahdi H, Singh DR, Bajpai VK, et al. Molecular medicine: free radical biomarkers. Proc of ICFR-2003 Feb 10-12, 2003:p. 1.