



Bio-Clinical Study of Envenomed victims in Osmania General Hospital, Hyderabad.

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ABSTRACT

Background & Objectives: Every year there are about one lakh cases of snake bite deaths all over the world, mostly either with cobra, viper or krait snakes. Nearly about 1/3rd of them are reported in India. Still most of the rural population of India needs awareness regarding envenomation management. About 40% of snake bitten victims are losing their lives ignorantly due to delayed hospitalization and indulging in unscientific management. Sole object of this study is to project and impress upon the scientific management through clinical evaluation, study of bio-clinical samples with subsequent treatment of envenomed victims.

Methods:

Various types of data concerning 276 snake bite victims both live and dead cases were collected from retrospective, prospective and follow-up cases for cobra bite detection during the period from 1999 to 2000. The live cases are followed clinically in the casualty, medical wards and ICU. Dead victims are examined in the mortuary of Osmania General Hospital, Hyderabad; A.P. Antibodies against cobra venom are raised while conducting animal experimentations (rabbits and goats) and subjected the anti body sera to ELISA test against bio-samples of victims. Live bio-clinical samples collected from the clinical wards are bitten area washings, blood, urine and cerebro spinal fluid (csf) for further investigations. From the mortuary snake bite victim's autopsy samples like skin bit, blood from right ventricle, liver and kidney are collected. All the samples are subjected to ELISA, histopathological examination and other routine investigations.

Results:

The results obtained in this study of envenomed victims of live and dead are analyzed, compiled and compared and they are found to be satisfied in their respective criterions. The same results are further strengthened with the observations of previous scientific studies mentioned later in the discussion part.

Conclusion

The clinical evaluation, biochemical and histopathological examination of all snake bite cases living and dead under the study have shown much convincing and corroborating evidences with one another. The results of the study are very much comparable and are consistent with previous scientific observations as expressed in discussions.

KEYWORDS : envenom victims, fang marks, snake bite deaths, ELISA, histopathological examination, neuromyolysis, cellulites, renal failure, cobra antibodies in rabbit.

MATERIALS AND METHODS



This is a three dimensional study involving retrospective study of 99 snake bite victims during 2000 to 2003 year and prospective study of 129 snake bite victims during 2003 year in addition to the follow up study of 38 snake bite victims during 2004 year. For retrospective studies of snake bite victims, the statistical data was collected from outpatient and inpatient records of Osmania General Hospital, Hyderabad, A.P. Analytical data of the dead bodies of snake bite victims was collected from the postmortem examination records of mortuary and Forensic department of Osmania Medical College, Hyderabad, A.P.



Bio-samples collected in sterile bottles with thimer salt solution

In the prospective study of 129 snake bites clinical evaluation and their laboratory reports of snake bite patients are collected from the wards of Osmania General Hospital. Autopsy details and their samples of investigations are collected from the mortuary of Osmania General Hospital, Hyderabad and rest of the research study is conducted in the Departments of Forensic Medicine and department of Biochemistry at University College of Sciences OU Hyderabad. Histopathological examination of autopsy samples (liver, Kidney, skin etc) collected from snake bite victims is carried in the dept. of pathology of Osmania Medical College, Hyderabad, A.P. Animal (rabbits & goats) experiments with cobra antigen, on the principles of sandwich Elisa tests were performed in the department of Biochemistry, University College of Sciences, O.U. Hyderabad, A.P.

ELISA Test:



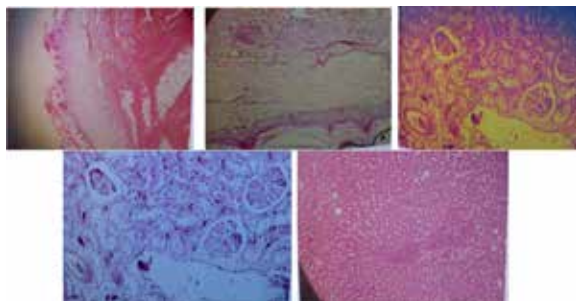
Animal (rabbit) is subcutaneously injected with cobra venom to get anti sera.

Indirect-competitive inhibition ELISA is based on the principle that affinity purified rabbit IgG raised against Cobra venom were incubated with a known amount of the antigen (reference standard: Cobra venom) or test sample (unknown). The amount of free antigen (Cobra venom) present in the standard or test sample competes with the immobilized antigen (Cobra venom) for the binding sites on the antibody. The detection system was based on labeled antibody (against rabbit IgG raised in goat).



The concentration of venom in the test sample was quantitated from the calibration curve using linear regression equation (Cobra venom: $y = 0.2581x + 0.4375$ ^(1, 2, 3)).

Histopathological Examination: Bio-samples of snakebite victims like skin bit, liver and kidney are collected and preserved in 10% formalin. After fixation and processing the tissue the micro slides are prepared for the following histopathological examination



Histopathological features of skin, kidney and liver

Skin: The features of hemorrhages and necrosis with hyper pyknosis and cellular infiltrations are the specific features.

Kidney: 1) Carticular tubular epithelial swelling.2) Congestion of Glomeruli.3) Thickening of Bowman's capsule .4) Signs of Mesangioly-sis.5) Cytoplasmic granulations and 6) Nuclear Pyknosis and Karyolysis are features of consideration.

Liver: Cytoplasmic granulations in hepatocytes, cloudy swelling, narrowing of sinusoidal spaces, inflammatory cells accumulation and evidences of kuffer's cells are visible in histopathological examination.

RESULTS

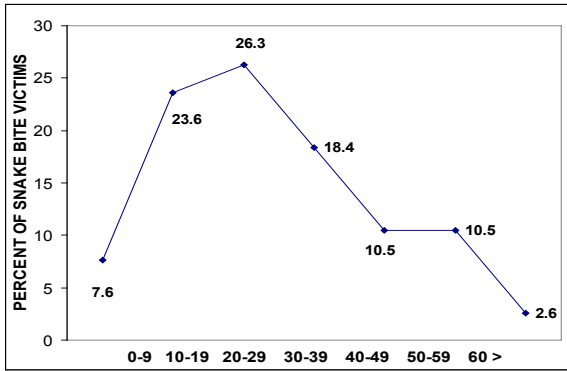
Analytical reports in this follow up study have revealed the following observations

- 1) Males were 55% and Female were 45% of total number of snake bite victims.
- 2) The maximum number of cases was found in the age group of 11 to 30 years.
- 3) In 78% of the total cases the snake was identified either by patients or by clinical examination.
- 4) The scene of snake bite was 57% in the houses and 27% was outside the houses.
- 5) Maximum number of cases (34%) was reported during the month of June i.e. at the onset of the monsoon season.
- 6) In local prevalence of snake bites, the krait bites were 29%, cobra bites were 24% and viper bites were 26% of all total cases.
- 7) In 72% of snake bite cases the time of bite is found to be more during the period of 8:00 P.M. to 8:00 A.M.
- 8) In 47.2% of cases bite marks are found in the lower limbs.
- 9) Clinically there were 53% of snake bite patients were with neuro paralytic symptoms and 36% of patients were with local cellulites and acute renal failure.
- 10) 23% of the total number of snake bite victims was admitted in the hospital within 3 hours of snake bite and another 36% of the total cases were admitted during 3 to 12 hours of snake bite.
- 11) 63% of the snake bite victims were survived with necessary treatment in the hospital.
- 12) Of all the samples of 38 snake bite victims, 15 cases (including two cases of cross reaction with krait) showed Elisa positive (39.5%) to the cobra venom.
- 13) In this study, the maximum quantity of cobra venom detected was 10 ngm/dl at the site of bite.
- 14) In this study the venom was detected in the samples as early as 2 hours of post bite interval and as late as 12 hours of snake bite.
- 15) In the majority of cases, enough amount of venom was still present at the site of snake bite was evident at the time of autopsy.
- 16) Survival chances of patients were better and significant in those who were admitted within 2 to 4 hours of snake bite having received adequate doses of ASV, when compared to those who

were admitted later i.e. after 8 hours of bite and received sub therapeutic doses of ASV.

17) The patients who have survived for long period have later succumbed due to complications involving the end organs like kidneys, brains, heart, liver and lungs.

Fig 63. Frequency distribution of snakebite victims in different age groups (Follow Up Study)



YEARS IN AGE GROUPS
n = 38

Fig 64. Month wise variations of snake bite during peak period of 2004

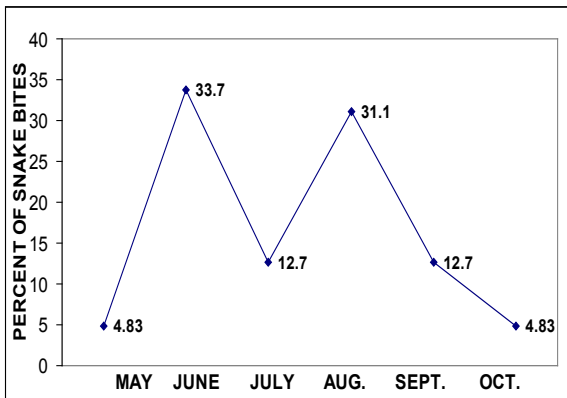


Fig 66. Identification of offending snake in the cases (Follow Up Study)

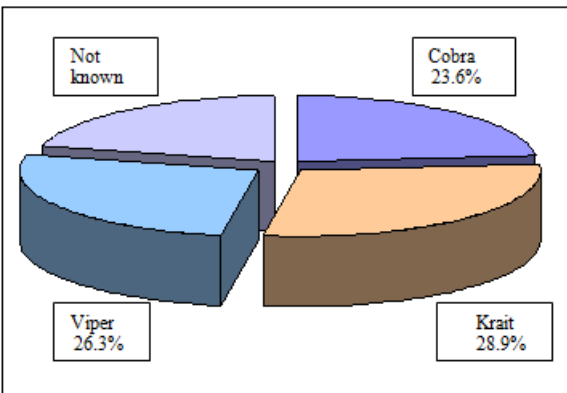
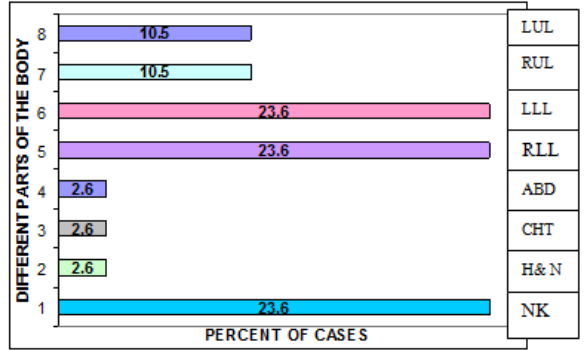
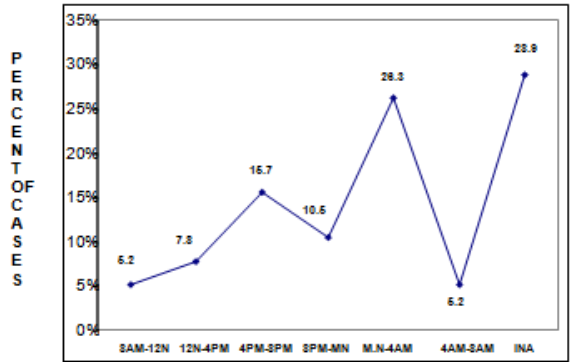


Fig 68. Frequency distribution of snake bites over different parts of the body (Follow Up Study)



n = 38
UK - Unknown, H &N - Head & Neck, CHT- Chest, ABD - Abdomen, RLL - Right Lower Limb, LLL - Left Lower Limb, RUL - Right Upper Limb, LUL - Left Upper Limb

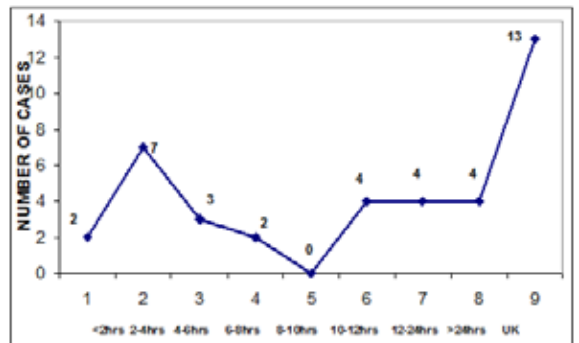
Fig 70: Frequency distribution of snakebite in different time group intervals of the day (Follow Up Study)



DIURNAL VARIATION IN TIME GROUP INTERVAL

n = 38

Fig 71: Frequency distribution of snakebite victims hospitalized in different time group interval (Follow Up Study)

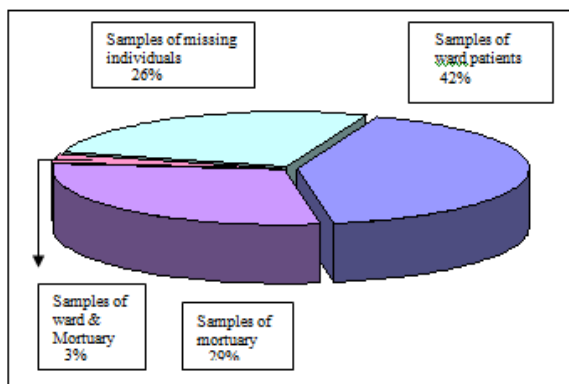


POST BITE PERIOD IN DIFFERENT TIME GROUP INTERVALS

n = 38

Fig 72: Collection of samples of snakebite victims from

different places of hospital (Follow Up Study)



n= 38

DISCUSSION

The follow up study involving 38 snake bite cases is purposefully carried in parallel to the retrospective and prospective studies totaling 239 cases of snake bites at Osmania General Hospital of Andhra Pradesh state, India, during 2000 to 2004 year, Sarangi. et. All has studied on 74 cases of snake bite victims with special reference to hemorrhages, renal and neurological abnormalities⁽⁴⁾. Dabo. Et all studied on 13 cases of snake bite victims selecting a village Bancomana in mali with Special reference to therapeutic evaluation⁽⁵⁾. Hung DZ. Et all have reported the clinical significance of venom in 31 samples from cobra victims⁽⁶⁾.

1) Epidemiological aspects:

Lal. P. Srihari et all has observed that there is steep rise in snake bite incidences from 2.9 to 5.2 per thousand from 1990 to 1996 year⁽⁷⁾. The observations in this study revealed the rise of incidence from 4.13 to 6.86. As per the present study there are 57% of total snake bite cases were recorded during monsoon (June to August). A.K.Hati et all conducted survey in Burdwan district of West Bengal and found the maximum incidence of snake bite victims during the months of July-August (rainy season)⁽⁸⁾. The same team further opined that maximum age incidence was reported to be in the group of 21 to 30 years, which is coinciding with the presented observations A.Bhardwaj et all of central research institute Kausali Himachal Pradesh and Lal.P.Srisailum et all have noted that the victims were mostly workers and laborers in the agriculture fields⁽⁹⁾. Similarly present observation has not differed in that the 47% of the victims were agriculture field workers dwelling in slums. Dabo A diawara et all conducted the survey and found out of seventeen cases there were 8 males and 9 females⁽⁵⁾. The present survey reveals the males to female ratio as 63% and 36% A.K.Hati. et. all reported that there were male to female ratio as 55% : 45%⁽⁸⁾.

2) Hospitalization of victims:

Amaral CF et all have recorded that 37.8% of victims in 37 cases were admitted in less than 4 hours of bite⁽¹⁰⁾. The same numbers were again admitted in less than 8 hours of bite. The present observations of 23% of 38 cases were admitted in less than 3 hours and another 36% of cases were admitted in 3 to 12 hours of post bite interval.

3) Clinical Study:

Present study observed the fang marks in 67% of the total cases, Sarangi, et all reported 76% of his total cases were presently with the fang marks over the body⁽⁴⁾. Hung DZ et all noted regional lymphadenitis within 2 to 3 hours of bite Intravascular hemorrhages in 3 out of 18 viper bite cases. 18 of 74 cases had no local or system feature of envenomation⁽⁶⁾. As per the present observation in majority cases depending upon dose of envenomation different grades of drowsiness, drooping of eyelids, dysphagia, dysphasia and dysnoea were observed. The final fatal events were paralysis, coma and death.

4) Fang marks:

Aud Ebert et all⁽¹¹⁾ have classified clinical features into 4 grades depending up to the venom transmitted into body. AH Hate, et all⁽⁸⁾ have reported 53% of total cases had fang marks over lower limbs.

Bhardwaj et all have found 50% of total victims had fang marks over lower limbs and in 45% cases fang marks were found on the left side when compared to right side. In this study the observations revealed 47% of total cases have fang marks on the lower limbs and 22% cases had an upper limbs, 46% of total cases had fang marks are left side of the body when compared to right side of the body (32%).

5) Investigations (ELISA and histopathological examination)

Aud Ebert, et all made use of Elisa test for diagnosis of type of snake bite and also revealed more than 20 ng/dl will produce moderate to severe envenomation⁽¹¹⁾. A.Bhardwaj. et all account that poisonous snake bite cases occur only in 10% of total cases of snake bite⁽⁹⁾. There were 50% of cases of krait, 25% of Russell's viper and 10% case of cobra in the total number of cases as reported by Hung DZ et all who, has conducted study on 31 samples of snake bite patients and recorded 127 ng/dl of maximum concentration of cobra antigen in the serum and also affirmed that the concentration was remained at same level till 48 hours in the absence of treatment⁽⁶⁾. Slevanayagam ZE et all detected the venom⁽²⁾ concentration as low as 100 pg/dl in the postmortem samples (blood, liver, kidney). They have also confirmed that the maximum concentration of saw scale viper venom was found at the site of bite⁽¹²⁾. Of the samples collected from 38 snake bite victims 15 of them found to be Elisa positive with cobra venom and the maximum concentration of 12ng/dl was detected at the site of bite. Mtom et all conducted the test on samples of viper bitten victims and opined that venom was mixture of variety of enzymes damaging the coagulation system of the victims. Farid T et all established the nature offending snake venom by rapid and reliable diffusion technique⁽¹³⁾. Trevelt, et all using the venom detection kit has diagnosed the nature of venom by taking the swabs over the skin bitten area and conducting the Elisa test. They emphasized the use of mano specific ASV thus saving the time, money and hazardous effect of polyvalent anti snake venom⁽¹⁴⁾. Sarangi et al studies on the victims of snake bite who were effected with toxic glomerulus's nephropathy⁽⁴⁾. In this study all the ante mortem and post mortem samples are subjected to Elisa test and histopathological examination in addition to the daily routine required clinical investigations. The samples collected within 2 hours of snake bite interval, were successfully giving Elisa positive test. Histopathological examination of samples confirmed the acute tubular necrosis and mesangiolytic of kidneys and hepato cellular necrosis with cloudy swelling and scattered hemorrhages in the liver. In the bitten skin bits, there are evidences of necrosis and hemorrhages in dermo epidermal layers.

6) Therapeutic Study:

De Rezende et, all ruled out that there are no dry bite cases and all victims must be treated empirically on the lines of envenomation⁽¹⁵⁾. But one of the American survey in this matter have concluded that 12% of 41 cases were treated with ASV, though they were not envenomed cases, thus exposing to the hazardous effect of ASV⁽¹⁵⁾. Forkes.T.P. have strongly recommended ASV to all snake bite victims including suspected cases. As per the present survey in the hospital all the alleged cases of snake bite with positive clinical manifestation were given ASV, improving the survival percentage, Lal, P.Srihari et, all revealed that 72% of 18 cases had local and systemic features of envenomation, rest of the cases proved to be non poisonous and few with doubtful status⁽⁷⁾. Hugh Alistair et, all proved that ASV is antidote to snake bite victims and non clotting of blood by pit viper bite is due to the induced defibrination by the venom⁽¹⁶⁾. Amaral, CF campolina et all, conducted Elisa tests in timely series over the ASV infused patients and opined that circulating venom antigen gets detoxified with its periodical administration⁽¹⁰⁾. The present follow up study regarding these 38 patients clearly revealed that the treatment is successful, if the ASV was administered within 2 to 4 hours of bite or even if little delayed an adequate dose of ASV is essential depending upon degree and delay of envenomation. Apart from the ASV infusion, endotracheal intubations, anti cholinergic drugs, iono tropic support are of equally important therapeutic eventualities

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