

A Follow up Study on Hospitalised Cases of Snake Bite Victims Osmania General Hospital, Hyderabad.

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Abstract: Background and objectives:

The Medical fraternity in certain places is still facing the problems of management in relation to the morbidity, mortality and the medico legal nature that are challenged in some of the cases of snakebites. This evidence based toxicological study comprises of clinical, biochemical and histo-pathological evaluations apart from the preservations of circumstantial and photographic evidences. In this study 23 out of 38 cases of snakebite victims are under the clinical study leaving the rest of the 15 cases to the postmortem examination at Osmania general hospital mortuary Hyderabad AP. This study has generated lot of interest in following the various valuable observations from the cases of living and dead victims of snakebite.

Material and Methods:

The following are some of the prospective study data collected in relation to material and methods.

1) Age and sex distribution, 2) Snake identification, 3) Scene of snake bite, 4) Seasonal variation, 5) Diurnal variation, 6) Frequency distribution of fang marks over the body, 7) Preparation of anti body sera from rabbit and goat animals, 8) Clinical statuses and treatment modalities, 9) Biochemical and Pathological examinations of the Bio-samples (skin washings, blood and csf) collected at the place of treatment in the hospital, 10) Collection and preservation of the postmortem samples (skin bit, blood, liver and kidney) of the snakebite victims at the mortuary, 11) Immuno analytical assay of the live and dead samples of snake bite victims by the ELISA test, 12) Histo-pathological examination of live and dead tissues in the microscope obtained from the live and dead snakebite victims.

Results:

The results of the above data is collected from the live and dead bodies and are analyzed, compiled and compared in their respective criterions. The scientific conclusions drawn on the observations of both anti-mortem and postmortem samples studies of snake bite victims were corroborating and consistent with the previous studies mentioned in the discussion part.

Conclusion:

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

Keywords: Elisa Test, Snake bite victims, Autopsy Samples, Clinical Samples, histo-pathological examination.

1. Introduction

In India there are 216 species of snakes. Out of which 52 species are reported to be poisonous and the most common, important and deadly poisonous are the "Big 4 snakes" (Cobra, Krait, Viper and Saw scale viper). The percentages of envenomation deaths are found to be 0.74% of overall autopsies done in Osmania General Hospital, Hyderabad during the period of this research study. As per the latest reports there are 15000 deaths to 20000 deaths due to snakebites in India every year. Dr.Aliaster Head of Liverpool School of Tropical Medicare has found that only about 15% to 18% of total snakebite cases end up with death and rest of the cases survive due to bite of the nonpoisonous snakes or due to dry snake bites or due to recovery from neurogenic shock. As per the investigation carried in this scientific activity there are

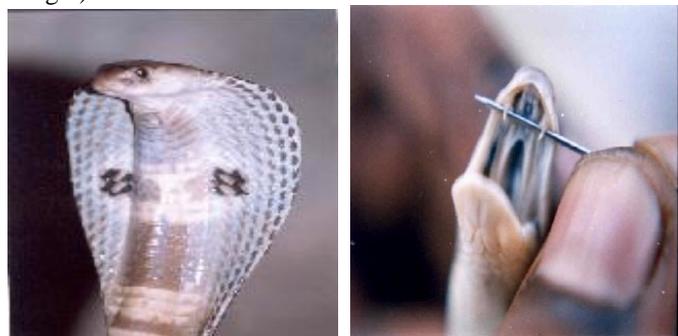
more than 65% of snake bitten patients were first taken to the traditional healers. In view of the monetary benefits given by the Government of Andhra Pradesh, India, under the scheme Apathbandhu to the dependants of those who die to snake-bite, several false cases of snake-bite have also been reported for claiming the compensation. Hence, it is of immense importance for the forensic experts to detect and quantify the snake venom residue in autopsy specimens of snake-bite victims so as to ascertain the exact cause of death and to prevent false claims⁽¹⁾.

2. EPIDEMIOLOGY

The following is the profile of snake bite cases in Andhra Pradesh state with the percent wise distribution in the districts^(2, 3).

Adilabad-1.75%, Nizamabad-9%, Medak-3.7%, Mahaboobnagar-17.5%, Rangareddy-6.25%, Nalgonda-6%, Kareemnagar-4%, Khammam-5.5%, Hyderabad-1.5%, Anathapur-1.5%, Chittoor-3%, Guntur-5.5%, E.Godavari-4.5%, W.Godavari-3.75%, Kudapah-1.75%, Krishna-5.75%, Kurnool-2%, Nellore-5%, Prakasham-3.75%, Sreekakulum-0.5%, Vijayanagaram-1%, Vizag-2.75%.

Venom:-The lethality of snake bite depends upon the dose of venom injected in to the human body, total storage capacity of venom sac and weight and length of the snake. Average adult cobra holds 8-10 drops of venom in its gland. The average quantity of poison measures about 0.5 to 1 gm by dry weight. When cobra bites, it usually injects 2-5 drops of poison but the fatal dose of cobra is about 1/2 drop only. The Krait contains totally 2-3 drops of venom in its sack but when it bites it injects 0.5 to 1 drop but the fatal dose of 1/10 the of the drop is sufficient. The Viper contains 15-20 drops of venom in its sack. When it bites it injects 4-5 drops. Fatal doses of common poisonous snakes are Cobra=12mgs, Viper=15mgs and Krait=6mgs (1 drop = 100 micro liters or 0.2gms by wet weight).



Clinical Manifestations:- Apprehension of impending death. Basing upon the dose of venom entered into the systemic circulation there are 4 grades of clinical evaluation of patients, like a) minimal, b) mild, c) severe and d) very severe envenomation. In brought dead cases, the victims are subjected to postmortem examination with the bio-chemical (ELISA) and histo-pathological examinations which are carried in the same institution and also in Dept. of Biochemistry O.U Science College.

1. Clinical evaluation of patient

1.1

1.1.1 Local Pain and swelling of bitten area with regional lymphadenopathy

1.1.2 Tachycardia and hypotension.

1.2 Neurological finding in progression are:

1.2.1 Drowsiness

1.2.2 Drooping of eyelid

1.2.3 Dysnoea with frothing

1.2.4 Dysphasia

1.2.5 Dysphagia

1.3 Paresis of neck, jaw and body muscles leading to Paralysis

1.4 Respiratory failure and finally cardiac arrest.

1.5 In viper bite victims there is a local cellulite with bleeding disorders internally as well as externally.

2. Postmortem Examination of snake bite victims:

On the external examination of dead bodies, there are features of either cyanosis or paleness with frothy discharge at the mouth. Bite marks are studied with magnifying lens, which appear like one, or two small abrasions marks or one to two small pinheads sized

punctured marks generally found with underlying ecchymosis and subsequent necrosis. In the internal examination of the dead bodies all the organs are congested with petechial hemorrhages and with collection of blood clot in the blood vessels DIC (Viper). The postmortem cardiac blood has been suggested to the most suitable specimen for venom detection⁽⁴⁾.

Earlier reports indicate that the preservation and examination of autopsy samples are important for exact quantification of venom^(5,1).



3. Materials and Methods:

1. Clinically, the patients are investigated for routine tests like complete blood picture with erythrocyte sedimentation rate, routine urine examination, random blood sugar, blood urea, serum creatinine, electro cardiogram, bleeding time, clotting time and SGPT etc. The anti-mortem samples of snake bite patients (blood, CSF samples along with skin scrapings and washings of bite area) obtained from the clinical wards and are subjected to Elisa test.
2. The various autopsy samples like vitreous humor, csf, blood from right ventricle, pieces of liver and kidney including the bitten skin bits of victims were collected from dead bodies and subjected them for both Elisa and histopathological examinations.
3. 42% of test samples of snake bite victims were collected from live patients from clinical wards and 29% samples were collected from the dead bodies while conducting postmortem examination.
4. Preservatives for clinical sample is prepared with 70% ethanol (95%vv) 2% glycerol + 28% 20mMPBS(PH-7.2) with 0.05% Thyme salt preservative for autopsy samples is prepared with 70% ethanol (95% v/v) + 2%

glycerin +28% 20m MPBS (PH-7.2) with 0.05% sodium azide salt. For fixing the bio sample tissue, a solution is made with 4% formaldehyde; 4gms of sodium phosphate, mono basic mono hydrate 4gms and sodium dibasic anhydrous 6.5gms and one liter of distill water. For preservation of autopsy samples a cocktail solution containing 70% + 2% glycerol +28%mm PBS (PH 7.2) and Theme salt is used ⁽¹⁾.

5. The autopsy of snake bite victims were immediately conducted for collection of investigatory test samples and preserved them in freshly prepared special solution of Thymer salt.
6. After collection of blood, csf, skin bite area scrapings, and its washings from the snake bite patients in the plastic container, the samples are preserved in Thymer salt solution.
7. Skin bits of snake bite area are also fixed in formalin for histopathological study.
8. The entire clinical as well as autopsy sample are preserved at -20⁰ C. The Immuno assay results of samples of 38 snake bite victims were analyzed in the department of Bio-Chemistry of University College of Science, O.U., Hyderabad, and 15 cases are found to be positive for cobra venom.(inclusive of cross reaction with 2 krait envenomed cases)^(6, 7).

Sources of Bio-sample collection from snakebite victims:

Previous reports have established the order of distribution of venom in different organs of the body which are: 1. Site of bite, 2. Heart, 3. Liver, 4. Kidneys, 5. Lungs, 6. Spleen and 7. Brain ⁽⁵⁾. In this study 5% of total cases, both the anti-mortem as well as the post mortem samples are collected and analyzed. In 42% of total samples comprising of skin scrapings, blood, CSF and viscera, are collected from the snake bite victims while they are undergoing treatment. 32% of total samples are collected from mortuary while doing post mortem examination. 26% of samples of snake bite victims are collected from the clinical wards which included discharged and absconded victims from the hospital.

Routine Bedside Clinical Investigations

In 55% cases complete investigations of snake bite victims could not be done various reasons like (a) Patient brought in gasping state and died immediately after admission, and also in some cases of absconding victims. In 45% of cases the minimum investigating like CBP with ESR, blood sugar, routine urine examination, blood urea, serum creatinine, bleeding time and clotting time, SGPT and SGOT and ECG etc are carried. In 52% of cases blood glucose is higher than 120mgs percent probably due to continuous glucose infusion. In 34% of cases of hospitalized snake bite victims, blood urea and serum creatinine are found to be elevated due to the effect of envenomation and progressive toxicity on the kidneys. Venom detection in tissues depends upon time laps between bite and death with extensive absorption redistribution and excretion ⁽⁸⁾ of venoms.

The post envenomation status:

39% of total cases are found to be of cobra bite in nature. In all cases of cobra bite, all samples had evidences of deposition of cobra antigen at the site of skin bite with evidences of cobra antigen even in the serum also. The largest quantity of venom over the skin bite area is noticed as 12ngm/ml. The largest quantity of venom found in the serum is 8ngm/ml. The average critical phase of survival is reported to be 8 hrs of post bite

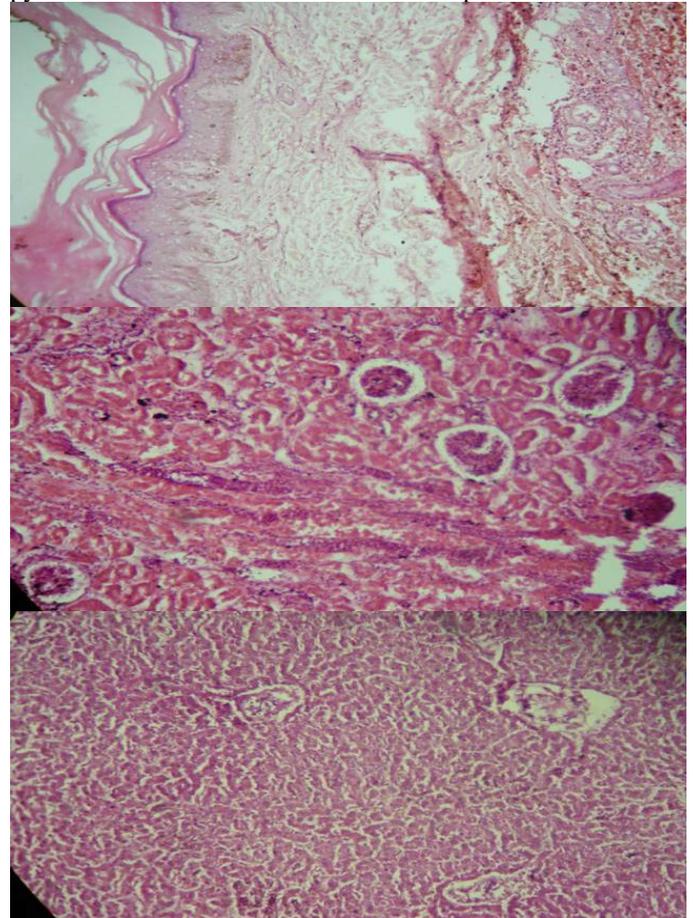
interval. Earliest period of detection of venom in the victims is 2hrs after bite. The longest hospital stay of the patient who has died was 10 days with the infusion of 26 anti-snake venom vials.

ELISA Test:

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: $y = 0.2581x + 0.4375$ ^(6, 7, 1)).

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.

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 Mesangiolyis. 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.

4. Results:

Survival status of snakebite victims

The total number of snake bites under study is =38

The number of snakebite patients admitted into the hospital=31(81%)

The number of snakebite victims brought dead to mortuary/causality=07(18%)

The number of snakebite patients absconded while undergoing treatment=02(5%)

The number of snakebite patients died while undergoing treatment=11(35%).

The following are the percentages of the multiple considerations involving the case studies.

Sex: The numbers of male victims are 55% and female are 45%. The number of male victims are more because of the close proximity with the snake's habitant while at work in the fields. In the previous studies reported from Ambajogai⁽⁹⁾ (Maharashtra) and Karnataka⁽¹⁰⁾, the male to female ratio was found to be 3.2:1 and 2:1, respectively. Studies from other countries also indicate male victim's preponderance. Male: female ratio was reported as 1.9:1 in Thailand and 1.3:1 in Pakistan⁽¹¹⁾. The predominance of male victims suggests a special risk of outdoor activity.

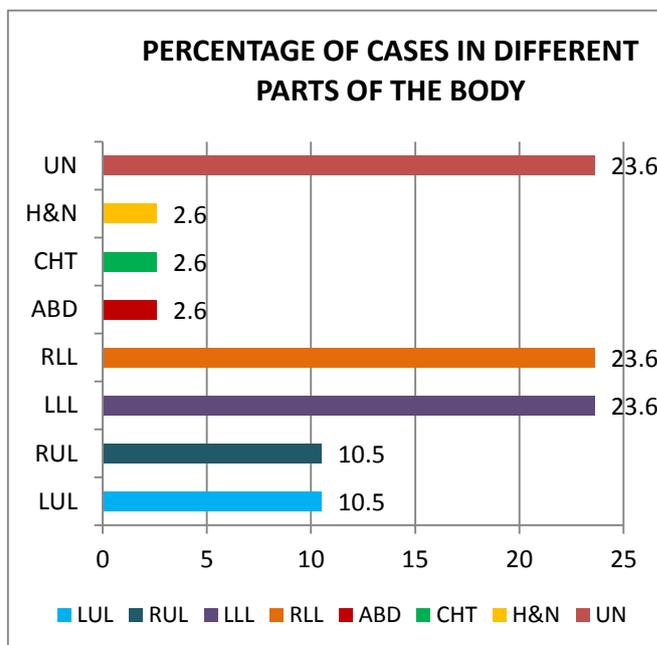
Age: The number of cases of victims are found to be more in the age group of 10-40(65%) because of their peak period of physical activities every day. The commonly affected age groups are also observed to be 10-40 years in Nepal⁽¹²⁾, 15-44 years in Pakistan⁽¹¹⁾ and 6-40 years in Zimbabwe⁽¹³⁾.

Seasonal Variation: Maximum numbers of snakebite cases are found during the period of June-September months (Monsoon period - 75% of total cases). The percentage is very less during the winter season as snakes are of cold blooded animals and they prefer to undergo hibernation. Highest incidences of snake bite cases are widely reported during the months of June to September of the year (JIPMER Hospital¹⁴).

Fang Marks: There are 29 snakebite cases, with the fang marks constituting 76% of the total cases. In the rest of cases the fang marks are not conspicuously visible.

Distribution of fang Marks

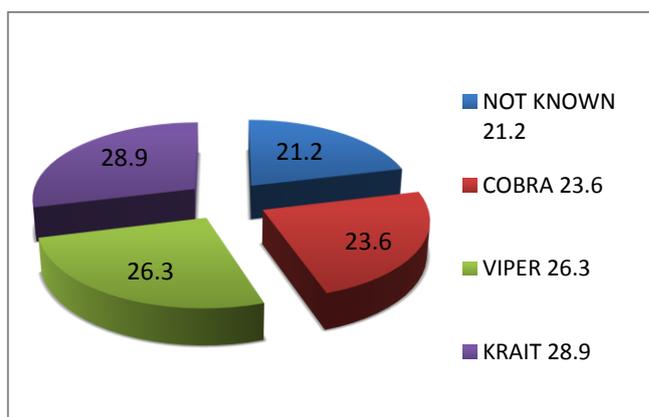
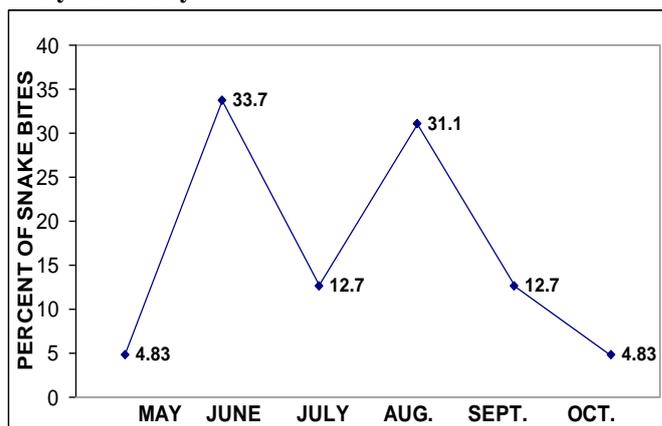
Majority of the cases showed the bite marks on the lower limbs, constituting 48% of the 38 total cases. Both side limbs are equally affected with snake bites sharing 24% on either side. Both upper limbs constitute 22% of 38 total cases of snake bite victims with equal sharing. Snake bites found over the least possible sites like abdomen, head & neck and chest constituting 3% each. In 24% of total cases the fang marks are of doubtful in nature with inconspicuous visibility.

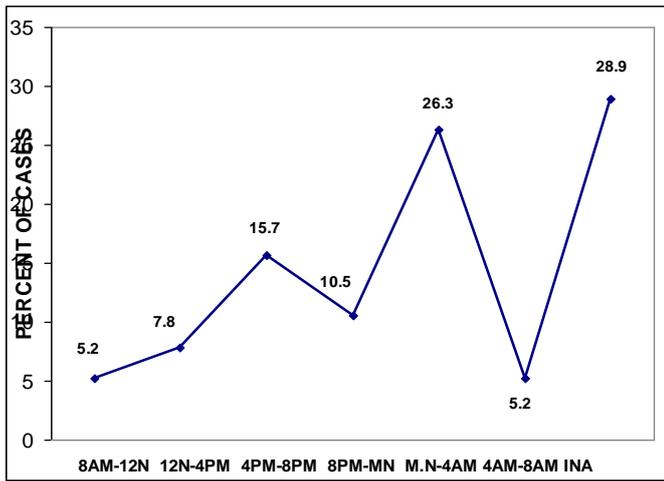


UN – Unknown, H &N – Head & Neck, CHT- Chest, ABD – Abdomen, RLL – Right Lower Limb, LLL – Left Lower Limb, RUL – Right Upper Limb, LUL – Left Upper Limb.

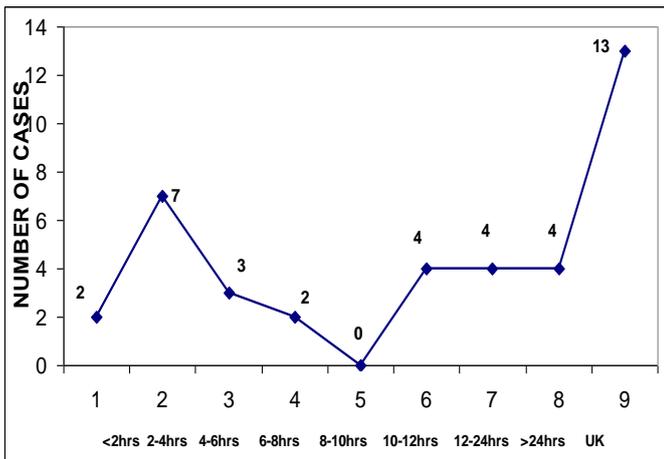
Scientific study on bio-clinical examination of snakebite victims:

Analytical Study:

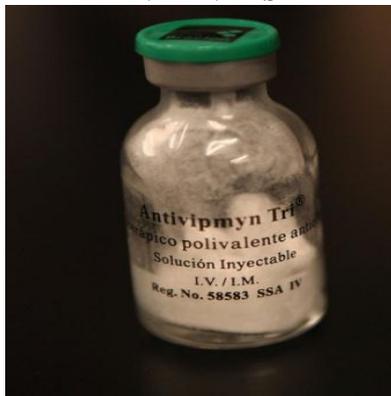




DIURNAL VARIATION IN TIME GROUP INTERVAL



POST BITE PERIOD IN DIFFERENT TIME GROUP INTERVALS



II. Analytical study on samples and survival of snakebite victims

Survived victims: Three cases are brought dead to casualty in total number of 38 cases of the snakebite victims. Out of 35 admitted cases 8% cases absconded on second day while undergoing treatment. 37% of the 35 cases have died while undergoing treatment consequent to envenomation. 54% successfully survived after treatment. Together with the 3 victims absconded (who are presumed to be alive), the total survivals of snake bite victims are 22 in number (66%).

5. Type of Clinical Presentation

The majority cases (53%) are found to have features of neurological envenomation (cobra and krait bites) with important findings of ptosis, dysphagia, dyskinesia and cholinergic secretions in the upper respiratory tract. In 26% of snake bite victims the cases are found to have bleeding and

clotting disorders (DIC) with local cellulites and clinical features of acute renal failure (viper bite). In 21% of snake bite victim the exact nature of envenomation could not be established even than conventional treatment is provided with ASV and other supportive treatment.

Identification of Offending Snake

The above study revealed Krait snakes took the 1st place (28.9%) by attacking the human beings. The next in the line are Viper (26.3%) and 2nd next is the Cobra (23.6%). Still there are good numbers of cases (21.2%) where the offending snakes could not be identified. Identification of Krait snake bite is more difficult and victim's awareness to its bite is less with more toxic manifestation comparatively because these snakes have a peculiar character of coming close to human habitat and staying near to their prey.

Scene of Snake bite:

Majority of snake biting cases are reported in the house to the frequency of 57% of total cases. 21% of these cases are found to have bitten by the snakes outside the houses. In 22% of rest of the cases scene of snake bite is not available.

Diurnal Variations of Snakebite:

72% Snake bite are commonly noticed during the night time, supporting the view that the snakes are more active, aggressive and reach the human surroundings during the period from 8:00 pm. to 8:00 am. In 28% cases of the envenomation took place in the day time when the human beings are busy and reach the snake habitat accidentally. There is a progressive increase in frequency of snake bites from 8:00 pm. to 8:00 am with sudden spurt of snake bite cases in the middle of the night. There is a slump in the biting cases from 4:00 am to 8:00 am.

Hospitalization with Post bite interval:

5% of cases which are admitted before 2 hours of snake bite period.

23% of total cases which are admitted around 3 hours of post bite interval.

34% cases are admitted before 24 hours of bite.

10% of cases which are admitted after 24 h of snake bite.

28% of cases information of time of bite is not clearly understood.

6. DISCUSSIONS:

As per statistical analysis, the awareness has increased in the society regarding the envenomation deaths and they are getting the assurances of the better survival status if, timely admitted. However still resorting to the unscientific treatment of snake bites has not yet been vanished from the minds of rural mass^(2, 3). The aim of this thesis primarily is to focus the overall collected risk factors impacting on the snake bite victims if medically not managed properly. The fatality due to snake bite is mostly an unnatural and an accidental event. In case of death medico legal autopsy has to be conducted over the dead bodies of the snake bite victims, because it clears the suspicious nature of death and grants the economical sanctions from the government⁽¹⁵⁾. There are some false cases on records presented for autopsy as cases of snake bites either with ignorance or with intentions. So postmortem examination of case of snake bite death is mandatory and is the rule medico legally. The following are the procedural conclusions in this follow up study of 38 snake bite victims.

1. Reporting of snake bite deaths are very much variable from region to region because of rural and urban factors^(16, 17) involving agricultural, economical,

educational, and social imbalances. Few decades back less than 20% of snake bite cases were reported to the hospital as per the statistical study

2. India has large number of snake bite victims, of which 65% are still consulting first the traditional unscientific healers in the villages.
3. In Andhra Pradesh deaths due to cobra and krait are more, unlike to coastal areas like Sri Lanka and Chennai, where bites are more with viper.
4. Worldwide incidences of snake bites are more during pre-monsoon, monsoon and summer season, because snakes are, active, hungry and aggressive when compared to the winter season. As snakes are warm blooded animals, they do not tolerate cold climate and go to hibernation during the winter period.
5. Incidences of snake bites is more in the males as is noticed in earlier studies, as was noticed in earlier

studies⁽¹⁰⁾, in this study also the males to females ratio is 5:4

6. As per the age groups study, the victims are in large number in the 10 to 39 years age group in support of earlier studies⁽¹⁾.
7. The offending snake is recognized by 21% of 38 snake bite victims.
8. Frequency distribution of fang marks in the snake bite victims are mostly confined to lower limbs and upper limbs
9. In 24% of cases, the fang marks could not be detected by naked eye

Frequency distributions of snake bite timing in these victims were found to be more during the period from 8pm to 8am explaining their nocturnal habitat⁽¹⁸⁾. Regarding hospitalization of the victims 23% of cases were admitted within 3 hours, 34% were admitted before 24 hours, and 10% of cases were admitted after 24 hours of snake bite⁽¹⁹⁾.

Table No.2: Analytical study on samples and survival of snakebite victims

S.No	Code number of the case	ELISA test (skin/ blood) Positive for cobra antigen	Concentration of cobra venom in ng.	Amount of ASV admission	Post bite interval before admission	Discharged alive or dead
Autopsy samples						
1	Case No.PCM 1991	+/+	7.0-9.0/5.0-8.0	20 vials	13 hrs	Died
2	Case No.GSR 2294	-/-	ND/ND	Spot death	8 hrs	Died
3	Case No.NGM 2760	-/-	ND/ND	2 vials	4 days	Died
4	Case No.RMN 53671/22919	+/-	8.0-10.0/ND	INA	INA	Died
5	Case No.CTR 5426	+/-	6.0-9.0/ND	20 vials	8 hrs	Died
Bio Samples						
6	Case No. YS 5425	+/+	9.0-11.0/ND	10 vials	5 hrs	Discharged
7	Case No.CTR2 5426	+/-	8.0-10.0/ND	20 vials	4 hrs	Discharged
8	Case No.VJA 20447	+/-	7.5-11.0/ND	6 vials	4 hrs	Discharged
9	Case No. VSN 19824/PM 1802	+/-	7.0-9.0/ND	INA	12 hrs	Died
10	Case No. RMH 26867	+/-	9.0-12.0/ND	10 vials	2 ^{1/2} hrs	Alive
11	Case No. VSN 19824/PM 1802	-/-	ND/ND	6 vials	INA	Alive
12	Case No. SHB 24798	+/-	6.0-8.0/ND	4 vials	6 hrs	Alive
13	Case No.HMG 22467	+/-	8.0-10.0/ND	4 vials	4 hrs	Alive
14	Case No. NKT 19337	+/-	3.0-5.0/ND	10 vials	12 hrs	Alive
15	Case No.YDG	+/-	10.0-12.0/ND	20 vials	2 hrs	Alive

Table No.1: Scientific study on bio-clinical examination of snakebite victims:

SUBJECT CODE NO. AGE/SEX	IP No. 24798 25/F (11)	IP No. 28332 23/M (15)	IP No. 34136 18/M (20)	IP No. 19091 55/F (1)	PME No. 1835/04 55/F (1)
1. Time of bite (TOB)	14/07/2004	10/08/2004 at 2:20 P.M.	20/09/2004 at 10:30 P.M.	03/06/2004 at 11 P.M.	14/06/2004
2. Time of Adm. (TOA)	14/07/2004	10/08/2004 at 4:25 P.M.	21/09/2004 at 11:15 P.M.	04/06/2004 at 2:45 P.M.	14/06/2004 at 2:45 A.M.
3. Time of disc./death (TOD)	23/07/2004	12/08/2004 at 2:45 P.M.	25/04/2004 at 3 P.M.	14/06/2004 at 10:15 P.M (Died)	14/06/2004 at 10:15 P.M.
Particulars of envenomation	Neuroparalysis (Cobra/Krait)	Bleeding stains over the bite	Neuroparalysis (Cobra/Krait)	Neuroparalysis (Cobra/Krait)	Neuroparalysis (Cobra or Krait)
Particulars of snake bite victim	-----	Bite over left hand – while working in the field	Bite over the left side of the abdomen	Lt calf - while sleeping	While in sleep in her house, in the night. Bite marks over right pop. fossa
Whether the victim 1. Survived 2. Died while undergoing treatment 3. Spot death	Survived	Survived	Survived	Died	Died while undergoing treatment
Date and source of sampling	Hospital ward: 14/07/2004 Blood, Skin, Scr.	Hospital ward: 10/08/2004 Blood, CSF	-----	Hospital ward: 14/06/2004 Blood, CSF, Skin, Scr.	Mortuary: Blood, CSF, Skin, Viscera
Hospital investigation	B. Sugar: 140 B. Urea: 24 S. Creat: 0.8mg	B. Sugar: 70 B. Urea: 24 S. Creat: 0.7mg C.T: 4.5 min B.T: 3.3 min	-----	B. Sugar: 90 B. Urea: 14 S. Creat. 0.8mg	B. Sugar: 340 B. Urea: 50 S. Creat: 0.7 SGPT: 260 ESR: 130/170
Treatment of including Ante snake venom	ASV 4 VIALS Manocef Metro Chymoralfort	ASV 1 VIAL IV Fluid	ASV 12 VIALS Atropin Neostigmine IV Fluid	ASV 20 VIALS Atropin Neostigmine IV. Fluid	ASV 20 VIALS IV Fluids Endotrachial IT Neostigmine Atropine Manocef
Type of snake venom detected on ELISA	Positive for cobra 6-8ng/dl (in skin)	-----	-----	Positive for cobra in 8- 10ng/dl (in skin)	Cobra positive 6-9ng/dl (in skin)

IP no: inpatient number, PME No: postmortem examination number, C.T: clotting time, B.T: bleeding time, ASV: anti snake venom, ELISA: *Enzyme linked immune sorbent assay*.

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