DISSERTATION

on

“STUDY OF HAEMATOLOGICAL AND COAGULATION PROFILE IN SNAKE BITE PATIENTS IN GMKMC, SALEM”

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requirements for

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THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI

GOVERNMENT MOHAN KUMARAMANGALAM

MEDICAL COLLEGE

SALEM

MAY 2020

CERTIFICATE

This is to certify that this dissertation entitled as **"STUDY OF HAEMATOLOGICAL AND COAGULATION PROFILE IN SNAKE BITE PATIENTS IN GMKMC SALEM",** is a bonafide work done by **Dr. B. ARUL VARUNI**, Post Graduate Student, Department of Pathology, Govt Mohan Kumaramangalam Medical College, Salem, in partial fulfillment of the university rules and regulations for the award of MD DEGREE in PATHOLOGY BRANCH-III, during the academic period from January 2018 to June 2019.

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DECLARATION

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ABBREVATIONS

APC : Activated protein c

APTT : Activated thromboplastin time

BT : bleeding time

EPCR : Endothelial cell protein c Receptor

3 FTX : Three finger toxin

GP1b : Glycoprotein 1 b

GP2a – 3b : Glycoprotein 2a – 3b

HMWK : FM GERALD

PAI -1 : Plasminogen activator inhibitor -1

pNA : para nitro aniline

PT : prothrombin time

PLA2 : Phospholipase A2

SERPINS : serine protease inhibitors

TT : Thrombin time

TFPI : Tissue factor pathway inhibitor

TLE : Thrombin like enzyme

TPA : Tissue plasminogen activator

UPA : Urokinase plasminogen activator

VWF : Von wiilibrand Factor

VICC : Venom induced consumption coagulopathy

WBCT : whole blood clotting time

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INTRODUCTION

Snakes are fascinating part of Nature. There are over 3000 species of snakes in the world, of which more than 200 snakes ranging in size from 100 mm long – worm snakes to 6 m long pythons are found in India.[53]

Snakes occur in most habitats from sea to deserts, from swamps , lakes and farmlands up in to the mountains. These predators play a major role in maintenance of ecosystem.[54]

Snake bite is a common emergency encountered in day to day practice. In tropical and subtropical countries, morbidity and mortality due to snakebite is a preventable health hazard. In India, as the major portion of the population is rural and dependant on agriculture, and due to prevailing climatic conditions snake bite is a major health problem.[54,55]

In India, 497 per 100,000 population has been estimated as the annual incidence of snake bite. Every year 15,000 die out of 2,00,000 snake bite in India , thus 7.5 % is the approximate death rate.

In India most common species found are Cobra (Naja Naja) , Common Krait (Bangarus caerulus) Saw-Scaled Viper (Echis carinatus) and rusell's Viper (Daboia Rusell). Following snake bite, the victim develops symptoms such as local pain, edema, systemic complications, acute renal failure, neurological abnormalities, hemorrhage, infarction, and ultimately resulting in death.

Morbidity appears to be high, even in the absence of death. One of the most common manifestations in these cases is coagulopathy. Abnormality in coagulation can be detected by blood coagulation tests. We have chosen a study to emphasis on the need of various coagulation tests and their role in the management of snake bite.

AIM AND OBJECTIVES OF THE STUDY

- To document the Age of Occurance of snake bite
- To document the Predominant sex affected in our study population
- To document the Predominant bite site involved
- To study the Species of snakes that commonly cause coagulation abnormality
- To Analyse the clinical presentation of the victims
- To Analyse the coagulation abnormality before and after Anti snake venom therapy
- To find out the Primary Mechanism involved in snake bite

REVIEW OF LITERATURE

- **NORMAL HEMOSTASIS**:

Normal hemostasis constitutes a series of regulated process that maintains blood in fluid state in normal vessels, also forms a localized haemostatic plug at the site of vascular injury. The three elements involved in hemostasis are vascular wall, platelets and coagulation cascade.[1]

- **STAGES OF HEMOSTASIS**:

Hemostasis occurs in three stages namely primary hemostasis,secondary hemostasis and fibrinolysis.[2]

- **Primary hemostasis:**

The formation of initial platelet plug is called primary hemostasis. Following vascular injury, reflex neurogenic mechanisms cause transient arteriolar vasoconstriction. Endothelial injury exposes subendothelial extracellular matrix [ECM] facilitating platelet adherence, activation and aggregation, thus forming the platelet plug. It is a rapid, short-lived process . [1]

- **ROLE OF INTACT ENDOTHELIAL CELLS** [anticoagulant properties]

The anticoagulant properties of intact endothelial cells are due to :

1] rhomboid cells [EC] presenting a smooth, continous surface secrets the eicosanoids, platelet inhibitor prostacyclin.

2] secretes vascular relaxing factor nitric oxide

3] secretes anticoagulant glycosaminoglycans ,heparin sulphate

4]secretes coagulation extrinsic pathway regulators,tissue factor pathway inhibitor

5]express endothelial protein c receptor

6] express cell membrane thrombomodulin,a protein c coagulation control system activator

7] secretes tissue plasminogen activator, thus ultimately inhibiting platelet adhesion , platelet aggregation.[3,14]

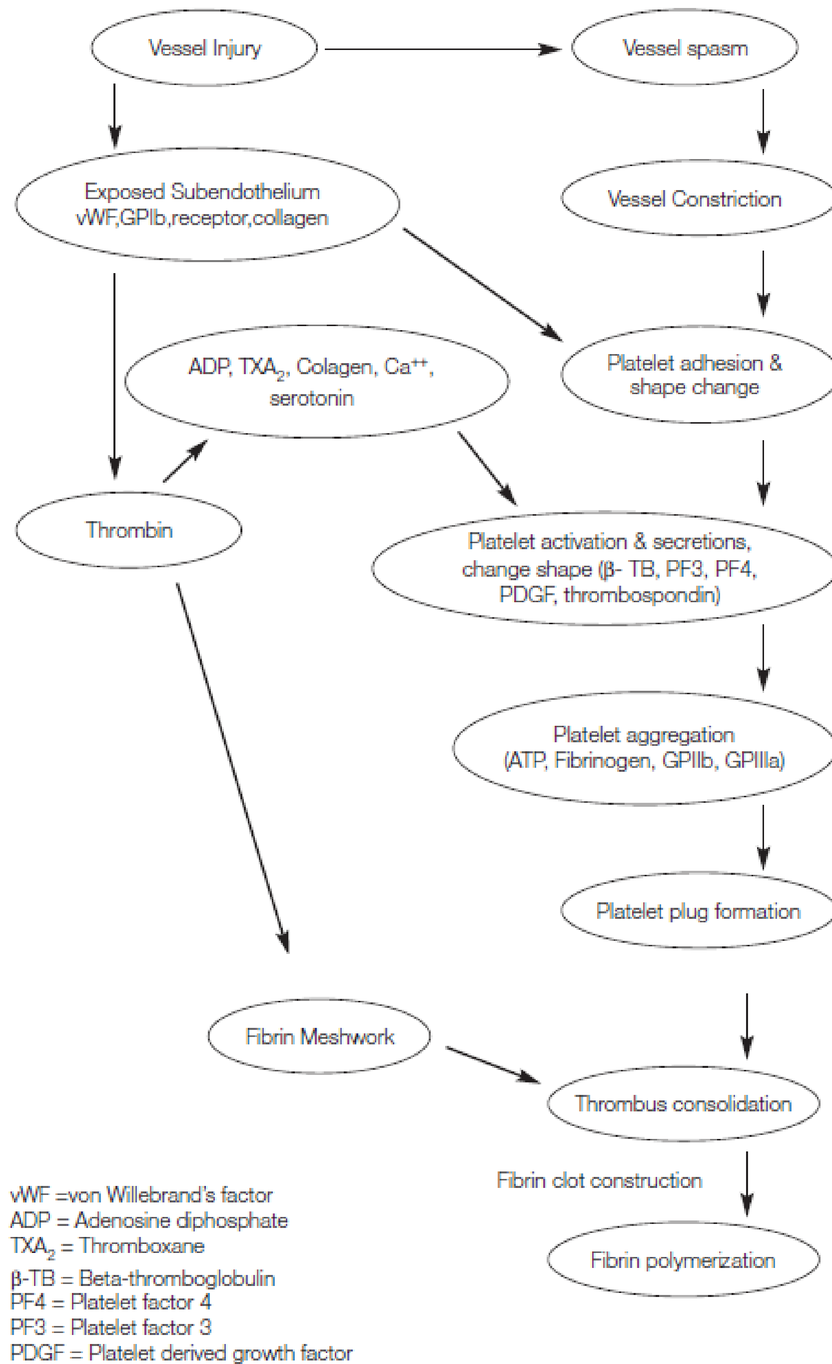
- **PROCOAGULANT PROPERTIES OF DAMAGED VASCULAR INTIMA**:

Vasoconstriction is induced by the smooth muscle cells in arterioles and arteries. Damaged endothelial cells secrete von willibrand factor[VWF] and adhesion molecules such as P-selectin, ICAMS, PECAM which helps in binding of VWF and platelets to exposed subendothelial collagen.Exposed smooth muscle cells and fibroblasts secrete tissue factor exposed on cell membrane, thus propagating secondary hemosatsis.[3,14]

- **ROLE OF PLATELETS IN PRIMARY HEMOSTASIS:**

Platelets are anucleate cell fragments produced by marrow megakaryocytes. Platelet surface has glycoproteins like GP1b-receptors

and GP2b -3a.They are essential for attachment of platelets to VWF which in turn attaches to vascular endothelial cells.[13]



Flowchart showing primary haemostasis

SECONDARY HEMOSTASIS:

Coagulation cascade is a successive series of enzymatic reactions. It is a interdependent, enzyme mediated reactions, resulting in the generation of thrombin,an enzyme which converts soluble protein fibrinogen to insoluble fibrin, finally stabilizing the platelet plug.

- **THE COMPONENTS OF SECONDARY HEMOSTASIS ARE :**

1] procoagulants / coagulation factors

2] coagulation cascade

3] fibrinolytic system or inhibitors of coagulation. (1)

- **COAGULATION CASCADE:**

Factor I [FIBRINOGEN]

Factor II [PROTHROMBIN]

Factor III [tissue factor]

Factor IV [calcium]

Factor V [Proaccelerin]

Factor VI [labile factor]

Factor VII [proconvertin stable factor]

Factor VIII [antihemophilic factor]

Factor IX [plasma thromboplastin factor]

Factor X [Stuart factor]

Factor XI [plasma thromboplastin antecedant]

Factor XII [Hageman factor]

Factor XIII[fibrin stabilizing factor]

HMWK FITZGERALD

PK Fletcher factor(1)

Fibrinogen :

It is a 340,000 dalton glycoprotein synthesized in the liver .The normal plasma concentration of fibrinogen ranges from 200 to 400 mg/dl. It is the primary substrate of thrombin which converts soluble fibrinogen to insoluble fibrin to produce a clot. Fibrinogen is also responsible for platelet aggregation and also a acute phase reactant protein.[3,20]

Prothrombin, thrombin:

It plays a vital role in the conversion of fibrinogen into cross linked fibrin.It also activates factor XIII, which stabilizes the hemostatic plug. Thrombin plays a potent role in the activation and aggregation of platelets. IT also has pro-inflammatory and anticoagulant effects.[1,10]

Function of thrombin :

- pro-inflammatory properties are due to the release of of endothelial cell cytoadhesion molecule, chemotactic factors, increased endothelialm production of IL-1 ,IL-6, IL-8

- cellular proliferative property is due increased production of platelet derived growth factor and transforming growth factor –beta from platelets.
- anticoagulant property is due to protein c activation, prostacycclin formation, endothelium derived relaxing factor, tissue plasminogen activator production.

Factor III (Tissue factor):

It is a cofactor which is found on fibroblasts and smooth muscle cells.It helps in the formation of extrinsic tenase complex .[3]

Factor IV (calcium) :

It is a cofactor with plasma concentration of 8.8 to 10.5 mg /dl.[3]

Factor V (proaccelarin):

It is a cofactor, a soluble plasma glycoprotein, activated by thrombin, inactivated by protein c. It forms the prothrombinase complex which increases thrombin generation.[3]

FACTOR VII (PROCONVERTIN STABLE FACTOR):

It is a single chain glycoprotein with a molecular weight of 50,000.It is a serine protease ,also a constituent of extrinsic tenase complex.[3]

Factor VIII (ANTIHEMOPHILIC FACTOR) :

It is a cofactor , soluble plasma protein, activated by thrombin and inactivated by protein c .It is produced by primary hepatocytes, circulated in bound form with vonwillibrand factor. It forms intrinsic tenase complex with Fac IXa and calcium.[3]

Factor IX (plasma thromboplastin antecedent):

It is a single chain glycoprotein with a molecular weight of 57,000, with a half life of 18 – 24 hours.It is a constituent of intrinsic tenase complex.[2]

Factor X (stuart factor) :

It is a two-chain glycoprotein with a molecular weight of 59,000.It is as serine protease and constituent of intrinsic prothrombinase complex.[2]

Factor XI (plasma thromboplastin antecedent):

It is a two chain glycoprotein with a molecular weight of 143,000.It is a serine protease which acts as a contact factor.[2]

Factor XII (Hageman factor):

It is a serine protease which acts as a contact factor.It is a single chain glycoprotein with a molecular weight of 80,000.[2]

Factor XIII:

It is a transglutaminase that catalyses the formation of covalent bonds between carboxyl terminals of gamma chains from adjacent D-domains in the

fibrin polymer.favtor XIIIa reacts with other plasma and cellular structural proteins and is essential to wound healing and tissue integrity.[3]

COAGULATION CASCADE:

The coagulation cascade occurs in two pathways namely intrinsic pathway and extrinsic pathway.[15,18]

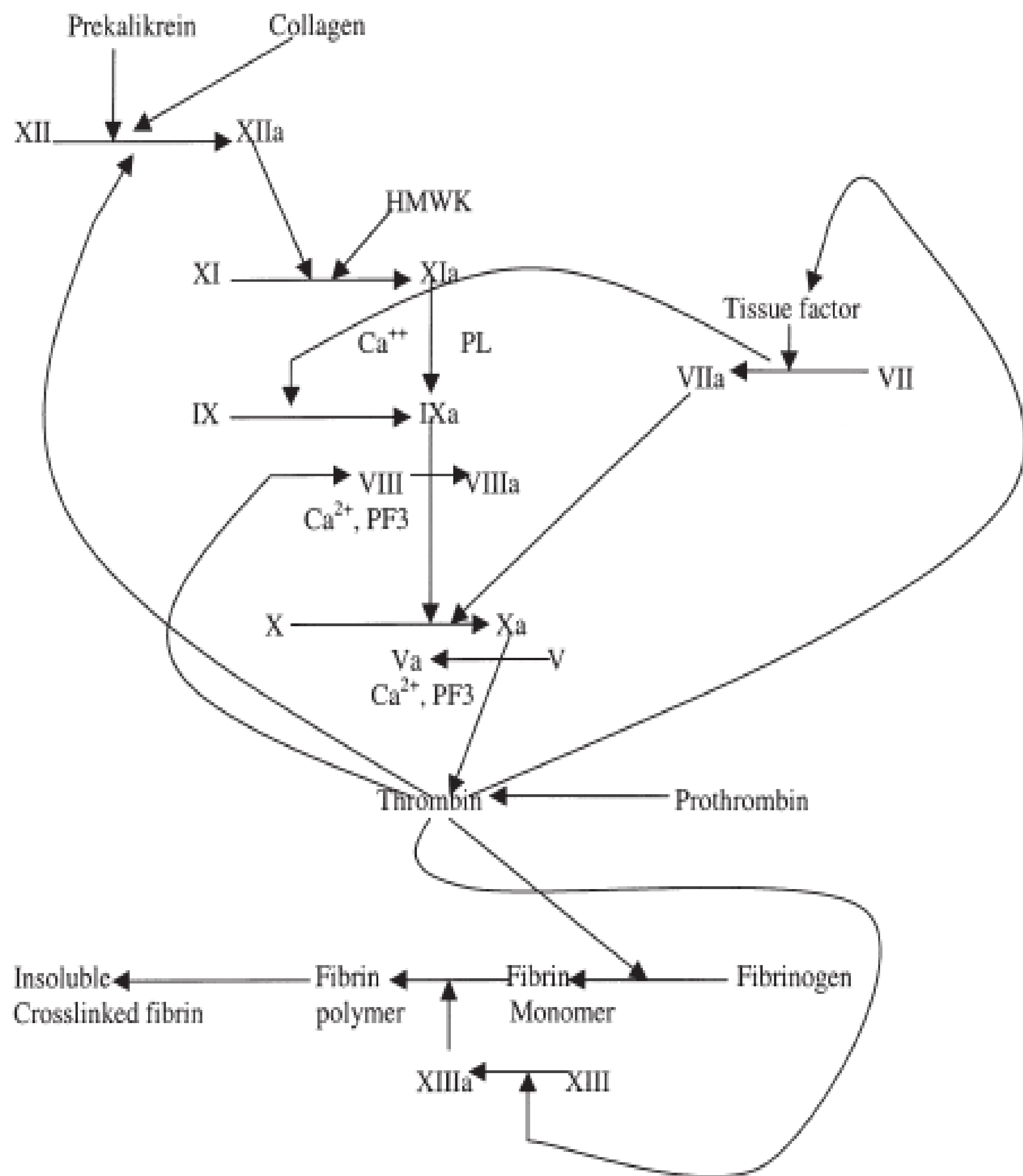
It takes place in two phases namely initiation and propagation phase.

INITIATION PHASE:

It occurs over cells expressing tissue factor such as fibroblast, subendothelial cells, resulting in the formation of extrinsic tenase complex and generation of small amount of factor Xa, IXa and thrombin. 3-5% of thrombin is generated in initiation phase. Additionally platelets, cofactors, and procoagulants become activated, fibrin formation begins and initial platelet plug is formed.[3,12]

PROPAGATION:

More than 95% thrombin generation occurs during propagation,occurring on the surface of activated platelet.these are refered to as COAT platelets-partially activated by collagen and thrombin.The activated platelets ,cofactors and procoagulants results in the formation of intrinsic tenase complex IXa: VIIIa, progressively activates prothrombinase complex,followed by cleavage of fibrinogen into fibrin clot, also activates factor XIII to stabilize the clot.[3,12]



Flowchart showing coagulation cascade

COAGULATION REGULATORY MECHANISM:

A delicate balance between thrombosis and abnormal bleeding is established by coagulation inhibitors and cofactors.These natural anticoagulants ,function to slow the activation of procoagulant and suppress thrombin production.The principle regulators are:

1. tissue factor pathway inhibitor
2. protein c regulatory system
3. antithrombin and other serine protease inhibitor.[2,26]

Tissue factor pathway inhibitor:

It is synthesized primarily by endothelial cells and also expressed on platelets.It inhibits coagulation in a two step process by first binding and inactivating Xa . The TFPI:Xa complex binds to TF:VIIIa, forming quaternary complex and preventing further activation of X and IX.Protein S acts as a cofactor for TFPI and enhances factor Xa inhibition by TFPI tenfold.[3,11]

Protein c regulatory system:

Thrombin produced as a result of coagulation cascade activation binds the endothelial cell membrane protein,thrombomodulin and triggers protein c anticoagulant system.Thrombin after binding with thrombomodulin together activates proteincp, which binds to endothelial cell protein c receptor[EPCR].free protein s binds and stabilizes activated protein c[APC].The APC /protein s complex digests and inactivates factor Va.(3)(23)(24) Protein s.[21,22]

Antithrombin and serine protease inhibitors[SERPINS]:

Antithrombin is a serine protease inhibitor which binds and neutralizes serine proteases like thrombin[fac IIa],factor IXa, Xa, Xia, XIIa, prekallikrein ,plasmin.Anti thrombin covalently binds thrombin forming an inactive thrombin-antithrombin complex [TAT], finally inhibiting clot formation.[3,17]

Fibrinolysis :

It is the final stage of coagulation,in which there is systematic ,accelerating hydrolysis of fibrin by bound plasmin.It begins a few hours after fibrin polymerization and cross linking.The two activators of fibrinolysis are tissue plasminogen activator [TPA] and urokinase plasminogen activator [UPA], which are released in response to inflammation and coagulation.

Tissue plasminogen activator [TPA] : it is secreted by endothelial cells. Circulating TPA is bound to inhibitors such as plasminogen activator inhibitor -1 and cleared from plasma.[16]

Urokinase plasminogen activator [UPA]: it is a intrinsic plasminogen activator secreted by urinary tract epithelial cells ,monocytes and macrophages.[3]

CONTROL OF FIBRINOLYSIS:

Plasminogen activator inhibitor 1 [PAI-1]:

It is a single chain glycoprotein serine protease inhibitor. It is produced by endothelial cells, megakaryocytes, smooth muscle cells, fibroblast, monocyte, adipocytes, hepatocytes and other cell types.It is the principal inhibitor of plasminogen activation, inactivating both TPA and UPA, thus preventing them from converting plasminogen to fibrinolytic enzyme plasmin.[27,19]

Alpha-2 antiplasmin:

It is the primary inhibitor of free plasmin , which is synthesized in the liver.[28]

Thrombin activatable fibrinolytic inhibitor:

It is synthesized in the liver, which is a plasma procarboxy pepetidase.It is activated by thrombin-thrombomodulin complex.It also blocks the formation of plasmin thereby inhibiting fibrinolysis.[29,30]

FIBRIN DEGRADATION PRODUCTS AND D-DIMER:

Fibrin,thus formed by coagulation cascade is cleaved by plasmin and produces a series of identifiable fibrin fragments X,Y,D,E and D-Dimer .Many of these factors inhibit hemostasis and contribute to hemorrhage by preventing platelet activation and by inhibiting fibrin polymerization.[3,31]

LABORATORY INVESTIGATIONS OF HEMOSTASIS

The quality of our results (PT, APTT, TT,FIBRINOGEN) were periodically assessed with external NABL accrediated standard lab.

Collection of venous blood samples and precautions to be undertaken:

- The patient must be relaxed and in warm surrounding ,as excessive stress and vigorous exercise will increase factor VIII ,VWF and Fibrinolysis.
- Venous blood should be collected by means of a clean venepuncture. The tourniquet must be removed as soon as the needle is in the vein.
- 21 guage number needle , length not exceeding 3.5 cms and a [lastic syringe is recommended.
- The venous blood is rapidly transferred to a plastic (polypropylene) or siliconized glass tube (13 x 100 mm) , containing 3.2 % trisodium citrate solution.
- The blood should be allowed to run down the side of the tube, should never be squirted into thetube.
- The tube should be screw capped .It should be quickly but gently mixed by inverting 5-7 times without producing frothing.
- The venous sample must be transported as quickly as possible.
- The venous sample must be centrifuged within 30 minutes of collection ,at 3500 to 4000 rpm for 15 minutes at 4 deg Celsius centrifuge.
- The centrifuged sample can be tested within 4 hours.[4,32,33]

Picture : 3.2% Trisodium citrate tube

Choice of Anticoagulant:

Trisodium citrate is the commonly used anticoagulant for coagulation samples. Here 9 volumes of blood is added to 1 volume of anticoagulant.[5,34]

Automated coagulation analyser:

The principle on which the hematology analyzers' work are Electromechanical clot detection system, Photo-optical, Chromogenic and immunologic method individually or a combination of these principles.[5,43]

Electromechanical clot detection system:

It is a viscosity based detection method. Here the instrument measures the motion of a steel ball inside a cuvette in a magnetic field .The viscocity of plasma increases as clot forms and the amplitude of ball motion decreases. This

method is insensitive to colored and hemolysed plasma, lipedemic plasma, bilirubin and turbid reagents.[5,43]

Photoelectric:

This method measures the increase in turbidity when fibrinogen is converted to insoluble fibrin.In this method reagents and plasma are pipette into cuvettes, after precise mixing and incubation phases, the change in plasma optical density and the end point of clot formation are measured.[5,43]

Chromogenic detection methods:

This method is based on the use of chromophore,a color specific generating substance.Para nitroaniline (pNA),a commonly used chromophore, has a maximum absorbance of 405 nm.In this method the chromophoe is conjugated to a colorless, synthetic oligopeptide substrate which mimics the target sequence of activated coagulation factor being measured.The chromogenic substrate is cleaved by coagulation protease at specific binding site in the amino acid sequence.The pNA is released, and a photodetector measures the optical density at 405 nm.[5,43]

Immunologic detection methods:

In this method the latex particles are coated with a specific antibody against the antigen which is to be determined.A monochromatic light beam with a wavelength larger than the diameter of suspension microparticles passes through latex microparticle suspension.An increase in agglutination results in

increased light absorbance.The antigen level is proportional to the amount of light absorbed.[5,43]

Picture : Semi automated coagulation analyser

Platelet count:

Platelet count were assessed by peripheral smear study under microscope.

Manual method: A rough estimate can be made by evaluating peripheral blood smear. There should be 8 to 20 platelet per oil immersion field or one platelet present for every 20 red blood cells.[5]

Bleeding time:(TEMPLATE METHOD)

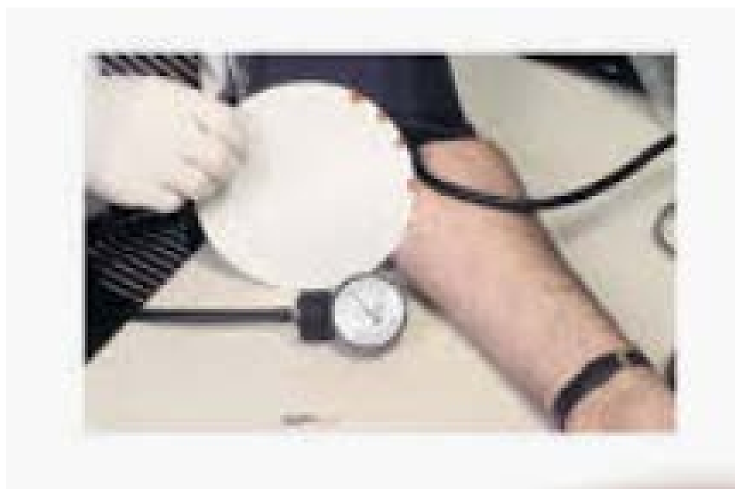
Principle:

It measures the time that elapses after infliction of a standard wound and the arrest of bleeding , which depends largely on the rate of formation of platelet plug, independent of fibrin forming coagulation mechanisms.

Procedure:

- Blood pressure cuff is placed around upper arm and inflated to a pressure of 40mmhg
- Skin of forearm is cleaned with spirit and allowed to dry.
- With the help of a scalpel blade an incision 9 mm in length and 1 mm depth is made in cephalo - caudal direction, about 5 cm distal to the crease of the elbow.
- Timer is started immediately
- Drops of blood are blotted with filter paper
- Repeat at 30 second intervals
- If, blood no longer stains the filter paper, timer is stopped, time is recorded.[4,42]

Normal bleeding time: 2-5 minute



Picture : Bleeding time (Template method)

CLOTTING TIME:

It is the time required for a sample of blood to coagulate in vitro under standard condition.

Indication: monitoring anticoagulant therapy

Prerequisites:

- Sterile dispersible pricking needle or lancet
- Stop watch
- Dry glass capillary tube(narrow diameter 2 cm,10 cm long)
- Cotton swab
- 70% ethyl alcohol

Procedure:(capillary tube method)

- Sterilize the finger tip with spirit and allow it to dry
- Punch the finger tip with a lancet
- Let the blood flow without squeezing
- Timer is started
- Introduce the capillary tube into the blood drop collected on the finger tip
- Blood enters the capillary tube by capillary suction
- Make sure that the capillary tubes are completely filled up
- Break the tube at 30 seconds interval until fibrin thread is formed
- The time from pricking to fibrin thread formation is clotting time
- Normal values: 8 -15 minutes

Figure : Clotting time (Capillary tube method)

PROTHROMBIN TIME [PT]: (**MANUAL**)

- It measures the efficiency of extrinsic pathway.

Reagents and equipments:

- Heat block 37 degree Celsius
- Thromboplastin calcium chloride mixture(thromborel)
- Control samples(normal and abnormal)
- Test tubes 12mm x 75mm
- Stopwatch

Specimen:

Citrated plasma-one part sodium citrate to nine parts whole blood

Principle:

The calcium in the whole blood is bound to sodium citrate, thus preventing coagulation. Tissue thromboplastin to which calcium has been added is mixed with plasma and the clotting time is noted.

Procedure:

Centrifuge anti coagulated blood at 2500 rpm for 10 minutes as soon as possible after collection.

Pipet 0.2 ml off thromboplastin- calcium mixture into a set (4-6) of 12 x 75 test tubes.

- Warm the test tubes in 37 degree Celsius heat block for atleast 1 minute, until they reach 37 degree Celsius
- Incubate a portion of plasma for approximately 2 to 3 minutes until it reaches 37 degree Celsius. Plasma should be incubated for no longer than 10 minutes after reaching 37 degree Celsius
- Forcibly inject 0.1 ml of patient,s plasma into the test tube containing0.2 ml of thromboplastin - calcium mixture band simultaneously start the stop watch
- Remove the tube from the heat block and mix the contents of the tube
- Hold the tube such that contents can be monitored for formation of clot

- Gently tilt the tube back and froth until a clot forms at which point timing is immediately stopped.[5]

PROTHROMBIN TIME -AUTOMATED

- ❖ Take 50 microlitre of plasma in cuvette
- ❖ Add 100 microlitre of thromborel-s mixture
- ❖ Wait for 120 seconds
- ❖ Note the reading

Normal range : 12 to 14 seconds.[5]

Method:

0.1 ml of plasma is taken in a glass tube and placed in a water bath, to which o.1 ml of thromboplastin is added.allow the mixture to warm for 1-3 minutes.then 0.1 ml of calcium chloride is added and time noted.[35]

Normal values: 12 – 14 seconds.[5]

ACTIVATED PARTIAL THROMBOPLASTIN TIME [APTT]

(MANUAL)

It measures the efficiency of intrinsic pathway.

Reagents and equipments:

- Heat block 37 degree Celsius
- Calcium chloride 0.025 M
- Partial thromboplastin containing activator

- Control samples(normal and abnormal)
- Test tube, 12 mm x 75 mm
- Stop watch

Specimen :

Citrated plasma –one part sodium citrate to nine parts whole blood.

Principle:

The calcium in whole blood is bound by sodium citrate thus preventing coagulation.the plasma after centrifugation contains all intrinsic coagulation factors except calcium and platelets.IN aptt, partial thromboplastin and an activator are added to plasma allowing coagulation cascade to begin.During incubation,factors x11,pk 11 are activated building up the levels of 11a in reaction tube. Once calcium chloride is added rest of coagulation cascade is allowed to continue and timing of the event is obtained. The time required for the plasma to clot is the activated partial thromboplastin time.

Procedure:

- Centrifuge anticoagulated blood at 2500rpm for 30 minutes as soon as possible after collection.
- Incubate calcium chloride at 37 degree Celsius for five minutes.
- Pipet 0.1 ml of actin fs into the test tube.
- Pipet 0.1 ml of patient’s plasma into the same test tube (containing actin fs).

- Mix the contents well.
- Incubate the contents at 37 degree Celsius for three minutes.
- Add 0.1 ml of preformed calcium chloride to the same test tube containing the mixture, simultaneously start the stop watch.
- Observe the clot formation.[5]

ACTIVATED PARTIAL THROMBOPLASTIN TIME (AUTOMATED)

- ❖ 50 micolitre of prewarmed platelet poor patient's plasma is added in the cuvette
- ❖ Add 50 microlitre of actin fs reagent in the same cuvette,followed by adding 50 microlitre of calcium chloride.
- ❖ Wait for 180 seconds
- ❖ Note the reading

Normal range : 28- 35 seconds

Method:

Equal volumes of phospholipid reagent and kaolin suspension is added to a glass tube and left in water bath at 37 degree Celsius.In another glass tube 0.1 ml of plasma is taken to which 0.2 ml of kaolin-phospholipid mixture is added and left in the waterbath for 10 minutes. To the second glass tube 0.1 ml of calcium chloride is added and stop watch started.[36]

Normal values: 28 – 35 seconds.[5]

THROMBIN TIME [TT]:(MANUAL)

Reagents

Platelet poor plasma from the patient and a control Thrombin solution- freshly diluted in barbitone buffered saline in a plastic tube.

Procedure:

- Add 100 microlitre thrombin solution to 200 microliter of control plasma in a glass tube at 37 degree Celsius
- Start the stop watch measure the clotting time and observe the nature of clot (whether transparent or opaque , firm or wispy)
- Repeat the procedure with two tubes containing patients plasma in duplicate and then with a second sample of control plasma

Normal range: 1 to 19 seconds+/- 2 seconds

THROMBIN TIME (AUTOMATED)

- 1.8 ml of citrated blood is centrifuged for 15 minutes at 2800rpm
- 100 microlitre of platelet poor plasma is added to cuvette
- To the same tube 50 microlitre of thrombin reagent is added
- Waith for 180 seconds
- Note the reading

Normal range: 12 -15 seconds.[5]

Method:

To 200 micro litre of plasma, 100 microlitre of thrombin solution is added in a glass tube at 37 degree celsius and stop watch is started, end-point noted. [(37) (41)]

Normal value: 12 – 15 seconds

Quantitative fibrinogen assay (Clauss technique):

It is a clot based functional measurement. In this thrombin is added to various dilutions of known concentrations of fibrinogen, which produces thrombin CT [(39) (40)]

Normal range : 150 – 400 mg /dl

IDENTIFICATION AND DISTRIBUTION OF SNAKES

There are about 2500 to 3000 species of snakes of which about 500 belong to the five families of venomous snakes, atractaspididae, elapidae, hydophidae, colubridae and viperidae. In india 236 different species of snakes are found out of which 50 species are reported to be poisonous. Poisonous snakes prevalent in india belong to four families, they are

- Elapidae - Includes Cobras and krait
- Viperidae – Russell Viper and Saw Scaled viper
- Colubridae - Green pit viper
- Hydrophidae or Sea Snakes.[53]

COBRAS:

Two species of cobras are found in india , common cobra[nalla pambu] and king cobra [raja nagam].Cobras vary in colour from black or dark brown to yellow white. The head is indistinct from the neck and the ribs in this region are movable and expand to form the hood.This hood on its dorsal aspect resembles spectacle showing a connected pair of rings.[2]

KING COBRA (RAJA NAAGAM)

KRAITS:

Two species of kraits are commonly found in india. Common krait[kattu viriyan] and banded krait [pattai kattu viriyan]. Common krait is steel blue or black with white bars on the back. Banded krait is larger and is jet black in colour with yellow bars. Kraits are usually found in pairs.

KRAIT (KATTU VIRIAN)

RUSSELL'S VIPER: [kannadi viriyan];

This is the larger snake measuring 6 feet and is stout , lazy looking and makes a loud hissing sound by expelling air through its large nostrils. It is brown or yellowish with dark round spots on the dorsum edged with white and black colour.

RUSSEL VIPER (KANNADI VIRIAN)

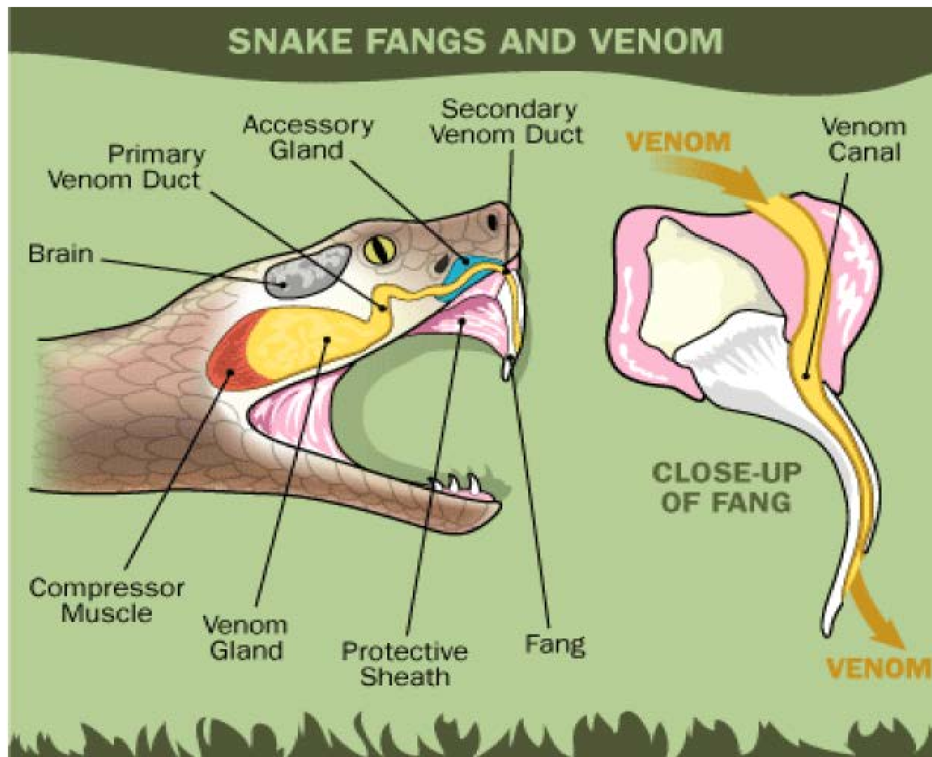
SAW-SCALED VIPER:[surutai pambu]:

A small snake (30 cm long) with brown or grayish dorsum showing zigzag pattern. It has a distinct cross or lance mark on the head. The ventral scales are rough. They produce a rasping sound by rubbing their coils together.

VENOM APPARATUS AND COMPOSITION:

In certain snakes [poisonous] the paired salivary gland has assumed a very significant function[venom apparatus]. They secrete venom, a powerful multipurpose enzyme fluid through the grooved teeth, the fangs.venom can be injected from the bottom of the fang(viper) or by an opening at the anterior aspect of fang,a few millimeters above the tip. [58,60]

Snake venom is a complex fluid used to immobilize the prey.venom is faint, transparent,yellowish slightly viscous and acidic. It is extremely heterogenous containing about 15 enzymes and 10 non-enzymatic proteins and peptides and a dozen of other substances.Variations in venom composition from species to species explains the varied clinical presentation of snake bite.[61]



VENOM APPARATUS

PATHOGENESIS OF SNAKE BITE HEMORRHAGE:

The pathogenesis of snake bite hemorrhage is due to venom induced consumption coagulopathy [VICC] which results from the consumption of clotting factors due to a procoagulant toxin in snake venom.The toxins have specific enzyme activation .

VICC:

VICC is charecterised by the activation of clotting pathway due to procoagulant toxins in snake venom.The procoagulant toxins are classically characterized by where they act on the clotting pathway into prothrombin activators ,factor v and factor X activators ,thrombin like enzymes[TLES] or fibrinogenase.

Prothrombin activators:

Toxins in viper are predominantly prothrombin activators. They are further classified into four groups based on the mechanism of action as group A and group B , which directly activate prothrombin.group C and group D , which are serine protease, resemble human prothrombinase or factor Xa Va complex which converts prothrombin to thrombin.

Factor X and V activation:

Russell's viper venom contains factor V and factor X activators.IT converts factor Xto Xa resulting in the formation of prothrombinase complex and subsequent conversion of fibrinogen to fibrin.

Thrombin like enzymes:

It is different from the other two, by not directly activating clotting pathway, instead it simply consumes fibrinogen.hence it produces isolated deficiency of fibrinogen.The zinc metalloproteinase snake venom has TLE activity. It cleaves the alpha chain of fibrinogen resulting in consumption of fibrinogen without production of fib

SNAKE VENOM:

Snake venoms are mixtures of numerous proteinaceous components. Snake bite victims present with a variety of life-threatening pathologies as the toxic constituents vary from species to species.

SNAKE VENOM SYSTEM :

It consists of a pair of venom secretory glands,on either side of the head on the upper jaw.These glands are connected to ducts,that transmits venom from gland to base of modified teeth (fangs) used for injection of venom.[58]

Snake venoms which are a complex mixture of proteinaceous components, has evolved from non-toxic house keeping genes by a variety of process like gene duplication, neofunctionalization, positive selection, alternative splicing, recombination.

Snake venoms can be broadly classified as haemotoxic, neurotoxic or cytotoxic.Certain snake species can cause combinations of different toxicities.[59]

Neurotoxic envenoming clinically presents as decending neuromuscular paralysis,such as paralysis of facial muscle, progressing to respiratory and generalized flaccid paralysis.[59]

PATHOGENESIS OF NEUROMUSCULAR TOXIN:

The toxins involved in neurotoxic envenoming belong to members of diverse phospholipase A2 (PLA2) and three finger toxin (3 FTX) families.These toxins act on pre and post synaptic junction resulting in blocking potassium orsodium channel, nicotinic or muscarinic receptor antagonists resulting in paralysis.[44,45]

CYTOTOXIC ENVENOMING:

The toxins involved in cytotoxic envenoming are Snake Venom Metalloproteinase

(SVMP),which are hydrolytic enzymes, phospholipase A2 (PLA2), non-enzymatic cytotoxic 3 FTX.Recently it was found that destruction of local tissue is due to snake venom inducing formation of neutrophil extracellular traps (NETS), which blocks blood vessels hence toxin is contained in bite site promoting cytotoxic pathology.They present with painful and progressive swelling at bite site ,blistering,bruising.Extensive local tissue damage.[44,45]

HEMOTOXIC SNAKE VENOM COMPONENTS:

The two main toxins involved are

- SNAKE VENOM METALLOPROTEINASE(SVMPs)
- SNAKE VENOM SERINE PROTEINASE (SVSPs).

The other toxins are

- Kunitz type SERINE PROTEASE INHIBITOR,
- BRADYKININ POTENTIATING PEPTIDE (BPP),
- C- TYPE LECTIN LIKE PROTEINS (CTLc).[50]

SNAKE VENOM METALLOPROTEINASE:

SVMPs has structural variants classified as P –I, P-II,P-III SVMPS,they exhibit multiple functional activites like hemorrhagic, fibrinogenolytic , Fac X or Prothrombin activation, inhibition of platelet aggregation.P- III has hemorrhagic activity, P- I is fibrinolytic , actively cleaves alpha chain of fibrinogen into fibrino peptides.SVMPs also have procoagulant properties by activating the clotting factors prothrombin or factor X.

Factor X activators are also isolated from venoms of Vipers,elapids ,Russel viper (RVV-X).

P-II SVMPs under goes proteolytic cleavage resulting in the formation of DISINTEGRINS,which are small cystein rich polypeptides ,their main action is integrin receptor antagonism.These integrins bind to alpha 2b beta3 integrin(

glycoprotein IIb /IIIa platelet fibrinogen receptor .there by preventing fibrinogen binding to platelets and hence inhibiting platelet aggregation.

Hence, SVMPs and related toxins exhibit various functions that impair haemostasis, including inducing hemorrhage, depleting various clotting factors and inhibiting platelet function.[50]

SNAKE VENOM SERINE PROTEINASE (SVSPs):

There are three toxins namely,

- THROMBIN LIKE ENZYME(TLE),
- GROUP C PROTHROMBIN ACTIVATOR,
- GROUP D PROTHROMBIN ACTIVATORS.

THROMBIN LIKE ENZYMES(TLE):

TLE exhibit thrombin like activities.TLE are selective and cleave either alpha chain or beta chain or both, resulting in polymerization of fibrin monomers. But TLEs do not stimulate FACTOR XIII , hence result in unstable clots that are readily dissolved by plasmin.Hence the continuous generation and destruction of fibrin leads to consumption coagulopathy.[50]

Other functions of TLEs:

OTHER TOXINS INVOLVED IN SNAKE VENOM APPARATUS :

KUNITE-TYPE SERINE PROTEASE INHIBITOR: They potentially inhibit plasmin and hence has antifibrinolytic property.

BRADYKININ POTENTIATING PEPTIDES(BPP) :

BPPs result in reduction of systemic blood pressure, as they inhibit angiotensin-convertig enzyme, preventing conversion of hormone angiotensin I in to angitensin II.BPPs effect is exacerbated by hemorrhagic SVMPs and SVSPs that exhibit Kallikrein- like function.[51,52]

C-TYPE LECTIN LIKE PROTEINS(CTLs):

CTLs affect platelet function, bind to Factor IX and FACTOR X thus inhibiting coagulation cascade. CTLs also inhibit binding of thrombin to fibrinogen.[51,52]

CLINICAL MANIFESTATION:

Fangs marks over the bitten area, inflammatory features like pain , redness and progressive edema. Nausea, vomiting, fatigability, perioral numbness or tingling of mouth ,palpitations, light headedness, hematemesis and hematuria, decreased platelet count and fasiculations. The swelling may be localized or spread throughout the entire limb. Respiratory insufficiency may occur due to edema near an airway or involving respiratory muscles.[47]

MANAGEMENT:

General measures:

- The victim must be reassured
- The bitten limb must be immobilized using a splint or sling

- ❖ Use of tourniquets is controversial as they may cause ischemia and gangrene, damage to peripheral nerves and increased local effects of venom. But in case of cobra or sea snake if medical therapy is to be delayed a firm crepe bandage can be applied
- ❖ Inj tetanus toxoid should be given

Antisnake venom therapy:

It is indicated in hemostatic abnormalities, neurotoxicity, generalized rhabdomyolysis, definite evidence of envenomation. It is contraindicated in atopic patients and those who had reactions to equine antiserum on previous occasions. It can be prevented by pretreatment with adrenaline, anti-histamine and corticosteroids.[56]

Types of Anti snake venom:

The very first ASV was prepared in india by Dr.Calmette against the viper bite.India has become the pioneer in the production of ASV.In India,ASV currently used is a polyvalent ASV which contains antibodies against Indian Cobra,Common Krait,Russell's viper and saw-scaled viper.[57]

ASV production is done by four institutions in India namely:

- Serum institute ,Pune
- King institute, Guindy
- Hofkin institute ,Mumbai
- Central research institute ,kasauli

The amount of venom which is neutralized ASV is 0.6 mg for cobra ,0.6 mg for Russell's Viper,0.45 mg for Krait,0.45 mg for Saw scaled Viper .

MECHANISM OF ACTION OF ASV:

ASV can neutralize the venom which is circulating in the blood .ASV cannot neutralize the venom attached to target organs like RBC's , platelets, muscle cells, vascular endothelium, neuro muscular receptors**.**The half life of ASV is 26 -96 hours, but the antigen may be present in circulation for 130 hours.Loading dose of ASV given initially , serves to neutralize the circulating venom .The subsequent low doses, given as slow microdrip infusion serves to neutralize the venom which is absorbed from the site of snake bite, hence acts as a depot.If ASV is administered in excess, it might prevent the subsequent development of immunity in victims who are bitten by the same species of the snake which is prevalent in that particular surrounding.[57]

ASV:

The dose of ASV is not a fixed one , because satisfactory data is yet to be available regarding the dose of ASV required to completely reverse the clinical manifestation of venom in a particular case.The amount of Venom which is neutralized by one ml of polyvalent ASV is:

- 0.6 mg for cobra
- 0.6 mg for Russell's Viper
- 0.45 mg for krait
- 0.45 mg for saw scaled viper respectively

Current empirical dose used for the snake bite is :

- 100 -800 ml for Russell's Viper
- 50 -360 ml for Krait and cobra bite
- 20 -450 ml in saw –scaled viper

Monoclonal ASV is the currently used in the treat victims of snake bite.

In case of cobra and krait bite envenomation , 100 ml of ASV is added to 200 ml of crystalloid solution and infused over sixty minutes. Venom present at the bite site is absorbed slowly, but it can be neutralized if ASV is given every sixth hourly.

Anaphylaxis to ASV include generalized itching, difficult breathing due to bronchospasm, urticaria, hypotension, palpitations and vomiting . Among these vomiting is the earliest symptom that the patient complaints of, swelling of lips and tongue are among other common symptoms. Early reactions are managed by 0.5 ml of 0.1 % adrenaline given subcutaneously and chlorpheniramine maleate 10 mg by intra venous route .Recently it has been reported that adrenaline can be used as prophylaxis before starting ASV. ASV gets precipitated on dilution, such samples should be discarded.[56,57]

MATERIALS AND METHODS

This is a prospective study carried out in the Department of Pathology on the patients admitted to Government Mohan kumaramangalam Medical college Hospital Salem under General Medicine Department during the period of January 2018 to June 2019.

Inclusion criteria:

Patients, both male and female, with history of snake bite with signs of envenomation are included in the study

Exclusion Criteria :

- Patients with history of snake bite treated outside with ASV
- Patients both male and female with pre-existing coagulaopathy
- Patients on anticoagulants, antiplatelet drugs
- Patients with history of renal disease
- Patient with chronic diseases like diabetes , hypertension , connective tissue diseases.

Sample size:

This study was conducted on 70 patients with bleeding and neurological signs and symptoms. The patients were selected as per the inclusion and exclusion criteria.

Sample collection:

Clinical details:

A detailed clinical history was obtained from the patient regarding the species of snake, site and time of bite, time of admission to the hospital, application of tourniquet, history of any chronic illness, bleeding or thrombotic tendency, history of drug intake ,symptoms ,signs relevant to snake bite.

Blood sample collection:

After obtaining informed consent from the patient, samples were collected under aseptic precautions. Bleeding time was done using a bloating paper , lancet and a stop watch. Whole blood clotting time was done using .Venous blood sample were collected using 23 gauge needle and syringe and were transferred to blue colour capped vaccutainers containing 3.2% citrate.The sample is then centrifuged at 2500 rpm for 15 minutes.

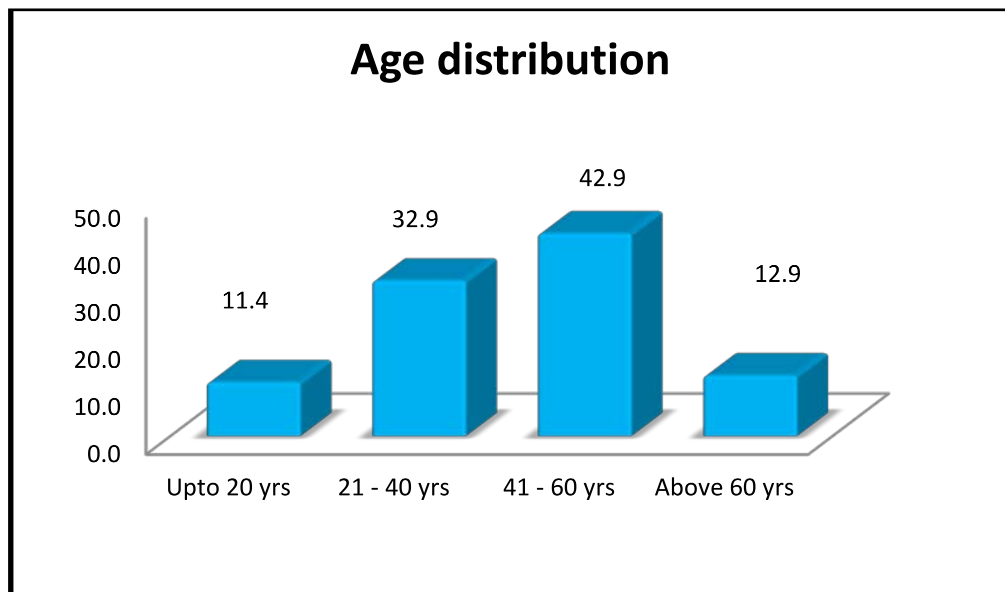
OBSERVATION AND RESULTS

In the present study , Among the 70 cases studied 11.4 % were below the age group of 20 years, 32.9% were in the age group of 21 to 40 years, 42.9% were in the age group of 41 to 60 years and 12.9 % were above 60 years.

Table No. 1: Age Distribution

S.No	Age Distribution	Frequency	Percent
1	Upto 20 Yerars	8	11.4
2	21-40 Years	23	32.9
3	41-60Years	30	42.9
4	Above 60 Years	9	12.9
	Total	70	100

Figure No.1 : Age Distribution

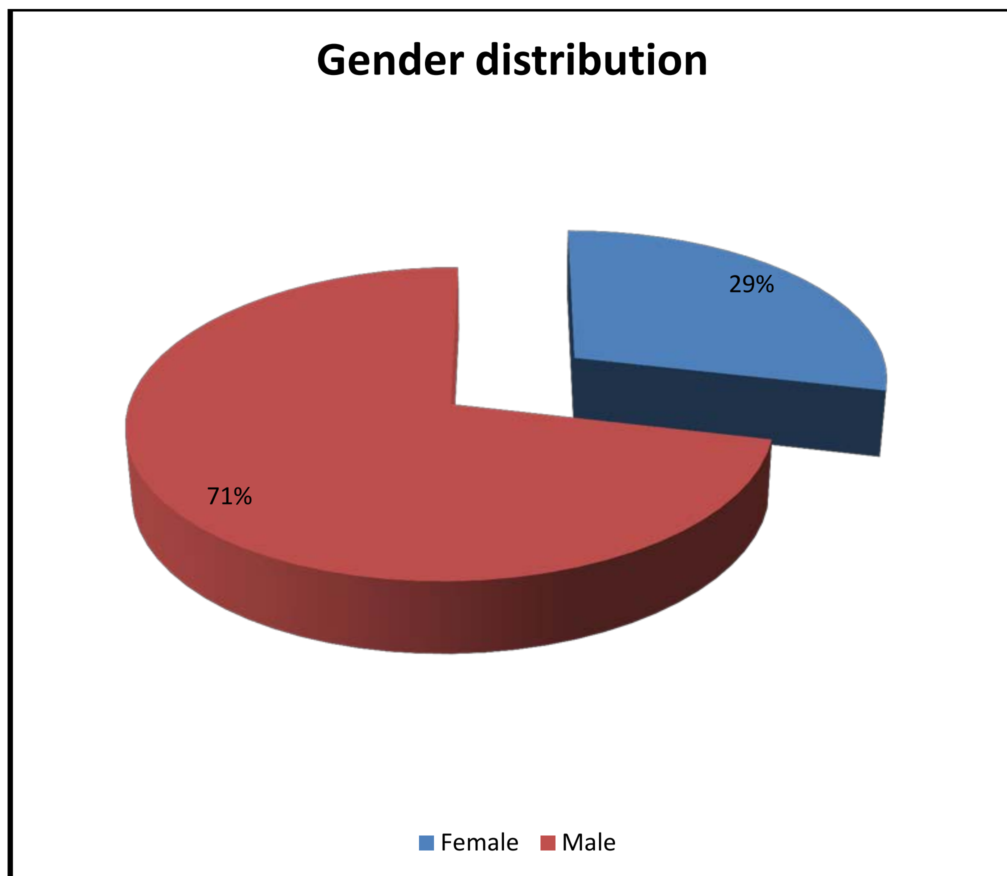


In the present study 71% were male and 29% were female.

Table No.2 : Gender Distribution

S.No	Sex	Frequency	Percent
1	Female	20	28.06
2	Male	50	71.4
	Total	**70**	**100**

Figure No.2: Gender Distribution

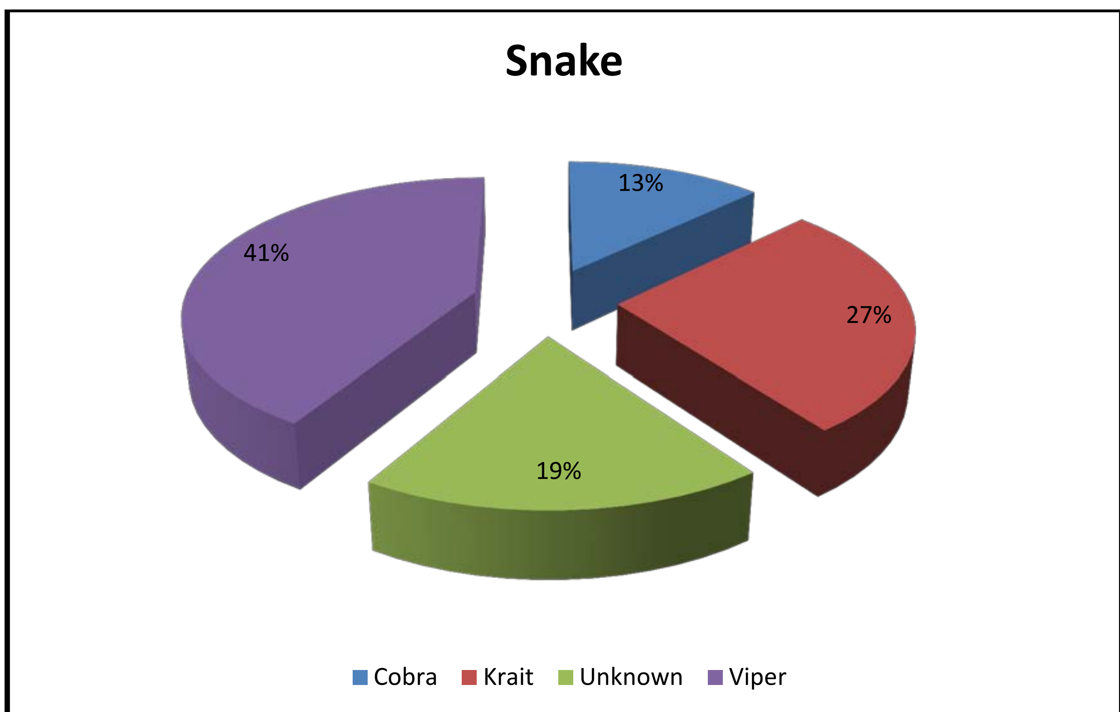


In the present study 41% were due to viper bite, 27% due to krait, 19% due to unknown bite, 13% due to cobra bite.

Table No 3 :Species of Snake

S.No	Species of Snake	Frequency	Percent
1	Cobra	9	12.9
2	Krait	19	27.1
3	Unknown	13	18.6
4	Viper	29	41.4
	Total	**70**	**100.00**

Figure No 3 : Species of Snake

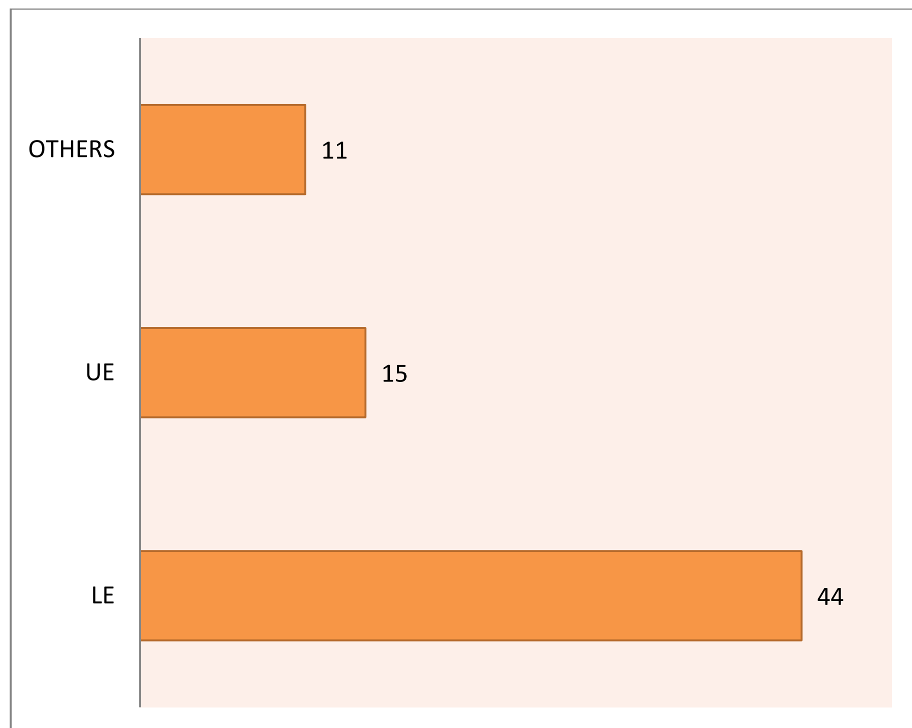


In the present study, lower extremity (44%) was the predominant bite site, followed by upper extremity (15%), other site (11%) which includes jaw, shoulder.

Table No 4: BITE-SITE DISTRIBUTION

S.No	BITE-SITE	Frequency
1	Lower Extremity	44
2	Upper Extremity	15
3	Others	11
	Total	**70**

Figure No 4: BITE-SITE DISTRIBUTION



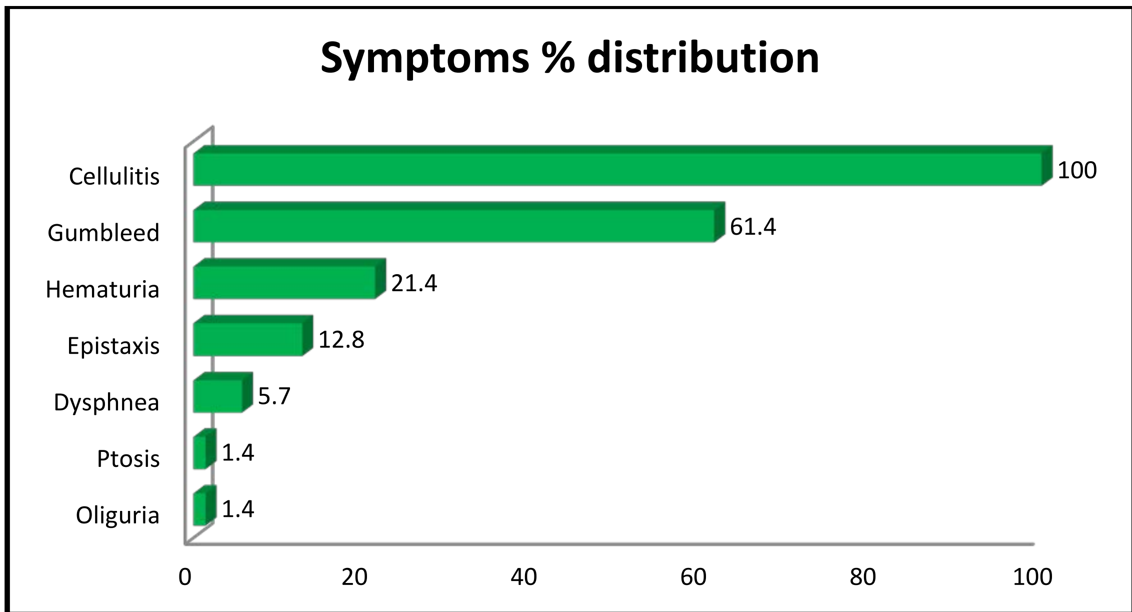
In the present study ,100% presented with cellulitis, 61.4 % presented with gumbleed , 21.4% presented with hematuria, 12.8% presented with pistaxis,5.7% presented with dysphnea, 1.4% presented with ptosis,1.4% presented with oliguria. It was also observed that 2 patients had fasciotomy done, 2 patients with neurotoxic features had ventilator support and 3 patients had dialysis.

`

Table No 5 : Symptoms Distribution

S.No	Symptoms	Frequency	Percent
1	Cellulitis	**6**	**8.6**
2	Cellulitis / Epistaxis/ Gumbleed	**1**	**1.4**
3	Cellulitis/Gumbleed	**34**	**48.6**
4	Cellulitis / Epistaxis	**5**	**7.1**
5	Cellulitis/Gumbleed/ Dysphnea	**1**	**1.4**
6	Cellulitis/Gumbleed/ Epistaxis	**3**	**4.3**
7	Cellulitis/Gumbleed/Hematuria	**3**	**4.3**
8	Cellulitis/Gumbleed/Oliguria	**1**	**1.4**
9	Cellulitis/Gumbleed/Ptosis	**1**	**1.4**
10	Cellulitis/Hematuria	**12**	**17.1**
11	Cellulitis/Dysphnea/Ptosis	**3**	**4.3**
	Total	**70**	**100.0**

Figure No 5: Symptoms Distribution



In the presence study average of 10 to 20 vial of ASV was given for 34.3 % of patients and less than 10 vial was given to 38.6% of patients and more than 20 vial was administered to 27.1% of patients.

Table No 6 : ASV (Vial) Administration

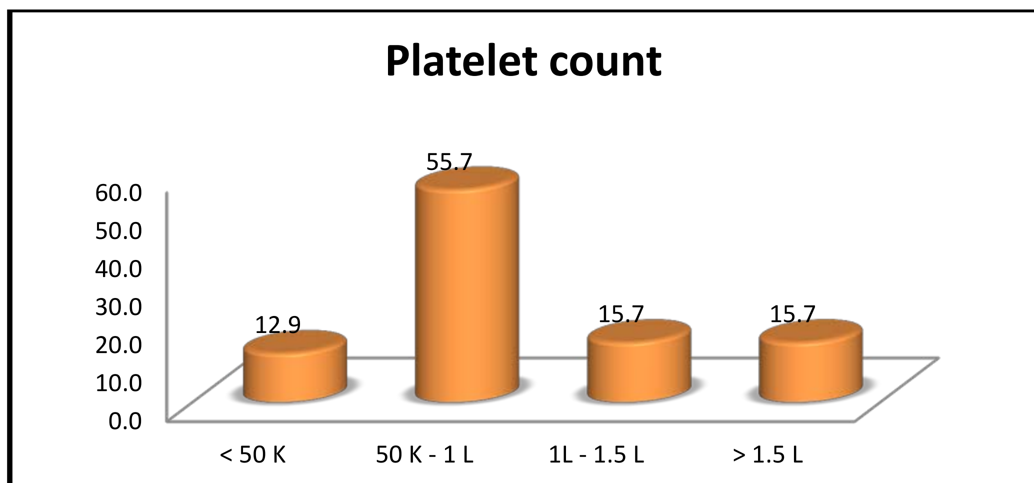
S.No	ASV (Vial)	Frequency	Percent
1	< 10 Vial	27	38.6
2	10-20 Vial	24	34.3
3	>20 Vial	19	27.1
	Total	**70**	**100.00**

Among the 70 patients studied, 12.9 % presented with platelet count less than 50,000 /cumm , 55.7% presented with platelet count 50,000 to 1,00,00 lakh/cumm , 15.7 % presented with 1,00,000 to 1,50,000 cells /cumm and 15.7% presented with 1,50,000 cells / cumm.

Table No 7 : Platelet Count Distribution

S.No	Platelet Count (Cells/dl)	Frequency	Percent
1	< 50000	9	12.9
2	50000 - 100000	39	55.7
3	100000 – 1,500000	11	15.7
4	>1,500000	11	15.7
	Total	**70**	**100**

Figure No 6: Platelet Count Distribution



Among the 70 patients studied , at the time of admission 2.9 % of patients had undetectable levels (NCD),71.4 % of patients had prolonged PT , 25.7% had normal PT.

Table No 8: Prothrombin Time(PT1) (Before Administration of ASV)

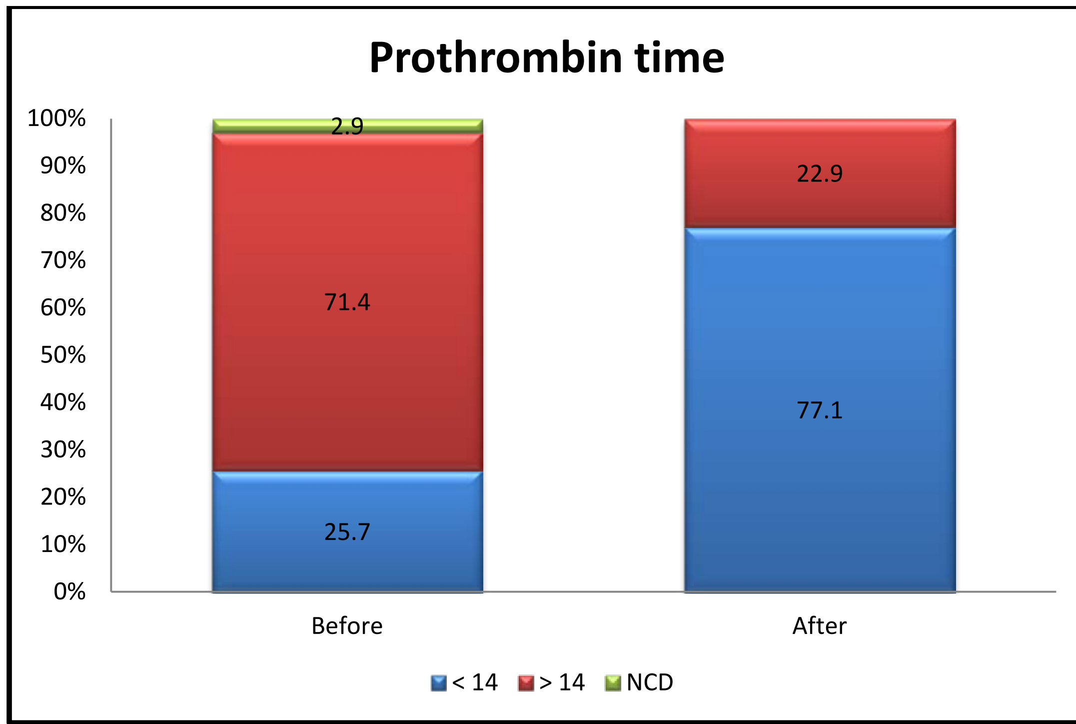
S.No	Prothrombin Time (PT1)	Frequency	Percent
1	< 14 Seconds	18	25.7
2	>14 Seconds	50	71.4
3	No Clot Detected (NCD)	2	2.9
	Total	**70**	**100**

After giving ASV Undetectable levels were not observed in any patients ,22.9 % had prolonged PT and 77.1% had normal PT.

Table No 9: Prothrombin Time (PT2)(After Administration of ASV)

S.No	Prothrombin Time (PT2)	Frequency	Percent
1	< 14 Seconds	54	77.1
2	>14 Seconds	16	22.9
3	No Clot Detected (NCD)	Nil	Nil
	Total	**70**	**100**

Figure No 7 : Comparison of Prothrombin Time (PT1 & PT2)



Among the 70 patients studied , at the time of admission 4.3 % presented with undetectable levels (NCD) ,94.3 % had prolonged APTT, only 1.4 % had normal APTT.

Table No 10 : Activated Partial Thromboplastin Time (APTT1)

(Before Administration of ASV)

S.No	APTT 1	Frequency	Percent
1	<28 Seconds	1	1.4
2	>28 Seconds	66	94.3
3	No Clot Detected (NCD)	3	4.3
	Total	**70**	**100**

After the administration of ASV , undetectable levels were not observed , but 95.7 % still showed prolonged APTT and 4.3 % showed normal APTT.

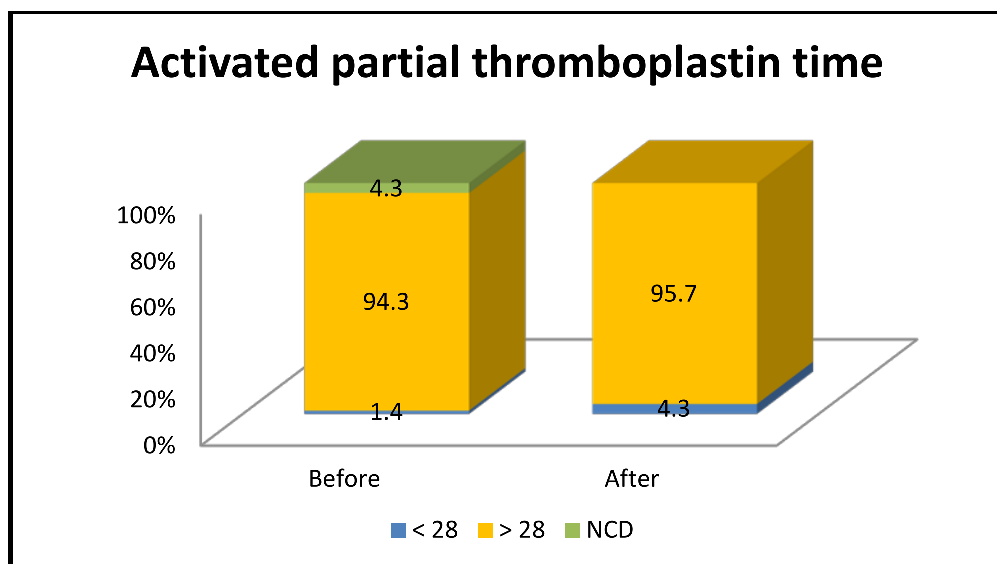
Table No 11 : Activated Partial Thromboplastin Time(APTT2)

(After Administration of ASV)

S.No	APTT 2	Frequency	Percent
1	<28 Seconds	3	4.3
2	>28 Seconds	67	95.7
3	No Clot Detected (NCD)	Nil	Nil
	Total	**70**	**100**

Figure No 8 : Compression of Activated Partial Thromboplastin Time

(APTT 1 & APTT 2)



Among the 70 patients studied ,8.6 % presented with undetectable levels (NCD), 42.9 % presented with prolonged TT, 48.6 % had normal TT.

Table No 12 : Thrombin Time (TTI) (Before Administration of ASV)

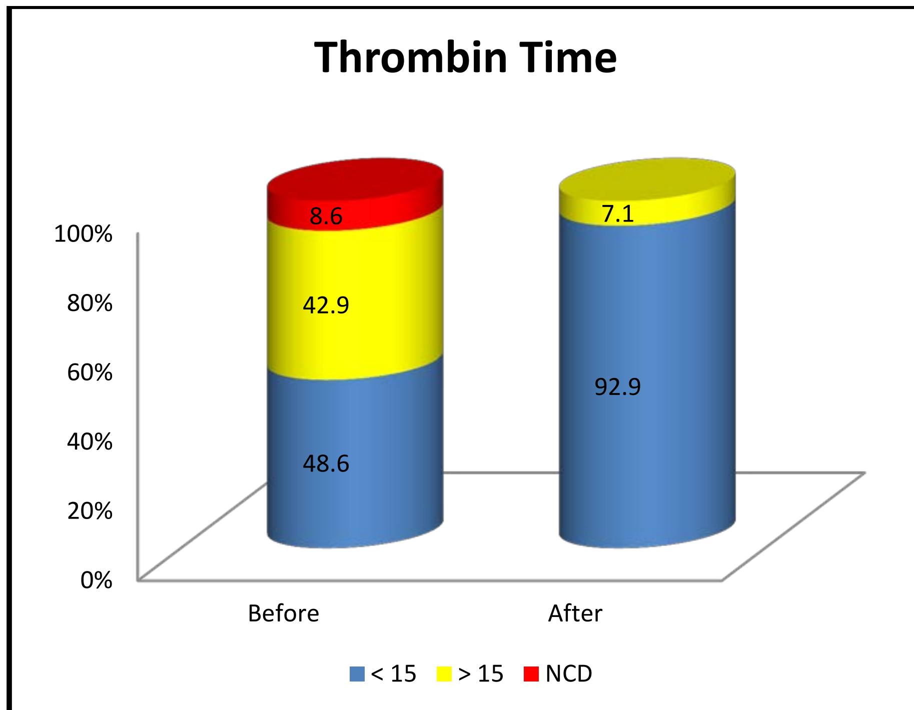
S.No	TT 1	Frequency	Percent
1	<15 Seconds	34	48.6
2	>15 Seconds	30	42.9
3	No Clot Detected (NCD)	6	8.6
	Total	**70**	**100**

After the administration of ASV, undetectable levels were not observed in any of the patients , 7.1 % presented with prolonged TT, 92.9 % presented with normal TT.

Table No 13 : Thrombin Time (TT2)(After Administration of ASV)

S.No	TT 2	Frequency	Percent
1	<15 Seconds	65	92.9
2	>15 Seconds	5	7.1
3	No Clot Detected (NCD)	Nil	Nil
	Total	**70**	**100**

Figure No 9 : Comparison of Thrombin Time (TT 1 & TT 2)



Among the 70 patients studied, 27.1% presented with undetectable levels, 71.4 % had Fibrinogen values in the range of 150 -400 mg /dl , 1.4 % had fibrinogen less than 150 mg/dl .

Table No 14 : Fibrinogen Assay (Before Administration of ASV)

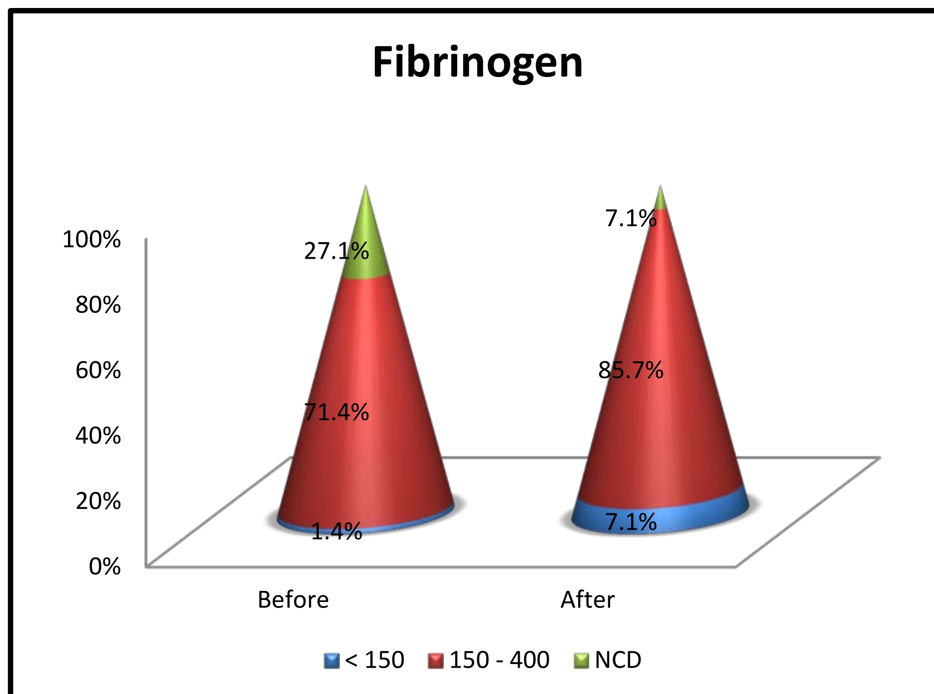
S.No	Fibrinogen Assay 1	Percent
1	<150 mg/dl	1.4
2	150-400 mg/dl	71.4
3	No Clot Detected (NCD)	27.1
	Total	**100**

After the administration of ASV 7.1% had undetectable levels (NCD) ,85.7 % had fibrinogen in the range 150-400 mg / dl , 7.1 % had fibrinogen below 150 mg / dl

Table No 15 : Fibrinogen Assay (After Administation of ASV)

S.No	Fibrinogen Assay 2	Percent
1	<150 mg/dl	7.1
2	150-400 mg/dl	85.7
3	No Clot Detected (NCD)	7.1
	Total	**100**

Figure No 10 : Comparison of Fibrinogen Assay (Assay 1 & Assay



DISCUSSION

In the present study,70 cases of snake bite were selected based on the inclusion and exclusion criteria. Clinical details were collected regarding the bite site, type of snake, and symptoms. Coagulation profile like bleeding time, clotting time, prothrombin time,activated partial thromboplastin time, thrombin time, fibrinogen assay were carried out on admission and after 12 hours of ASV administration using semiautomated ERBA Coagulation Analyser ,with commercially available reagents.

AGE DISTRIBUTION :

In the present study ,42.9 % were in the age group of 41 – 60 years, 32.9 % were in the age group of 21 -40 years, 12.9 % were above 60 years and 11.4 % were below 20 years. Hence it is inferred that middle aged people are more commonly affected , which is in accordance with the study done by Shubham Agarwal(6) and Suma Dasaraju (7).In the study done by sagar biradar et al ,27.8 % were in the age group of 30 -39 years.(8)

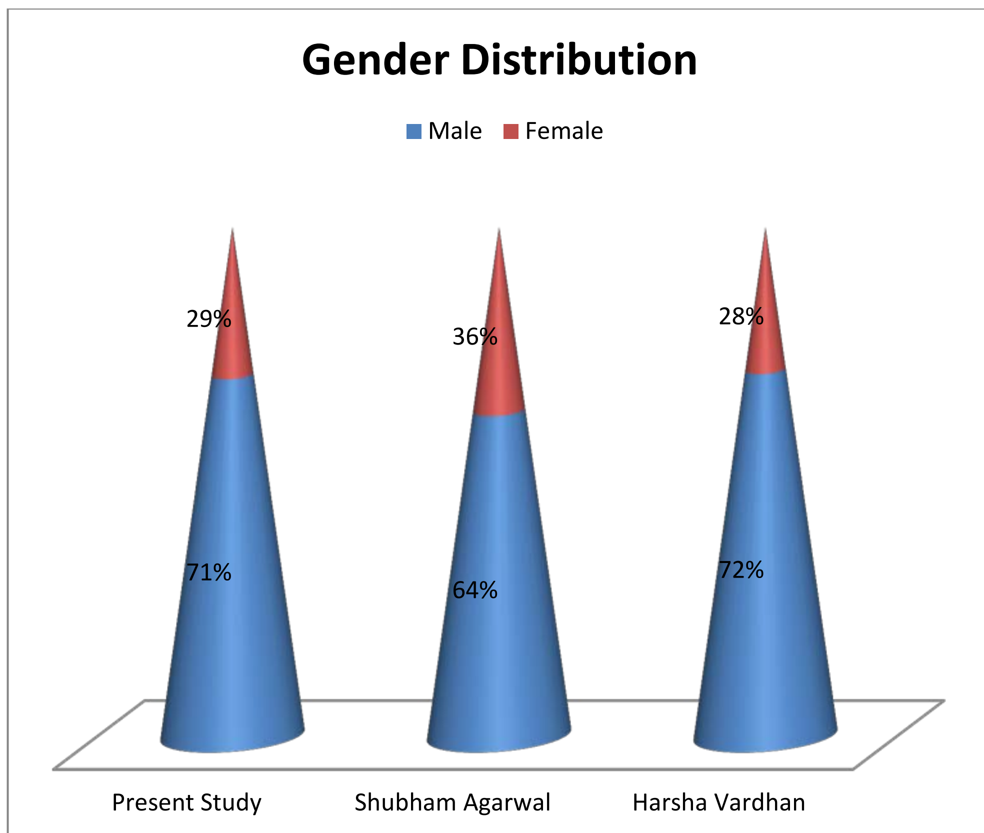
GENDER:

In the present study, snake bite was more predominant in male constituting 71 % and only 29 % of female population were affected which is in accordance with the study done by Shubham Agarwal (6) in which males (64 %) showed higher incidence than females (36%) and also in the study done by Harsha vardhan (9)in which 72 % male and 28 % female were affected.

Table No 16 : Comparison of Sex Incidence

Gender	Present Study	Shubham Agarwal	Harsha Vardhan
Male	71%	64%	72%
Female	29%	36%	28%

Figure No 11 : Sex Incidence



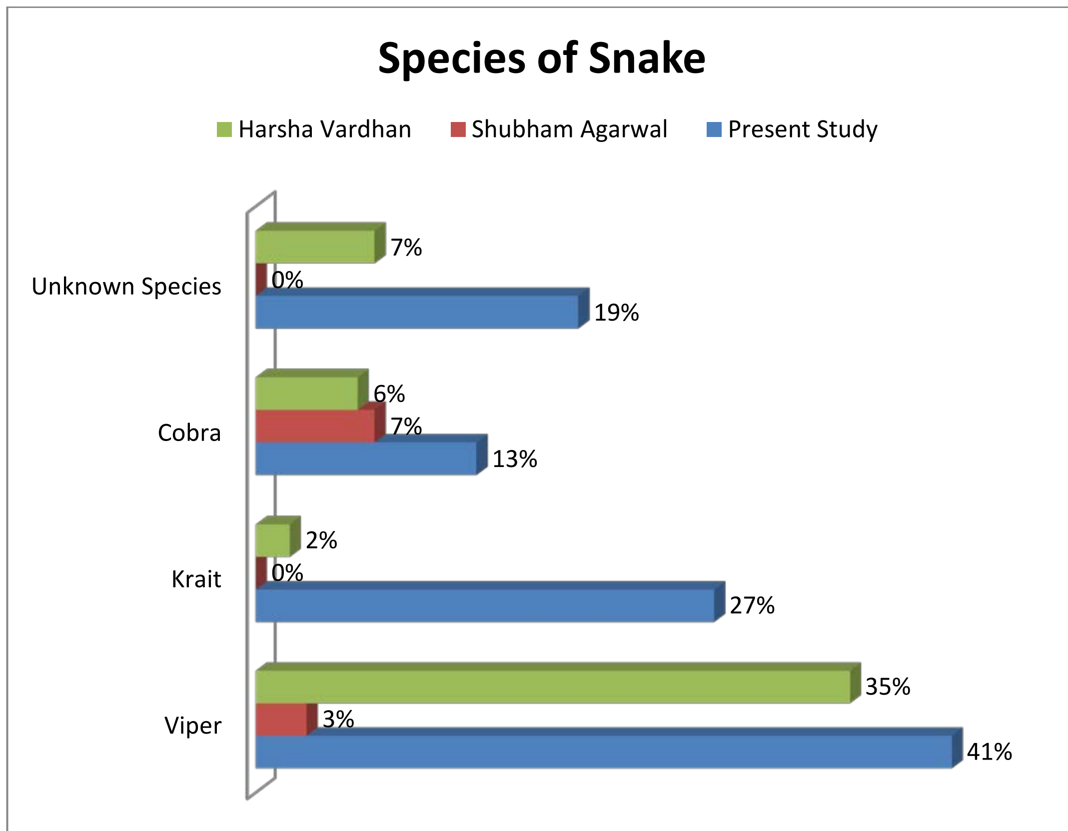
TYPE OF SNAKE :

In the present study, viper bite was most common which constitutes 41 % of viper, kraits being the second most common constituting 27 % ,cobra bite constituting 13% and 19% due to unknown species. In the study done by Shubham Agarwal (6),among 53 patients studied, 7 patients developed neurotoxicity and 3 patients developed both hemotoxic and neurotoxic features.(3)In the study done by Harsha vardhan (9),35 patient had viper bite ,6 patients had cobra bite , 2 % had been bitten by krait and in 7 % patients snake was not identified.

Table No 17 : Comparison of Incidence of type of Snake

Species of Snake	Present Study	Shubham Agarwal	Harsha Vardhan
Viper	**41%**	**3%**	**35%**
Krait	**27%**	**0%**	**2%**
Cobra	**13%**	**7%**	**6%**
Unknown Species	**19%**	**0%**	**7%**

Figure No 12 : Comparison with Shubham Agarwal & Harsha Vardhan



Bite site distribution :

In the present study , predominant site of bite . In a study done by Sagar Biradar maximum number of bites occurred on lower extremities.[22,66]

SYMPTOM DISTRIBUTION :

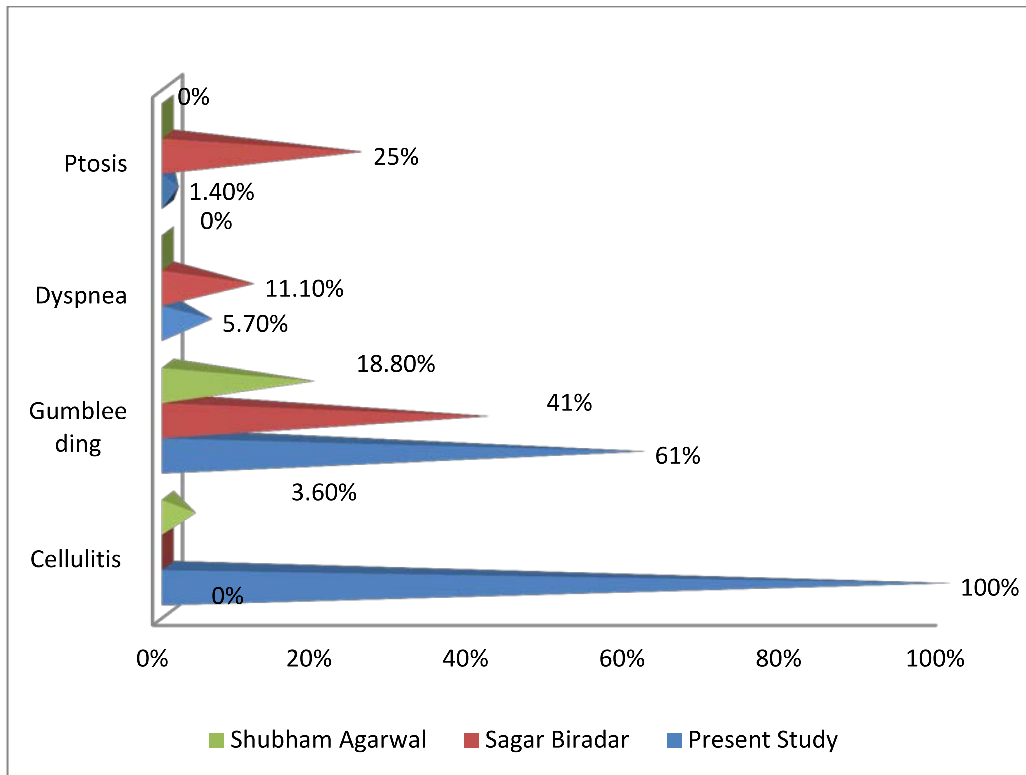
In the present study , all the patients had cellulitis invariable of the type of snake . Among the 87 % of patients with features of hemotoxic manifestation 61.4 % had gumbleeding along with cellulitis.In the study done by Sagar Biradar gum bleeding was seen in only 16 % of patients ,while 41 % presented with

bleed at the bite site(8). Among the 12.9 % of patients bitten by cobra, all of them presented with cellulitis, 5.7 % had dyspnea and 1.4 % had ptosis.In the study done by Sagar Biradar, among the patients bitten by cobra 25 % presented with ptosis , 11.1 % presented with respiratory difficulty(8). In the study done by Shubham Agarwal , 3.6% presented with cellulitis , 18.8 % presented with bleeding.[6]

Table No 18 : Comparison of Symptom distribution

Symptom	Present Study	Sagar Biradar	Shubham Agarwal
Cellulitis	100%	0%	3.6%
Gumbleeding	61%	41%	18.8%
Dyspnea	5.7%	11.1%	0%
Ptosis	1.4%	25%	0%

Figure No 13 : Comparesion with Sagar Biradar & Shubham Agarwal



BLEEDING TIME :

In the present study, bleeding time was within norml range irrespective of the type of snake , which contrasts with the study done by Suma Dasaraju , in which bleeding time was prolonged in 16 % of patients.[7]

Table No 19 : Comparison of Bleeding Time

Bleeding Time	Present Study	Suma Dasaraju
Prolonged BT	0%	16%

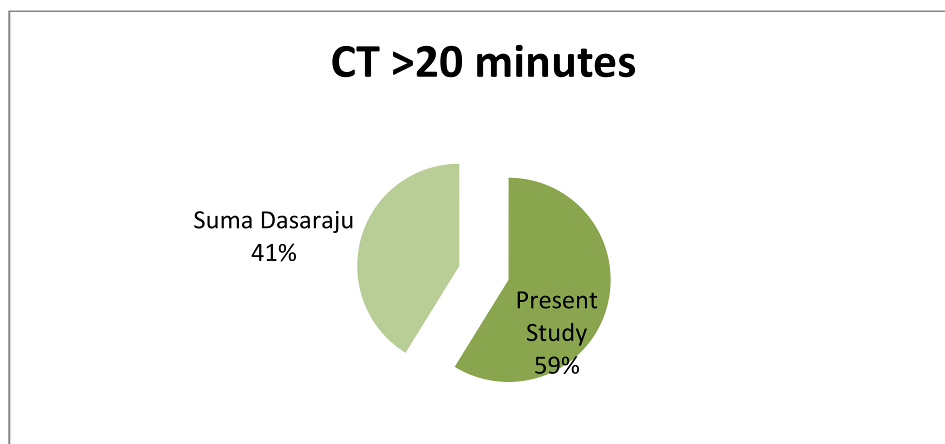
CLOTTING TIME:

In our study , 70 patients who were included in the study , invariable of the type of snake , with hemotoxic manifestation and neurotoxic manifestation had prolonged whole blood clotting time (WBCT < 20 mins) which is in accordance with the study done by Suma Dasaraju , in which 70% of patients had prolonged WBCT .[7]

Table No 20 : Comparison of Clotting Time

Clotting Time	Present Study	Suma Dasaraju
>20 minutes	100%	70%

Figure No 14 : Comparison with Suma Dasaraju



PLATELET COUNT:

In the present study, among the 70 patients studied, with both hemotoxic and neurotoxic manifestation , we observed that patients presented

predominantly with platelet count less in the range between 50,000 to 1,00,000 (55.7%) which contrasts with the study done by Harshavardhan(9) 48 % had platelets more than 1,00,000.Our study also contrasts with the study done by Suma Dasaraju (7)in which 74 % presented with platelets more than 1, 50,000 cells / cumm.

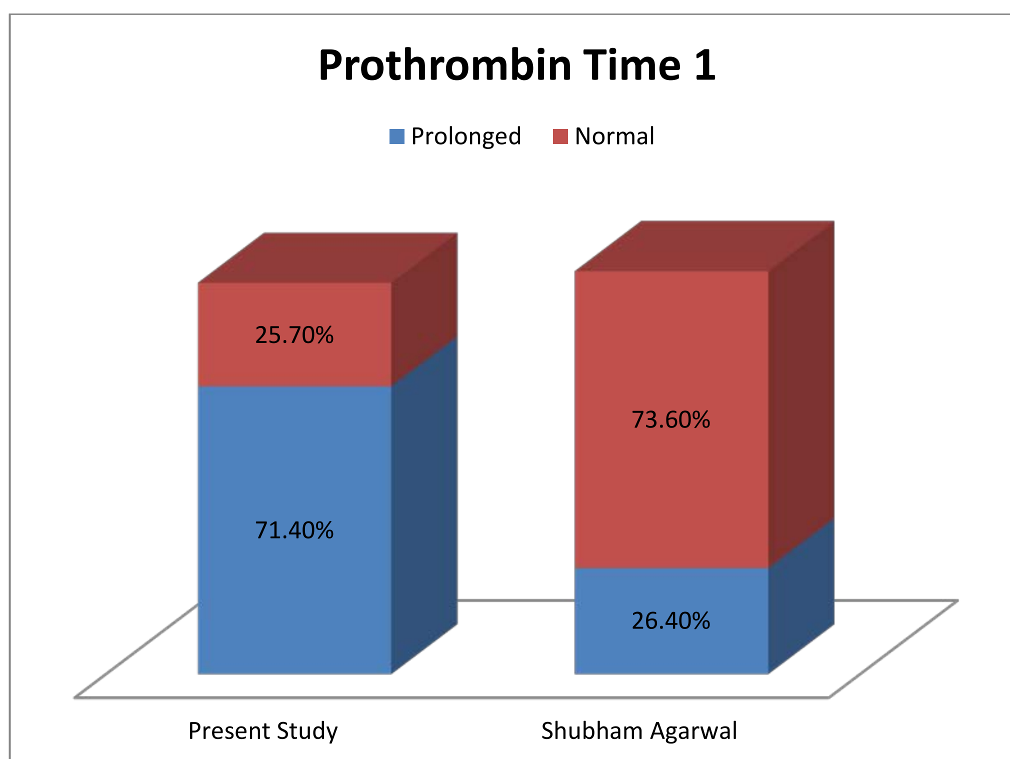
PROTHROMBIN TIME:

In the present study , among the 70 patients studied with prolonged whole blood Clotting time 71.4 % of cases had prolonged prothrombin time,2.9 % with non detectable levels (NCD) and 25.7 % had prothrombin time within normal limits which contrasts with the study done by shubham agarwal in which 73.6 % had normal prothrombin time during admission and 26 .4% of patients had prolonged prothrombin time. [6]

Table No 21 : Comparison of prothrombin Time (PT1)

PT 1	Present Study	Shubham Agarwal
Prolonged	71.4 %	26.4 %
Normal	25.7 %	73.6 %

Figer No 15 : Comparison with Shubham Agarwal

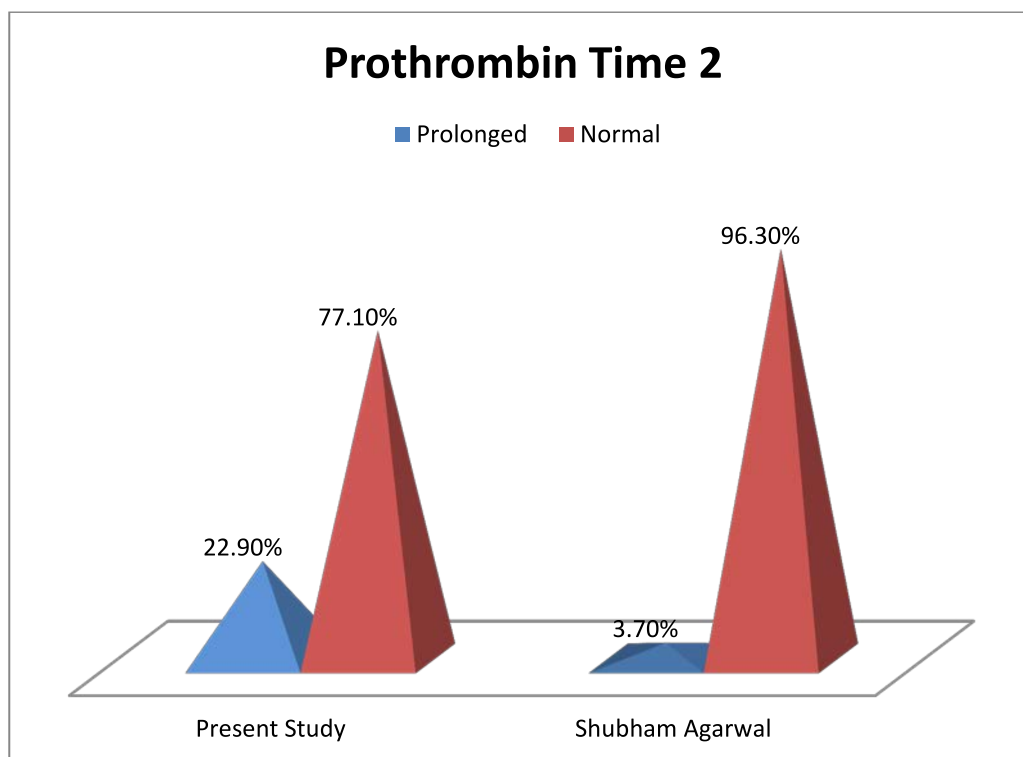


Prothrombin time was repeated 12 hours after initiation of ASV .We observed that 77.1% had normal prothrombin time and 22.9 % had prolonged prothrombin time, who are more prone for rebound coagulopathy, hence must be followed up. Our results are in accordance with the study done by Shubham Agarwal in which 96.3% had normal prothrombin time and 3.7 % had prolonged prothrombin time which was repeated after 12 hours.[6]

Table No 22 : Comparison of prothrombin Time (PT2)

PT 2	Present Study	Shubham Agarwal
Prolonged	22.9 %	3.7 %
Normal	77.1 %	96.3 %

Figure No 16 : Comparison with Shubham Agarwal



It is inferred from our observation that prolonged Whole blood clotting time which has been taken as the only criteria for the administration of ASV in majority of hospital is not reliable because in those patient with normal prothrombin time WBCT was prolonged .

ACTIVATED PARTIAL THROMBOPLASTIN TIME:

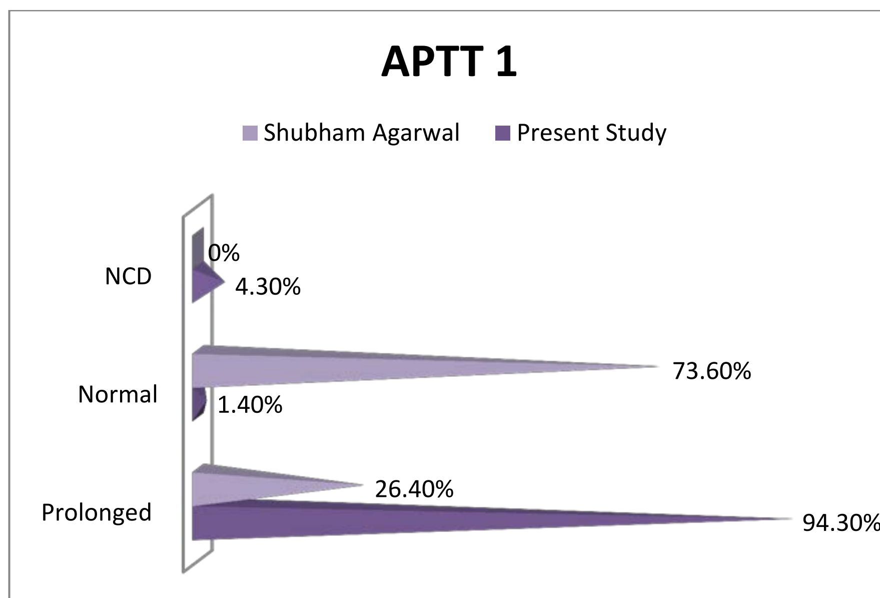
In our study,among all the patient with prolonged clotting time 94.3 % showed prolonged APTT which is a measure of intrinsic coagulation pathway,4.3 % show non detectable levels (NCD), which again confirms the effects of envenomation.It was observed that 1.4 % had normal APTT ,although WBCT was prolonged,which again emphasis the importance of coagulation

profile. Our study contrasts with the study done by Shubham Agarwal in which 73.6 % had normal APTT, 26.4% had prolonged APTT on admission .[6]

Table No 23 : Comparison of Activated Partial Thromboplastin Time (APTT1)

APTT 1	Present Study	Shubham Agarwal
Prolonged	94.3 %	26.4 %
Normal	1.4 %	73.6 %
NCD	4.3 %	0 %

Figure No 17 : Comparison with Shubham Agarwal

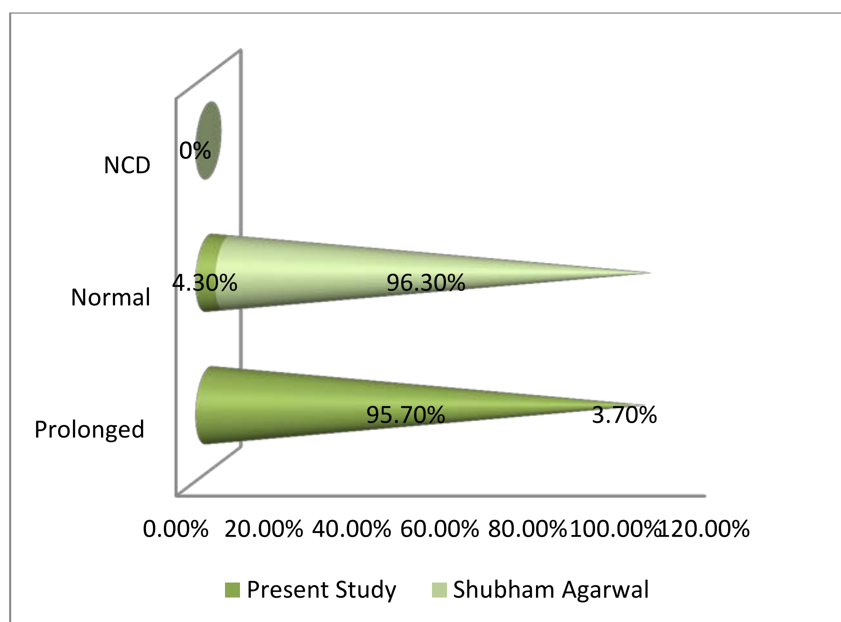


APTT was repeated in the patients after 12 hours of ASV and it was observed that 95.7% still had prolonged APTT. In the study done by Shubham Agarwal , APTT was repeated after 12 hours which showed 96.3 % had normal APTT and only 3.7 % had prolonged APTT.[6]

Table No 24 : Comparison of Activated Partial Thromboplastin Time (APTT2)

APTT 2	Present Study	Shubham Agarwal
Prolonged	95.7 %	3.7 %
Normal	4.3 %	96.3 %
NCD	0 %	0 %

Figure No 18 : Comparison with Shubham Agarwal



It is inferred from the observation that APTT has not reversed back in most of the patients which could be due to various composition of snake venom which could result in consumption of factors involved in intrinsic pathway and hence can be further evaluated with clot based factor assay and mixing studies.

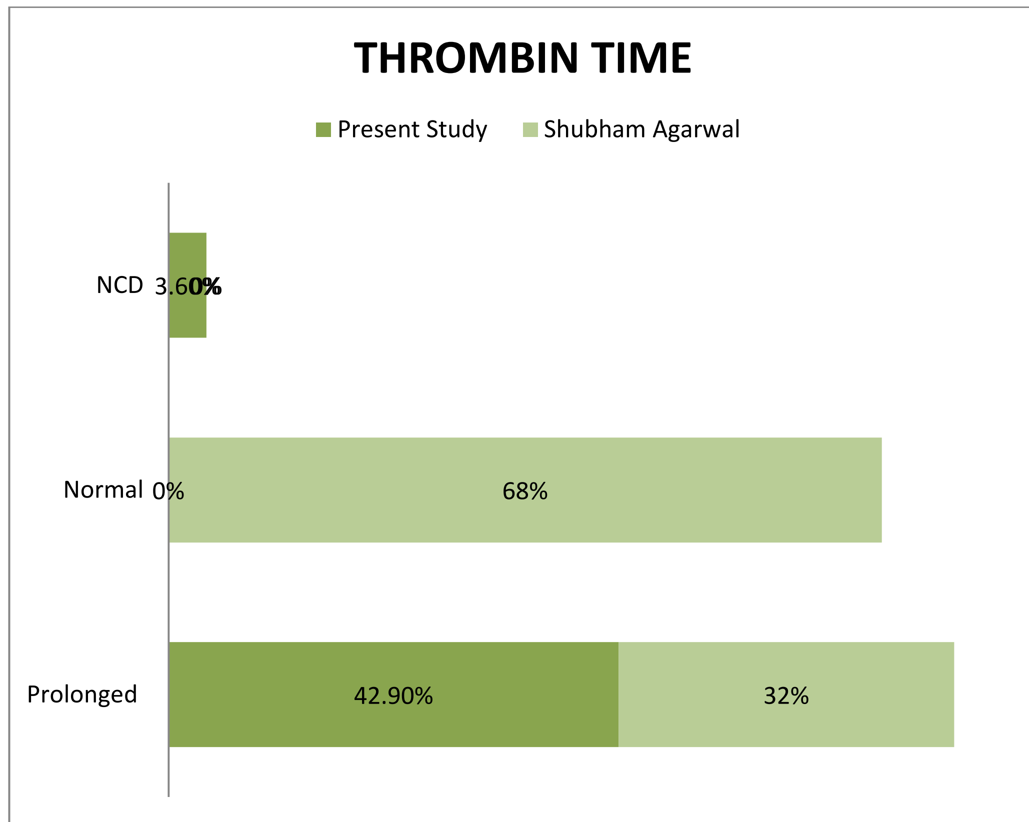
THROMBIN TIME :

In our study, among all the 70 patients with prolonged WBCT, 42.9 % had prolonged TT and 3.6 % had non detectable levels (NCD) which contrast with the study done by Shubham Agarwal , in which 68% showed normal thrombin time, 32 % showed prolonged thrombin time(6). It was observed that after the administration of ASV , 92.9% has normal thrombin time, only 7.1 % had prolonged but detectable levels of thrombin,which is in accordance with the study done by Shubham Agarwal.

Table No 25 : Comparison of Thrombin Time 1 (TT1)

TT 1	**Present Study**	**Shubham Agarwal**
Prolonged	42.9 %	32 %
Normal	0 %	68 %
NCD	3.6 %	0 %

Figure No 19 : Comparison with Shubham Agarwal

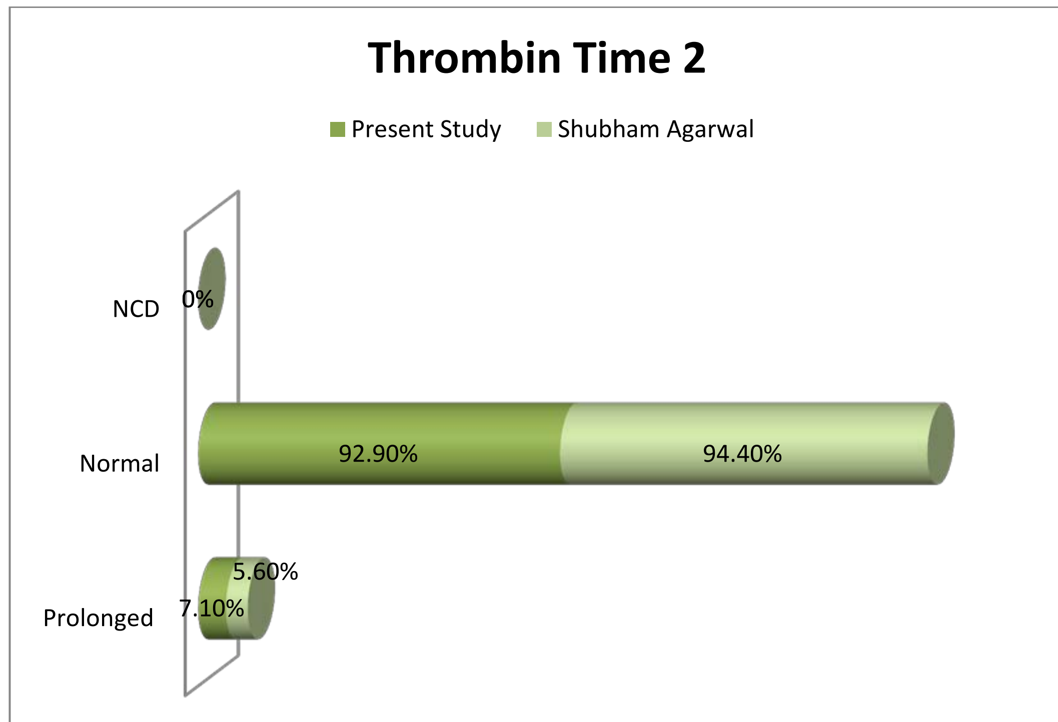


TT which was repeated after 12 hours showed that 94.4 % showed normal TT and 5.6% showed prolonged TT.[6]

Table No 26 : Comparison of Thrombin Time 2 (TT2)

TT 2	Present Study	Shubham Agarwal
Prolonged	7.1 %	5.6 %
Normal	92.9 %	94.4 %
NCD	0 %	0 %

Figure No 20 : Comparison with Shubham Agarwal



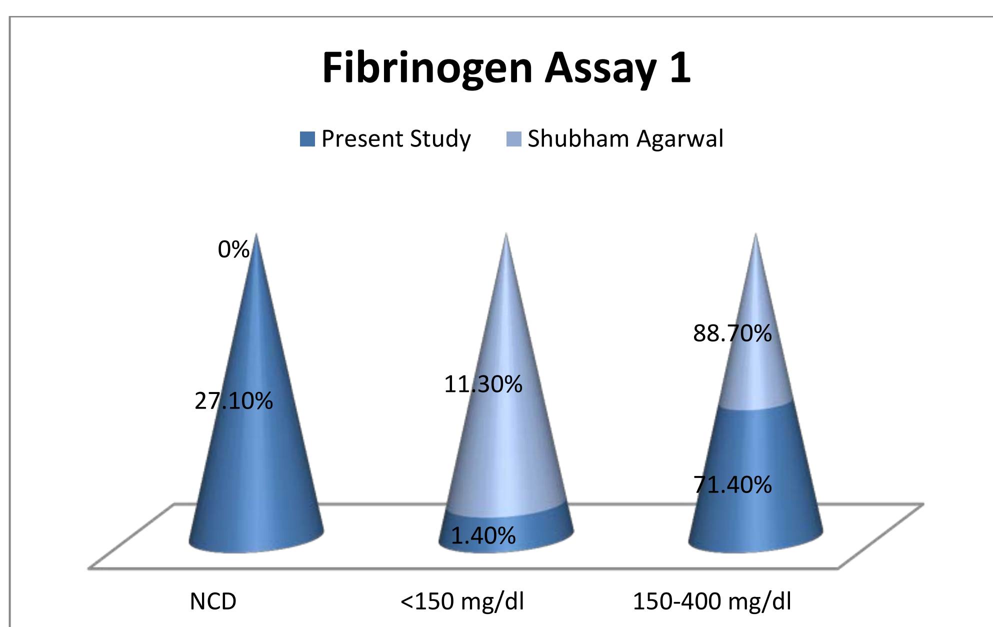
FIBRINOGEN ASSAY :

In our study, this quantitative assay was performed and it shows 27.1% with non detectable levels of fibrinogen, 1.4 % had fibrinogen less than 150 mg / dl . Our study contrasts with the study done by Shubham agarwal in which 11.3% had decreased levels of fibrinogen on admission ,88.7 % had fibrinogen within normal limits.[6]

Table No 27 : Comparison of Fibrinogen Assay 1

Fibrinogen Assay 1	Present Study	Shubham Agarwal
NCD	27.1 %	0%
<150 mg/dl	1.4 %	11.3 %
150-400 mg/dl	71.4 %	88.7 %

Figure No 21 : Comparison with Shubham Agarwal

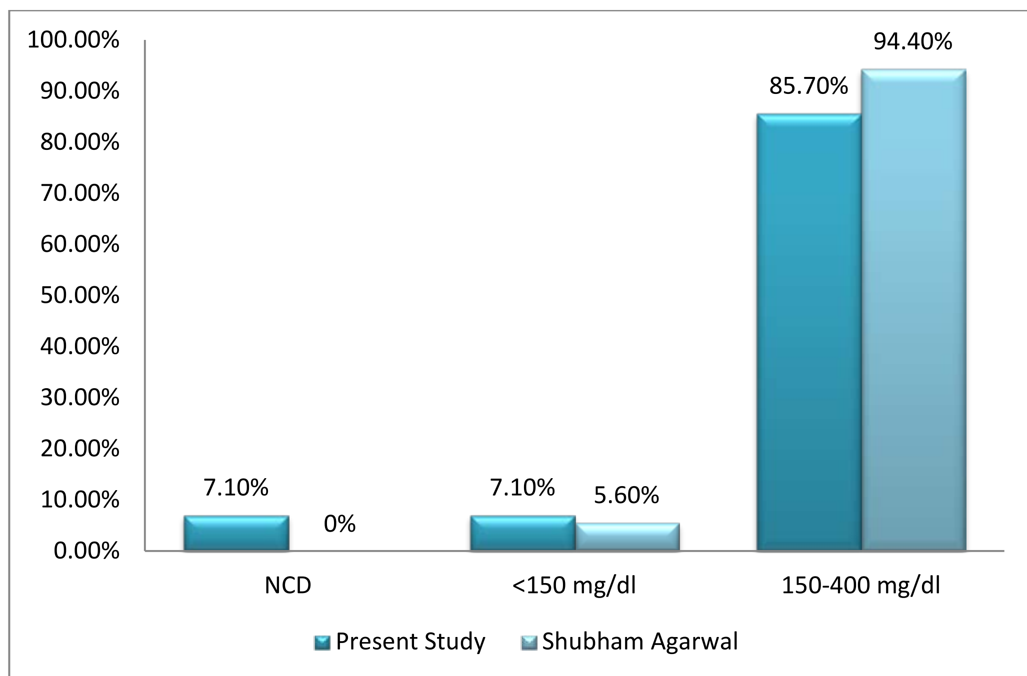


In Our study ,Fibrinogen was repeated after 12 hours after initiating ASV and was observed that only 7.1 % had non detectable levels and 7.1 % had decreased levels of fibrinogen which signifies the prolonged action of the venom which contrasts with the study done by Shubham Agarwal in which ,Fibrinogen Assay which was repeated after 12 hours showed 94.4 % had normal levels of fibrinogen and 5.6 % had decreased levels of fibrinogen.[6]

Table No 28 : Comparison of Fibrinogen Assay 2

Fibrinogen Assay 2	Present Study	Shubham Agarwal
NCD	7.1 %	0 %
<150 mg/dl	7.1 %	5.6 %
150-400 mg/dl	85.7 %	94.4 %

Figure No 22 : Fibrinogen Assay 2



SUMMARY

In the prospective study conducted from January 2018 to June 2019, 70 patients who presented to medical accident and emergency ward , based on our inclusion and exclusion criteria were enrolled.

In the present study, the predominant age group affected was 41 -60 years.Male gender was predominantly affected.

In the present study viper was the commonly encountered species and lower extremity was the predominant bite site, other unusual bite site like Jaw was also observed.

Irrespective of the type of snake all the patient presented with cellulitis , which is due to metalloproteinase action at the bite site. With our laboratory studies we can conclude that action of the venom mainly affects the secondary hemostasis , as clotting time is prolonged in all cases. It is also observed that venom predominantly involves the intrinsic pathway and common pathway , because even after the administration of ASV prolonged APTT, TT, Fibrinogen was prolonged .

In the present study , it is also seen that even after the administration of ASV, patients had prolonged PT, APTT, TT, Fibrinogen which serves as indicator of venom induced consumption coagulopathy.

CONCLUSION

To conclude snake bite is a common medical emergency in the study area. The primary mechanism involved was observed to be Venom induced consumption coagulopathy. Though 20 minute whole blood clotting test is simple, rapid and reliable test of coagulopathy , first line coagulation markers (PT, APTT, TT, fibrinogen) should be considered as first line of investigations . These coagulation markers helps us to assess the severity of the venom and to pin point the level of defect . 12 hours of observation was the safe period to rule out any complications following envenomation.The coagulation markers can also be considered in snake bite with neurotoxic manifestations. Futher investigation like Mixing studies , Clot based factor assays may help us to conclude on the particular factor which is deficient

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Annexure

Normal values :

PLATELET COUNT : 1.5 – 4 Lakhs / mm3

BLEDDING TIME : 2 - 5 minutes

CLOTTING TIME : 8 - 15 minutes

PROTHROMBIN TIME : 12 - 14 seconds

ACTIVATED PARTIAL THROMBOPLASTIN TIME : 28 - 35 seconds

THROMBIN TIME : 12 - 15 seconds

FIBRINOGEN ASSAY : 150 - 400 mg /dl

Key to Master Chart

M : Male

F : Female

L LE : Left Lower Extremity

R LE : Right Lower Extremity

L UE : Left upper Extremity

R UE : Right upper Extremity

L S Right : Left Shoulder

R S : Right Shoulder

PL COUNT : platelet count

BT : Bleeding time

CT : Clotting Time

PT 1 : Prothrombin Time before administration of ASV

APTT 1 : Activated Partial Thromboplastin Time before administration of ASV

TT 1 : Thrombin Time before administration of ASV

FIB 1 : Fibrinogen Assay before administration of ASV

B-A INT : Bite time and ASV administration interval

ASV : Anti Snake Venom

PT 2 : Prothrombin Time after administration of ASV

APTT 2 : Activated Partial Thromboplastin Time after administration of ASV

TT 2 : Thrombin Time after administration of ASV

FIB 2 : Fibrinogen Assay after administration of ASV

C: Cellulitis

GB : Gum Bleeding

HM : Hemorrhage

E: Epistaxis

OLIG: oliguria

PTO : Ptosis

DYS : Dyspnea

S.No	AGE	SEX	BITE SITE	SNAKE	SYMPTOM	PL COUNT BT	BT	CT	PT 1	APTT 1	TT 1	FIB 1	B-A int	ASV	PT 2	APTT 2	FIB 2	TT 2
1	25	M	L LE	Viper	c/gb/hm	26,000	2'30"	28'25"	22.6	41.1	13.6	137.7	5	36	12.2	28.2	138.2	12.3
2	44	M	R LE	Viper	c/e/gb	1,38,000	2'35"	25'30"	NCD	NCD	ncd	ncd	3	24	12.2	28	152	13.2
3	28	M	L LE	krait	c/hm	3,23,000	3'4"	23'22"	21.52	36.16	ncd	ncd	3	32	13.2	28.6	160	14.4
4	24	M	R UE	unknown	c/gb/olig	1,96,000	2'5"	18'22"	18.04	34.28	20.2	321.7	4	26	13.6	32.2	162	14.3
5	38	F	L LE	Viper	c/gb	90,000	3'8"	>20'	18.7	NCD	12.2	ncd	2	33	13.8	29.6	171.2	12.6
6	35	F	R UE	unknown	c	80,000	2'30"	>20'	18.4	45	13.6	254.8	4	21	12.4	28.6	172.2	12.4
7	27	F	R LE	cobra	c/gb	96,000	2'30"	>20'	14	35	14.2	234.7	2	10	12.4	28.6	142.2	13.4
8	16	M	L S	krait	c	2,73,000	2'20"	11'16"	13.4	28	15.04	195.9	5	15	12.6	30.2	162.2	13.4
9	64	M	L UE	krait	c/hm	1,96,000	2'30"	22'30"	14.4	29.92	15.48	ncd	3	10	11.4	32.2	154.2	12.5
10	50	F	R LE	Viper	c/hm	80,000	2'30"	24'30"	14.52	32.96	14	254.6	12	15	11.6	30.8	188.2	13.5
11	25	M	L LE	cobra	c/gb/pto	10,000	3'30"	26'30"	18.6	48	13.72	318.3	6	30	12.6	31.2	189.2	13.6
12	28	M	R LE	krait	c/gb/hm	76,000	2' 45 "	25'30"	24.7	30.72	20.04	135.1	6	20	12.8	31.2	190.2	14.3
13	39	M	LS	krait	c	60,000	2'8"	23'15"	19.4	41.72	13.2	90	6	25	13	30.8	152.2	12.4
14	60	F	R LE	krait	c/gb/epi	70,000	2'22"	27'30"	14.2	36	13.2	262.8	2	22	14	29.2	154	12.4
15	23	M	R UE	unknown	c/gb	55,000	2'20"	24'30"	18.2	42	14	ncd	6	16	12.6	28.2	156.3	13.5
16	66	M	L LE	Viper	c/epi	95,000	2'22"	26'30"	18	36	12.6	312.6	5	22	12.6	28.7	190.2	13.6
17	54	M	R LE	krait	c/gb	1,00,000	3'20"	25'30"	14.2	33	18	282.4	10	15	12.8	27.4	172.2	14.2
18	7	M	L LE	Viper	c/gb	1,10,000	2'35"	23'15"	24	42	13.2	ncd	3	35	12.6	28.4	180.2	13.7
19	35	F	R LE	Viper	c/gb	80,000	2'18"	27'30"	16	38	12	312.6	5	32	12.8	28.6	182.2	12.4
20	45	M	R LE	Viper	c	80,000	2'34"	24'30"	16	38	12	282.4	5	24	13.2	29.2	176.2	12.6
21	20	M	R LE	unknown	c/gb/dysp	1,25,000	2'30"	28'30"	16	37	13	ncd	12	20	13.4	29.4	156.2	13.6
22	57	M	L LE	cobra	c/gb	2,26,000	3'22"	22'30"	16	34	14	276.3	2	30	14	29.6	154.2	13.3
23	60	F	L LE	Viper	c/gb	76,000	2'10"	26'30"	16	38	16	268.2	4	17	13.8	28.4	178.2	13.4
24	46	F	L LE	krait	c/gb	66,000	2'12"	26'2"	16	42	14	273.2	12	10	12.6	28.4	156.2	14.5
25	32	F	L S	unknown	c/gb	1,18,000	2'4"	18'22"	16	36	10	162.8	6	15	12.6	28.4	162.2	15.2
26	40	M	L LE	krait	c/hm	2,00,000	2'40"	15'30"	16	36	12	382.2	10	28	14.2	30.4	182.2	13.2
27	58	M	R LE	Viper	c/hm	2,00,000	2'3"	19'20"	16	36	16	352.6	4	32	12.6	30.2	184.2	14.2
28	35	M	R LE	krait	c/gb	70,000	2'5"	16'22"	16	38	16	396.2	12	25	12.7	30.2	189.2	12.5
29	46	M	R UE	Viper	c	25,000	2'33"	28'30"	16	46	22	162.2	6	18	12.3	32.6	162.2	12.6
30	55	M	R UE	Viper	c/gb	1,10,000	2'5"	27'30"	16	38.4	22.56	138.6	6	16	12.2	32.6	163.2	14.2
31	94	M	R S	cobra	c/pto/dys	2,36,000	3'35"	23'10"	16	32.7	14.4	346.2	8	14	12.2	34.2	159.2	14.6
32	23	M	R LE	unkown	c/gb	1LAKH	2'8"	24'30"	16	32.4	18.4	ncd	12	16	14.6	34.2	158.2	12.4
33	44	M	R LE	Viper	c/gb/hm	90,000	2'4"	23'10"	16	39.4	15.9	282.3	3	4	14.8	30.4	156.2	13.6
34	50	M	R S	krait	c/gb	80,000	2'5"	25'10"	16	39.5	10.2	ncd	3	5	13.6	24.4	142.6	13.3
35	62	M	JAW	Viper	c/gb	45,000	2'8"	21'13"	16	42.2	11.6	183.5	4	5	14.8	28.7	170.4	14.2
36	17	M	L LE	Viper	c/epi	60,000	2' 43 "	26'14"	16	36.2	12.3	218.3	5	6	14.6	32.4	162.3	15.6
37	18	M	R LE	krait	c	1,20,000	2' 34"	24'18"	16	36.2	ncd	195.3	5	56	12.2	32.2	ncd	14.6
38	46	M	R UE	unknown	c/gb	1,51,000	2' 23"	28'36"	16	54.2	ncd	283.4	6	3	14.6	28.5	179.8	13.2
39	49	M	L LE	cobra	c/gb	25,000	2' 34"	23'45"	16	50.2	11.6	195.3	5	3	13.6	28.3	173.2	12.4
40	47	F	L LE	krait	c/gb	78,000	2'42"	18'20"	16	36.2	13.2	ncd	6	105	14.6	28.6	170.3	12.6
41	72	M	L UE	krait	c/hm	60,000	3' 23"	17'23"	16	48.2	18.2	ncd	3	4	13.6	32.4	165.4	13.5
42	47	F	R UE	krait	c/gb/epi	1LAKH	2' 32"	24'34"	16	39.6	18.2	198.2	3	6	12.2	30.6	176.2	13.7
43	17	M	L LE	unknown	c/hm	10,00,000	2' 34"	23'18"	16	36.2	10.2	283.3	10	12	14.5	32.8	ncd	14.2
44	32	F	R LE	cobra	c/pto/dys	1LAKH	2' 36"	27'12"	16	NCD	ncd	267.3	5	4	12.2	36.2	179.2	14.6
45	28	M	L UE	Viper	c/gb	1,25,000	2' 35"	21'34"	16	38.2	11.6	181.2	4	10	14.8	34.5	168.3	13.3
46	20	M	R LE	Viper	c/gb	2,25	2' 36"	22'36"	16	36.2	18.2	184.3	6	14	13.6	37.8	165.81	13.5
47	20	M	R LE	unknown	c/gb	20	2' 34"	23'43"	16	32.2	12.2	194.2	12	6	14.8	32.5	148.2	13.6

S.No	AGE	SEX	BITE SITE	SNAKE	SYMPTOM	PL COUNT BT	BT	CT	PT 1	APTT 1	TT 1	FIB 1	B-A int	ASV	PT 2	APTT 2	FIB 2	TT 2
48	50	M	L UE	Viper	c/gb	1	2' 54"	23'54"	16	48.2	17.2	196.2	4	12	14.8	33.6	ncd	13.3
49	26	F	R LE	cobra	c/gb	75	2' 32"	22'13"	16	36.2	15.2	211.2	10	4	12.2	32.5	170.3	14.5
50	60	M	JAW	krait	c/hm	60	2'36"	23'18"	16	38.2	20.2	226.2	14	3	13.6	36.2	168.2	13.5
51	66	M	L S	Viper	c/hm	68	2' 46"	24'18"	16	36.2	12.2	ncd	6	8	14.8	34.2	158.1	15.5
52	65	F	R LE	unknown	c/gb	75,000	3' 32"	27'17"	16	36.2	15.2	ncd	12	5	13.2	35.2	ncd	13.4
53	50	F	JAW	unknown	c/gb	80,000	3' 43"	24'15"	16	38.2	13.2	ncd	4	4	13.6	28.3	173.4	13.6
54	45	F	R LE	Viper	c/gb/epi	1,25,000	2' 32"	25'23"	16	38.2	19.2	ncd	3	5	12.4	28.4	182.2	12.5
55	44	M	R LE	Viper	c/gb	20,000	2' 34"	21'12"	16	38.4	12.3	232.1	8	10	12.6	28.4	162.4	15.6
56	41	M	L UE	cobra	c/gb	75,000	2' 38"	21'27"	16	32.2	19.2	186.2	5	12	13.2	30.5	169.2	15.7
57	23	M	L LE	krait	c/gb	1,25,000	2' 37"	25'24"	16	38.8	19.2	312.2	4	6	12.4	34.2	142.2	13.5
58	45	F	R S	krait	c/epi	1,35,000	3' 12"	24'32"	16	44.6	18.2	268.2	5	12	14.6	28.6	189.6	12.4
59	76	M	JAW	cobra	c/pto/dys	2,25,000	3' 15"	17'32"	16	32.8	11.2	ncd	10	12	12.4	28.2	182.2	12.5
60	43	M	L LE	Viper	c/hm	25,000	2' 23"	18'23"	16	40.4	14.2	ncd	12	4	12.3	28.2	164.4	12.5
61	28	F	R LE	Viper	c/gb	1,15,000	2' 32"	18'44"	16	35.2	14.2	168.3	6	4	12.8	34.8	180.2	12.4
62	25	M	L UE	Viper	c/gb	1,65,000	2' 45"	19'34"	16	36.2	15.6	ncd	12	10	14.6	36.2	189.6	13.6
63	42	M	R LE	Viper	c/gb	85,000	2' 34"	24'18"	16	40.2	16.6	ncd	12	4	14.6	34.2	169.2	13.8
64	47	M	R UE	Viper	c/gb	85,000	2' 32"	23'18"	16	40	20.6	168.3	4	4	12.2	28.8	192.3	13.6
65	35	M	L LE	unknown	c/epi	90,000	2' 35"	22'9"	16	32.2	14.8	ncd	4	5	12.8	28.8	187.2	13.5
66	46	M	L LE	Viper	c/hm	1,26,000	3'43"	25'15"	16	37.3	15.6	199.2	10	4	12.6	28.6	176.6	13.4
67	32	M	R LE	krait	c/gb	76,000	2' 34"	23'26"	16	38.2	16.2	232.2	4	4	12.4	28.4	182.6	13.5
68	45	F	R LE	Viper	c/epi	50,000	2' 34"	24'37"	16	38.2	17.2	211.6	4	7	13.2	28.7	162.6	12.4
69	49	F	R UE	Viper	c/gb	65,000	3'11"	26'32"	16	40.2	17.2	226.2	6	6	13.4	32.6	153.2	12.6
70	68	M	JAW	unknown	c/hm	95,000	2' 13"	26'13"	16	42.3	15.2	196.2	4	6	12.6	34.4	178.6	13.4