Characterisation of Venom Induced Consumption Coagulopathy (VICC) in patients with Haemotoxic Snake Bite and the effects of Blood Products on Coagulation Parameters.



DISSERTATION SUBMITTED IN PART FULFILLMENT OF THE REQUIREMENTS FOR THE M.D. DEGREE BRANCH XXI (TRANSFUSION MEDICINE AND IMMUNOHAEMATOLOGY) EXAMINATION OF THE TAMIL NADU DR.M.G.R.MEDICAL UNIVERSITY CHENNAI TO BE HELD IN MAY 2019.

This is to certify that this dissertation titled "Characterisation of Venom Induced Consumption Coagulopathy (VICC) in patients with Haemotoxic Snake Bite and the effects of Blood Products on Coagulation Parameters" is a bonafide work done by Dr. Jess Elizabeth Rasalam, in part fulfillment of rules and regulations from the M.D. BRANCH XXI (Transfusion Medicine and Immunohaematology) Degree examination of the Tamil Nadu Dr. M.G.R Medical University, to be held in May 2019.

I have independently reviewed the literature, standardized the data collection methodology and carried out the evaluation towards completion of the thesis.

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LIST OF ABBREVIATIONS

3 FTX	3 Finger Toxin
ACE	Angiotensin Converting Enzyme
ACT	Activated Clotting Time
ADAMTS 13	A Disintegrin And Metalloproteinase with A ThromboSpondin type 1 motif member 13
ANP	A Type Natriuretic Peptide
APTT	Activated Partial Thromboplastin Time
ASV	Anti-Snake Venom
BPP	Bradykinin Potentiating Peptide
CNP	C Type Natriuretic Peptide
CRISP	Cysteine Rich Secretory Proteins
DIC	Disseminated Intravascular Coagulation
FFP	Fresh Frozen Plasma
HRP	Horse Radish Peroxidase
HUS	Hemolytic Uremic Syndrome
INR	International Normalized Ratio
LAAO	L Amino Acid Oxidase
NEGF	Nerve Endothelial Growth Factor
NTD	Neglected Tropical Diseases
PLA2	Phospholipase A2
РТ	Prothrombin Time
SVMP	Snake Venom Metalloprotease
SVTLE	Snake Venom Thrombin Like Enzyme
TMA	Thrombotic Microangiopathy
TTP	Thrombotic Thrombocytopenic Purpura
VEGF	Vascular Endothelial Growth Factor
VEMAC T	Vellore Manually Activated Clotting Time
VICC	Venom Induced Consumption Coagulopathy
vWF	von Willebrand Factor
WBCT20	Whole Blood Clotting Time20
WHO	World Health Organization

ABSTRACT

Introduction

Snake bite is one of the most important "Neglected Tropical Diseases" in terms of both incidence and severity, and its clinical characteristics. Venom-Induced Consumption Coagulopathy (VICC) is the core pathogenic mechanism in haemotoxic snake bites. The common derangements seen are prolonged Prothrombin time (PT), prolonged Activated Partial Thromboplastin Time (APTT), low/undetectable fibrinogen. VICC is characterized by reduction of coagulation factors and the absence of systemic microthrombi and end-organ damage. The time course in VICC is rapid- occurring within a few hours of envenomation and resolution within 24-48 hours, if treated appropriately.

Objectives

The primary objective of this study is to identify patients with haemotoxic snake bite and to characterize Venom Induced Consumption Coagulopathy (VICC) in these patients. The secondary objectives are to study the coagulation profile in patients with haemotoxic snake bite and their response to treatment with Anti Snake Venom (ASV) and/or blood products. Comparison of sensitivity and specificity of the new test Vellore **Manually Activated Clotting Time** (Vemac Time) against a composite diagnosis of VICC. A review of retrospective data spanning a five-year period of the clinical and lab parameters of patients with haemotoxic snake who received blood products transfusion has also been undertaken.

Methods and Materials

This is an observational study with a retrospective arm comprising patients who were admitted between year 2012-2017 and a prospective arm for patients admitted between 2017 - 2018 at Christian Medical College, Vellore.

Results

Data from 280 patients who had a haemotoxic snake bite were analysed. Among these 47(16.8%) patients were transfused blood and plasma products. There was a male preponderance among patients (70.2%). Isolated haemotoxic features and local reaction were seen in 8.5%. Coexisting neurological and/or renal manifestations were seen in more than 90% patients. The average dose of ASV received per patient was 18.9 \pm 7.75 vials. Baseline INR more than 1.5 was seen in 87.2% and an elevated APTT ratio was seen in 55.3%. All components were transfused: platelet concentrates (30%), FFP (66%), cryoprecipitate (32%) and cryosupernatant (11%). Mortality was 10.6% among this patient group with transfusions.

Among the prospective follow up group pure haemotoxicity was seen in 24.3% (n=9) patients. Combinations of haemotoxicity with renal and/or neurological manifestations were seen in 75.7% (n=28).

The new test Vellore Manually Activated Clotting Time (Vemac Time) was compared against Prothrombin Time and also a composite diagnosis of VICC. The sensitivity of the test was found to be 81.82% and specificity was 100% when compared with PT. Positive predictive value of the test was 100% when compared with PT. When compared to a composite diagnosis of VICC it was found to have positive predictive value of 100%.

Discussion and conclusion

Snake bite was and still remains a problem that can easily be tackled, if diagnosis and treatment is given in a timely manner. The role of a good haemostasis laboratory in detecting VICC and management of the patient is emphasized by this study. Antivenom is the major treatment for VICC. Treatment focuses on neutralization of venom effects with antivenom and waiting for the replenishment of coagulation factors. Antivenom is not risk free and adverse reactions can be quite common and potentially severe. Patients should be observed in hospital until clotting function has normalised.

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INTRODUCTION

Snake bite is a one of the common medical emergencies and also an occupational hazard, which is frequently encountered in tropical India. More than 2,000 species of snakes are known worldwide. These includes almost 400 poisonous snakes. In India, Naja naja commonly known as spectacled cobra, Bungarus caeruleus or the common krait, Echis carinatus or the saw-scaled viper and Daboia russelii or the Russell's viper have long been recognised as the most important. Other species which may cause fatal envenomation are distributed in certain regions. These includes central Asian cobra (*Naja oxiana*) in the far north-west, monocellate cobra (*N. kaouthia*) and greater black krait (B. niger) in north-east, Wall's and Sind kraits (B. walli and B. sindanus) in the east and west and hump nosed pit-viper (*Hypnale hypnale*) in the south-west coast and Western Ghats. These snakes belong to the four families namely *Elapidae*, *Viperidae*, Hydrophiidae and Colubridae. The Russell's viper (Daboia russelii) commonly inhabits Southern Asian countries. Russell's bite is considered as an occupational hazard for the farming communities in India. Regardless of the fact that India is neither the home for venomous snakes nor there a shortage of anti-snake venom, every year there are 50,000 deaths from 2,50,000 incidents of snake bite. (1).

There is a paucity of data on the epidemiological profile of snake bite from the Indian subcontinent. Commonly rural people are victims of snake bite and use traditional healers. These cases go unidentified. Rural people are not well informed about the risks of harmful practice and how simple measures can prevent or treat snake bites. They adopt harmful practices such as tourniquets, cutting & suction, herbal remedies, quackery etc. These are not only inadequate but also hazardous(2). Only the cases with symptoms of severe envenomation reach proper health care services.

Snake venoms are rich collections of enzymes, proteins, peptides and other components that can cause a wide range of physiological, neurological and haemostatic effects on their prey. Among these effects, the venom components that affect mammalian haemostasis have been most well studied for more than 150 years. They have added to the explanation for the extensive mechanisms of the haemostasis process (e.g. platelet aggregation and inhibition, mechanism of defibrination, DIC, various coagulopathies, etc), elucidation of various clinical disorders (e.g. congenital haemorrhagic disorder, various blood factor deficiencies, etc), development of many diagnostics (e.g. Reptilase time) and therapeutics (e.g. batroxobin). Therefore, venoms have been regarded as 'gold mines' for researchers, pharmaceutical companies, clinical analysts and medical practitioners (3).

Qualitatively and quantitatively variation in venom is appreciable in different families of snakes (4)(5). In general, snake venoms varies mostly in composition of soluble polypeptides, but may also vary in amount of carbohydrates, lipids, metal ions, and other organic compounds, including amines and purines (6).

Almost up to 90% of the dry weight of most venom is comprised of polypeptides of three size classes: low molecular weight components (< 1.5 kDa), polypeptide toxins (5 to 10 kDa), and enzymes (10 to 150 kDa) (3).

AIM

The aim of the study was to characterize the effects of snake bite on the haemostatic system, transfusion requirements and effect of transfusion on haemostatic parameters.

OBJECTIVES

The primary objective of our study was:

1.To identify patients with haemotoxic snake bite and to characterize Venom Induced Consumption Coagulopathy (VICC) in these patients.

The secondary objectives were:

- 1. To study the coagulation profile in patients with haemotoxic snake bite and their response to treatment with Anti Snake Venom (ASV) and/or blood products.
- 2. Comparing sensitivity and specificity of the new in house designed test named Vellore

Manually Activated Clotting Time (Vemac Time) against a composite diagnosis of

VICC.

3.To retrospectively study for a period of five years the clinical and lab parameters of patients with haemotoxic snake who received blood products transfusion.

METHODS AND MATERIALS

SETTING

This study was carried out in the Departments of Transfusion Medicine and Immunohaematology along with the Departments of Internal Medicine, Accident and Emergency and the Medical Intensive Care Unit, at Christian Medical College Hospital, Vellore.

The study was approved by the Institutional Review Board of the Institute IRB no 10467 dated 05/01/2017. For the part of the study being conducted retrospectively, all patient identifying information was completely delinked from the study.

SAMPLE SIZE CALCULATION

This was an observational study which was conducted over a period of 15 months. The National Snake Bite study from Christian Medical College (CMC), Vellore completed in 2016 showed about 170 patients over a period of 2 years. 60-70 patients were expected to be recruited in this study. However, only 37 patients with snake bite fulfilled the inclusion criteria for this study.

PARTICIPANTS

Inclusion Criteria for the prospective study

1.All consenting patients above 16 years of age presenting to CMC Hospital with haemotoxic snake bite within 24 hours.

2.Patients who have an abnormal Whole Blood clotting time and/or PT with INR ≥ 1.2 with or without bleeding manifestations.

Exclusion Criteria for the prospective study

1.Children (<16 years)

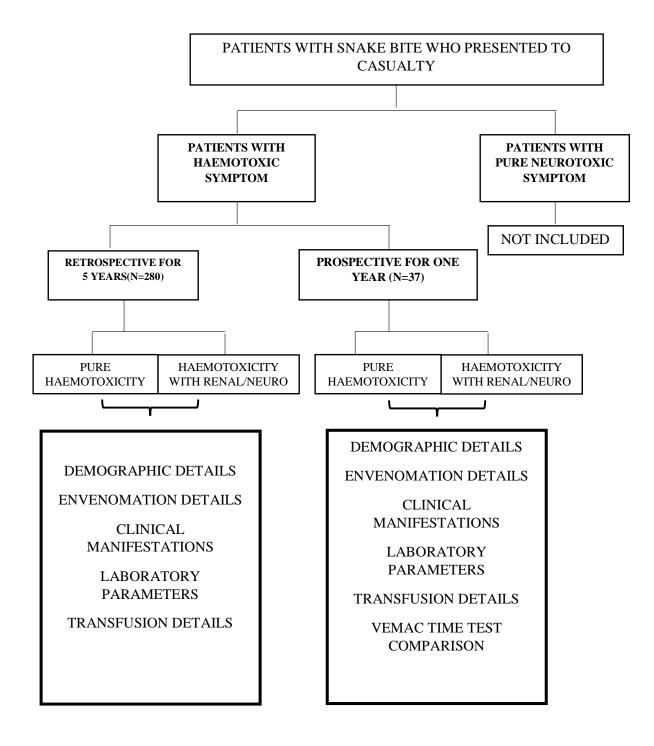
2. Cases of snake bite with no evidence of envenomation and blood tests remain normal.

3.Non-consenting patients.

The patients had the standard investigations and treatment for snake bite as it is currently practised in CMC. The results from the various blood tests and the clinical course including transfusion requirements of the patient were observed. These were analysed to see whether they fulfil the diagnostic criteria described in the literature for VICC.

All categorical clinical profile variables were expressed as frequencies and percentages. All continuous variables which were approximately symmetric were presented using mean with SD. If not symmetric, median with IQR were presented.

FLOWCHART FOR DATA COLLECTION:



REVIEW OF LITERATURE

GENERAL FACTS ABOUT SNAKES

WHAT ARE SNAKES?

Snakes are squamate animals and part of the lizard phylogeny. They are a very diverse and specialized group of limbless lizards. Snakes are members of the second most speciose group of living reptiles (Reptile Database: http://www.reptiledatabase.org/).

"Limblessness" has evolved quite commonly among lizards and, including snakes, this feature has evolved independently at least 25 times (7). However, no group of limbless lizards are as successful as snakes. There are approximately 3,150 species of snake and they occur in nearly every habitat and on every continent except Antarctica.

Snakes are cold blooded animals without ears or tympanic membranes. They react to vibrations received through the surface on which they rest and are very sensitive to vibrations from movement. Snakes do not have good visual acuity and they do not generally associate stationary objects with danger. They have a good sense of smell and snakes are well known for the vomeronasal system of odour detection. Most of the land snakes feed on small animals such as mice, rats and frogs. Kraits and Cobras are exceptional and mainly eat other snakes.

EPIDEMIOLOGICAL FEATURES OF SNAKE BITE

Snake bites are common emergencies encountered in clinical practice. The estimated number of persons affected by snake bite is not accurately known, but, it is assumed

that more than 2,00,000 persons are annually bitten by snakes in India and about 15,000 of these turn out to be fatal (8).

In India, the prevailing climatic conditions and the fact that a major portion of the population is rural and agrarian, results in snake bite being a major health problem. Snake bite morbidities can be a preventable health hazard in these most affected population.

The death rate from snake bite is estimated to be 5% of bites. However, this figure is based on hospital statistics. In practice however, most rural patients are treated by local traditional methods and do not reach proper health care facility (9).

India is a country with more than 60 species of venomous snakes. Spectacled cobra (*Naja naja*), common krait (*Bungarus caeruleus*), saw-scaled viper (*Echis carinatus*) and Russell's viper (*Daboia russelii*) are the well identified venomous snakes. However, there are other species in particular regions, for example, the hump nosed pit-viper (*Hypnale hypnale*) in the south-west coast and Western Ghats (9) which lead to fatal envenomation.

Almost 26 species belong to the family of true vipers (subfamily *Viperinae*) and pit vipers (*Crotalinae*). Among them, Russell's viper (*Daboia russelii*) envenomation leads to the highest morbidity and mortality. Saw scaled viper (*Echis carinatus*) is commonly seen in western and southern India. They are also found in the dry coastal parts of northern Sri Lanka. Pit vipers have generally been considered to be less of a problem in South Asia. However, these snakes occur in diverse habitat types from wet mangroves to high mountains. These are also common in domestic gardens and agricultural areas.

Recent literatures from South India have reported high morbidity among plantation workers due to bites by the much smaller species, the Malabar pit viper (*Trimeresurus malabaricus*). Hump-nosed pit vipers (*Hypnale* and *H. nepa*) are also being identified as medically dangerous species in these regions, and can potentially cause renal failure and haemostatic abnormalities. Severe fatalities have been documented in India and Sri lanka due to *H. hypnale* envenoming (10).

The "Big 4 Snakes of Medical Importance," namely the Russell's viper, the saw scaled viper, the Indian or spectacled cobra, and the common krait are believed to cause the vast majority, of fatalities due to snake bite (1). Venomous snake bite was listed as NTD- "Neglected Tropical Disease", by the WHO in 2009 and it was removed from the list in 2013. It was again recognised by the World Health Organization (WHO) in the category A of the Neglected Tropical Diseases on June 9th, 2017 (11). It indicates that snake bite is prevalent in tropical and subtropical conditions in 149 countries affecting more than a billion people, costing developing economies billions of dollars of loss every year. Worldwide, approximately 4,21,000 envenoming and 20,000 deaths occur annually from snake bites. Figures are reported to be as high as 1,841,000 bites with envenomation, leading to almost 94,000 deaths (12). Not all snake bites lead to envenomation. In general one third of snake bites causes envenomation(10).

SOCIO DEMOGRAPHIC FACTORS

The increase in population numbers and population spread leads to encroachment by people into reptile habitats which increases the likelihood of contact with reptiles. Inadequate infrastructure in villages, including poor lighting, open sewerage systems, roads, lack of in-house water supply may all co-contribute to bites especially occurring

at night. Improper sanitation contributes to increase in rodent population and therefore snake presence. Poor transport facilities in rural areas leads to significant and at times, fatal delays in transportation of patients to medical care facilities (13).

SOCIO-CULTURAL

Most people who live in villages lacks protective foot wear, and sleep on the ground. Livestock are also present in close proximity to houses which draw rats to these places and therefore their predators too. Lack of toilet facilities leading to open defecation, quite often after sunset are all important socio-cultural contributory factors (13).

MEDICAL

Medical awareness among victims and their families, of the immediate measures to be followed when bitten by a snake is lacking. Precious time is lost when alternate or traditional forms of treatment are sought. Inadequate first aid measures leads to systemic envenomation and added complications (13).

LEGISLATIVE / GOVERNMENTAL

The process of manufacture of Anti-Snake Venom (ASV) and also its standardization lacks centralised quality control. The venom used for the manufacture of ASV for the entire country is from one or two sources and is limited to a small geographical area. Research has shown regional variation in venom constituents and chemical properties, as well as intra-species variation. Regional/zonal venom centres with the facilities to manufacture ASV for a particular region or zone are therefore important to ensure appropriate ASV availability (13). Characterisation of Venom Induced Consumption Coagulopathy (VICC) in patients with Haemotoxic Snake Bite and the effects of Blood Products on Coagulation Parameters.

TAXONOMY, 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, Hydrophidae, Colubridae* [some species] and *Viperidae*. In India 236 different species of snakes are seen, of which 50 species are identified as being poisonous. Among the non-venomous snakes only the giant constrictors are potentially dangerous to man – these include the South African and Asian pythons, and the South American anaconda .

Poisonous snakes prevalent in India belong to four families.

They are

- 1. *Elapidae* includes cobras & krait
- 2. *Viperidae* (true vipers) includes Russell's viper & saw scaled viper.
- 3. *Colubridae* (pit vipers) includes green pit viper.
- 4. *Hydrophidae* (or) sea snakes.

Elapidae, *Viperidae* and *Colubridae* are three families of venomous snakes in South-East Asia:

Elapidae: This family of snakes have relatively short fixed front fangs. It includes cobras, king cobra, kraits, coral snakes, Australasian snakes and sea snakes. They are relatively long, thin and uniformly-coloured snakes. They possess large smooth symmetrical scales on the dorsum of the head. They lack loreal scale between their pre-ocular and nasal scales. Two species of cobras are found in India, common cobra (*Naja naja*) and king cobra (*Ophiophagus hannah*). The head of the cobra is indistinct from the neck and the ribs in this area are movable and can expand to form the hood. This

hood on its dorsal aspect has a marking which resembles a spectacle showing a connected pair of rings. King Cobras are found in dense forests and can be up to a length of 18 feet. They are usually black in colour.

Two species of Kraits are commonly found in India. The Common Krait and Banded krait - Common krait is steel blue or black with white bands on the back. Banded krait is larger and is jet black in colour with yellow bands.

Viperidae: In contrast to above family these snakes have relatively long fangs. These fangs are normally folded flat against their upper jaw. When they bite, it becomes erected. They are further divided into two subfamilies, true vipers (*Viperinae*) and pit-vipers (*Crotalinae*).

Russell's viper is a larger snake measuring 6 feet and makes a loud hissing sound by expelling air through its large nostrils. It can appear sluggish and stout. It is brown or yellowish with dark round spots on the dorsum with white and black edges (14). Saw scaled viper a small snake (30cm long) with brown or grayish dorsum showing a zig zag pattern. It has a distinct cross or lance shaped mark on the head. The ventral scales are rough. They can produce a grating sound by rubbing their coils together. The other sub family *Crotalinae* possess a well formed infra-red heat-sensing organ, the loreal pit organ, to detect their warm-blooded prey. This is situated between their nostril and the eye.

Colubridae: The third group among the venomous snakes include colubrids. They make up the largest group of snakes and almost include 75 percent of all the world's snake species. Most of the these possess wide scales on their bellies and, usually, nine large scales on the tops of their heads. They also have glands behind each eye. These glands release a mixture of chemicals. Their venom usually takes minutes to act unlike the cobras and vipers, whose fast-acting venom can kill their prey in moments.

Non-venomous snakes

Several species of non-venomous snakes are responsible for bites. These commonly are found in urban and rural gardens. Humans are liable to their bite when approached too closely. Notably *Lycodon* and *Dryocalamus* mimics krait, resulting in unnecessary medical treatment (15).

Identification of venomous snakes

There is no simple general rule for identifying a venomous snake. Some harmless snakes have evolved to look almost identical to venomous ones. Various species of *Lycodon, Dryocalamus* and *Cercaspis* mimic the appearance of the kraits *B. candidus, B. caeruleus* and *B. ceylonicus*. The tail raising display and colouring of *Cylindropis ruffus*, may mimic coral snakes (*Calliophis* species). Most of the venomous snakes can be recognized by their size, shape, colour, pattern of markings, characteristic behaviours and sounds made when they feel threatened. When a cobra is threatened they spread a hood, hiss and make continuous strikes. There can be vast differences in the colour pattern between the same species of snakes. However distinct identification can still be made, like the possession of longitudinal rows of large, dark-rimmed, pale-centred spots of the Russell's vipers, or the alternating black and yellow circumferential bands of the banded krait. Snakes can even be distinguished by other characteristics like blowing hiss of the Russell's viper or a grating rasp of the saw-scaled viper (16).

THE VENOM APPARATUS:

The evolution of altered teeth capable of injecting venom evolved over millions years ago (16). Among the venomous snakes *Elapidae* and *Viperidae*, venom glands are situated behind the eye, surrounded by compressor muscles. The duct from these glands opens at the base of the fang. The venom flows through a groove to the tip of the fang(16). In *Elapidae* fangs are seated in the front of the mouth. In *Viperidae* fangs are seated on a rotatable maxilla which can be folded flat against the roof of the mouth. In *Colubridae* venom secreted by Duvernoy's (supralabial) glands which are positioned at the posterior end of the maxilla (17). Fangs allow the snake to inject venom deep into the tissues of its prey. Venom is generally injected subcutaneously or intramuscularly. Spitting cobras ejects the venom from the tips of their fangs in a skilful spray aimed towards the eyes of an intruder (16).

SNAKE VENOM

90% of the dry weight of snake venom is composed of more than a hundred varieties of proteins comprising of enzymes, non-enzymatic polypeptide toxins, and several other non-toxic proteins. The enzymes include digestive hydrolases, hyaluronidase (spreading factor), yellow L-amino acid oxidases, phospholipases A2, and peptidases (16).

Snake venom metalloproteases (SVMPs) may cause damage to the basement membrane resulting in severe endothelial damage causing spontaneous systemic bleed. Procoagulant enzymes includes activators of factors V, X, prothrombin and other clotting factors, causing Disseminated Intravascular coagulation (DIC) or incoagulable blood.

Phospholipases A2 can damage intracellular organelles and may produce wide spread toxicity which may include presynaptic neurotoxicity, cardiotoxicity, myotoxicity; tissue damage resulting in necrosis, hypotension, haemolysis, anti-coagulation, haemorrhage, plasma leakage (oedema formation) and release of histamine and other autacoids. Polypeptide postsynaptic (α) neurotoxins bind to acetylcholine receptors at the motor endplate. Presynaptic (β) neurotoxins are phospholipases that permanently damage nerve endings.

The chemical content and antigenicity of snake venoms varies strikingly between and within species, as these snakes mature, depending on season, between sexes, and in their geographical distribution. Thus, it is very important to note that envenoming by a particular species of snake in certain part of its geographical range may not be showing repose to an antivenom prepared against venom from the same species in some else location.

PATHOPHYSIOLOGY OF HUMAN ENVENOMING

Snake bite causes swelling and bruising locally as a result of the venom. This causes increased vascular permeability. Tissue necrosis can be seen because of thrombosis, tight tourniquets applied as first-aid, fascial compartments with swollen muscles (16). Hypovolaemia due to leakage of plasma may lead to severe hypotension. Other released oligopeptides like ACE inhibitors and bradykinin-potentiating peptides (BPPs) which are vasodilating autacoids can cause early transient hypotension. Injection of procoagulant enzymes lead to defibrinogenation, or a consumptive coagulopathy. Platelet activation/inhibition and sequestration leads to profound thrombocytopenia. Certain metalloproteinase example zinc metalloproteases (haemorrhagins) are reasons for spontaneous systemic bleeding.

Complement activation can directly affect platelets, blood coagulation and another humoral activator. Venoms from *elapid* and some *colubroid* activate the alternative pathway, while the *viperid* venoms activates the classical complement pathway. Neurotoxic polypeptides and PLA2s injection manifests as paralysis by blocking transmission at neuromuscular junctions. Descending paralysis affects bulbar and respiratory muscles leading to upper respiratory airway obstruction, aspiration, or even respiratory paralysis. Anticholinesterase drugs (e.g. neostigmine) can be used for the treatment of these manifestations. Generalized rhabdomyolysis, myoglobinaemia, myoglobinuria leading to acute kidney injury can be seen in PLA2 myotoxins and metalloproteases in the venoms of sea snakes, terrestrial Australasian elapids and some kraits.

Russell's vipers quite commonly cause acute kidney injury. The histopathological features seen include acute tubular necrosis, proliferative glomerulonephritis, bilateral renal cortical necrosis, acute interstitial nephritis, toxic mesangiolysis with platelet agglutination, fibrin deposition and ischaemic changes. Kidney injury may be direct result of hypotension, hypovolaemia, direct nephrotoxicity or secondary to haemoglobinuria, myoglobinuria, and hyperkalaemia.

DIC may result in inappropriate deposition of fibrin that has been stimulated by metalloproteases on the vascular endothelium, producing microangiopathic haemolysis

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and thrombotic microangiopathy (TMA). These manifestations resemble haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP), but interestingly A Disintegrin And Metalloproteinase with a ThromboSpondin Type 1 Motif, Member13 (ADAMTS13) levels are never low.

ENZYMATIC EFFECTS OF SNAKE VENOM

The most important and common enzymes in snake venoms are:

a) Phospholipase A2s (PLA2s),

b) Serine proteinases, metalloproteinases,

c) Acetylcholinesterases (AChEs),

d) L-amino acid oxidases,

e) Nucleotidases (5-nucleotidases, ATPases, phosphodiesterases and DNases)

f) Hyaluronidases.

There are several other non-enzymatic polypeptides which include cysteine-rich secretory proteins (CRiSPs), nerve growth factors, PLA2-based neurotoxins, non-PLA2 myotoxins, C-type lectins, disintegrins, bradykinin potentiators, and tripeptide inhibitors.

Acetylcholinesterase (AChE):

The exsistence of AChE in cobra venom was first demonstrated in 1938. Large amounts of AChE can be quantitated from the venom of snakes, specifically in species belonging to the family *Elapidae*, with *Dendroaspis* species as an exception. In contrast, AChE is not found in venoms of snakes belonging to the *Viperidae* and *Crotalidae* families. Incidentally, snake venom AChEs are also more active than Torpedo and mammalian AChEs in hydrolyzing AChVenom affects a wide range of physiological functions in the envenomated organism, and each individual venom component may have a distinctive function.

Phospholipases A2 (PLA2):

Almost all snake venoms contain phospholipases A2 (PLA2), which acts through celldestruction leading to oedema, lipolysis, or myolysis (18).

Nucleotidases:

These includes 5-nucleotidases. ATPases, phosphodiesterases DNases. and Phosphodiesterases are a group of enzymes, which causes hydrolysis of phosphodiester bonds. This catalysis is mostly seen as a breakdown of nucleic acids, including DNA and both types of ribonucleic acids (RNAs), the ribosomal RNA and the transfer RNA. These enzymes can also affect many other nucleotides and nucleic acids as well (19). Although the overall effect has not been fully evaluated, it would appear that the depletion of such nucleotides results in hypotension and/or shock. 5'- nucleotidases attack nucleic acid at the 5' carbon position, degrading the sugar moieties of both DNA and RNA. The overall effect of 5'- nucleotidases is to release nucleosides from nucleic acids. One group that affects nucleic acids is the alkaline phosphomonoesterases, which hydrolyze phosphomonoesterases at pH above neutral. The biological effect of the alkaline phosphomonoesterases is not entirely clear, but these three groups of enzymes attack nucleotides in different manners, and all three are present in viperid venoms.

Hyaluronidases:

Hyaluronidases are also commonly called "spreading factors" because of their capacity to degrade hyaluronic acid. Hyaluronic acid is a ubiquitous component of the extracellular matrix of tissues, and is in part responsible for cementing cells together (20). Hyaluronidases have been found in the venom of all *elapidae* and *viperidae* venom (21). After degradation of hyaluronic acid by hyaluronidase, the extracellular matrix breaks down allowing the remaining venom components to spread through the tissue, since their movement is not restricted. This can lead to both localized necrosis, as nearby cells are destroyed by other venom components. Systemic effects also occur as other venom components spread into the blood stream through blood vessels made permeable or leaky by the hyaluronidase.

L-amino acid oxidases:

L-Amino acid oxidases (LAAOs) catalyse the oxidation of L-amino acids via a twostep deamination process. As a result of the deamination process there is a general deterioration of amino acids that leads in cell death (22).

Proteases:

Proteolytic enzymes are those enzymes that lead to the degradation of structural proteins into component peptides or amino acids. They have great digestive capability and can hydrolyze proteins in their native (non-denatured) state through cleavage of peptide and ester bonds. Metalloproteinases (because of their reliance on metal ion co-factors) and serine proteases (because of their similarity to blood factors) are two of the major subgroups among these enzymes in venom. Metalloproteinases cause haemorrhage and necrosis, but may also have a function in the digestion of prey.

Serine proteases:

Serine proteases disrupt hemostasis and may do so through multiple mechanisms. Serine proteases are generally placed into three categories, depending on their mode of action (23). The **thrombin-like serine proteases** cleave fibrinogen at the same position as thrombin. This leads to a rapid depletion of fibrinogen. The resulting fibrin, is not coagulable due to a lack of fibrin stabilizing factor leading to an overall anti-coagulation effect (24) (25) (26). **Kallikrein-like serine proteases** release bradykinin from high molecular weight kininogen and causes degradation of angiotensin. This leads to a drop in blood pressure (27). Arginine esterases have enzymatic activities against peptide and ester substrates, but their biological effects are not fully understood (28).

CRiSPs have varied functions in different species It ranges from disruption of potassium or calcium currents in the neurons to the induction of hypothermia (29). The functions of many similar CRiSPs in other species have not been demonstrated yet. Nerve growth factors stimulate the growth of nerve cells and are found in venoms of both *viperidae* and *elapidae*. PLA2-based presynaptic neurotoxins act by blocking release of acetylcholine from axon terminals leading to a flaccid paralysis. They have strong neurotoxic functions and include the Mojave toxin found in certain rattlesnake species. The non-PLA2-based myotoxins, disrupt voltage-sensitive sodium channels, causing immobilization of the prey and myonecrosis. C-type lectins and disintegrins alter the coagulation cascade, but in different manners. C-type lectins bind to platelets. This can cause initial clotting or it can cause platelets to be removed from forming clots, depending on the specific form of the lectin (30).

Disintegrins disrupts platelet aggregation, and they are often found grouped with a metalloproteinase. Some disintegrins stop the initial aggregation, while others work by disrupting the already formed aggregates (31). Bradykinin potentiating peptides (BPPs) act by being an effective inhibitor of angiotensin converting enzyme (ACE). ACE converts angiotensin I into angiotensin II. Angiotensin II causes vasoconstriction. BPPs

block ACE activity and also may have a direct effect on angiotensin II. Both these actions lead to a reduction in vasoconstriction thereby allowing bradykinin to cause vasodilation and hypotension. Snake venoms also contain factors that help stabilize these other components. These include tripeptide inhibitors and citrate (32). The action of these seem to be to inhibit the enzyme interactions in the venom. This is to prevent the biological functions from occurring till the venom has been injected into potential prey and dispersed.

Many other venom components have been characterized including many other toxins, blood clot disruption factors, and some enzymes.

CLINICAL FEATURES OF SNAKE BITE

Dry bites: Only about 50% of venomous snakebites lead to envenomation.

Local features of Envenomation-

Fang marks: One must not exclude envenomation because of the lack of fang marks. Certain bites can be difficult to note, even just after the bite.

Pain: Pain develops immediately generally spread upwards. The lymph nodes draining the bitten areas may become painful.

Local swelling: Intense local reaction is seen with viper bites than any other snakes. Swelling can be visualized within 15 minutes and may remain up to 3 weeks. It spreads rapidly and often involve the whole limb. Lymph node swelling may also develop. Necrosis develops in the limb with tight fascial compartments.

Local necrosis: Local necrosis is seen commonly with viper bites.

Secondary infection: This may happen secondary to bacterial colonies in snakes.

General features: General symptoms include flushing, breathlessness, palpitations, and dizziness. Apart from these the other early symptoms in elapid bites include vomiting, heaviness of eyelids, blurring of vision, hypersalivation, congested conjunctivae. Abdominal discomfort is often followed by diarrhoea. Nausea and vomiting may be a common feature of all severe envenomation (33).

Systemic features-

Clotting defects and haemolysis: These are common features seen in envenoming by *Viperidae*. Continuous bleeding from the bite site, injection sites, and partially healed wounds hints that the blood is haemostatically unstable. Spontaneous systemic haemorrhage may occur and sometimes intravascular haemolysis can be observed.

Neurotoxicity: Elapid and sea snake venoms cause significant neurotoxicity. Ptosis and external ophthalmoplegia appear develops within minutes of the bite. Sometimes there can be a delayed onset of symptoms. Later on, laryngeal muscles may become paralysed. All these predisposes to respiratory failure. These symptoms are completely reversible on treatment to anti-venom or spontaneously reverts in a week time.

Myotoxicity: Sea snake venom causes myopathy and rhabdomyolysis. Trismus is also commonly seen. Rhabdomyolysis leading to myoglobinuria appears within hours of the bite.

Cardiotoxicity: Viper and elapid venom can cause severe myocardial damage.

Nephrotoxicity: Acute tubular necrosis, bilateral cortical necrosis and renal failure is secondary to ischaemia, nephrotoxic effect of venom, pigment nephropathy associated with rhabdomyolysis or intravascular haemolysis in Viper bites (especially Russell's viper).

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Shock: Shock precipitates because of hypovolemia, myocardial compromise, and adrenal or pituitary haemorrhage (33).

FEATURES OF ENVENOMATION BY DIFFERENT FAMILIES OF SNAKES

Elapidae (krait / cobra) - Dominant manifestation is neurotoxicity. Local blisters and

necrosis can occur. Australian *Elapides* can also cause bleeding manifestations.

Viperidae (Russell's viper / Saw scaled viper) - Local swelling, cellulitis, regional

lymphadenitis and bleeding manifestations.

Hydrophiidae (sea snake) - Rhabdomyolysis

Colubridae - Bleeding manifestations and renal failure.

DIAGNOSTIC APPLICATIONS OF SNAKE VENOM

There are several diagnostic application of snake venoms in the haemostasis laboratory. It can be utilized in assay for every coagulation factor or factor involved in fibrinolysis.(34).

Snake venom as thrombin-like enzyme

Snake venom thrombin-like enzyme (SVTLE) includes several serine proteases. Several snake species have been known to possess more than 100 thrombin-like enzymes which resemble thrombin factor both functionally and structurally (35). The term thrombin-like enzyme refers their capacity to trigger the clotting of fibrinogen. The sources of thrombin-like enzymes are from the pit viper family, like the *Agkistrodon, Crotalus, Lachesis* and *Trimeresurus* from the true viper family. Snake venom thrombin-like enzymes can be obtained from *Bitis gabonica, Cerastes vipera, Dispholidus typus* and from the *Colubridae* family (36). Thrombin cleaves fibrinogen into fibrinopeptide alpha (FPA) and fibrinopeptide beta (FPB). Some of these enzymes cleave fibrinogen and are known as Snake venom thrombin-like enzyme.

Snake venom thrombin-like enzymes are used to determine the presence of fibrin degradation product, dysfibrinogenaemias and defects in fibrin polymerisation. This test is commonly known as the Reptilase time. This test sometimes can be used as an alternative to more commonly used thrombin time. Dysfibrinogenaemia will manifest a prolonged reptilase time. If the prolongation in reptilase time is lesser compared to that of the thrombin time, it indicates the presence of fibrin degradation products (34).

Snake venom as prothrombin activators

Many snake venoms are composed of prothrombin activators and are utilized in prothrombin assays, to demonstrate dysprothrombinaemias (37).

Meizothrombin an intermediate product in the clotting of whole blood is produced as a conversion of prothrombin to thrombin in systems which are composed of purified factor Xa and factor Va that are quantitatively assembled on an anionic phospholipid surface(38). Ecarin from saw-scaled viper (*Echis carinatus*) venom, textarin from the Australian brown snake (*Pseudonaja textilis*) and the enzyme from the taipan (Oscutarin) are commercially available prothrombin activators (39). Snake venom prothrombin activators are categorized into four groups based on the structural and functional properties in prothrombin activation (40).

Group I activators

These activators are not affected by the non-enzymatic cofactors of the prothrombinase complex (CaCl2, factor Va and phospholipid). They can competently transform prothrombin into meizothrombin.

Group II activators

They require only calcium.

Group III activators

They require calcium and phospholipids. They do not depend on factor V at all.

Group IV

They require all the cofactors including Ca2+, factor V and phospholipid.

The primary product from digesting prothrombin with ecarin is meizothrombin, which was very rapidly converted to thrombin. The in vivo activation of prothrombin to thrombin occurs when a complex involving a serine proteinase factor Xa and factor V which is a cofactor (prothrombinase complex) assemble on a negatively charged phospholipid membrane.

The factor Xa-like snake venom proteases are seen in **trocarin, oscutarin and ecarin.** These venom proteins convert prothrombin to thrombin (40).

Snake venom proteins as factor V activators:

Thrombin activates factor V (FV) into FVa and a thousand-fold increase in catalysing power of factor Xa. Under normal physiological state the cofactor activity of factor Va in prothrombin activation is dampened by activated protein C. The venoms of *Daboia russelli* and *Daboia lebetina* contain a serine protease that accurately activates Factor V. The enzyme can be used for routine assay of FV due to the ability of the venom protein to activate factor V.

Snake venom as factors VII and X activators

A protease that activates factor VII has been isolated from the venom of *Oxyuranus scutellatus* (taipan snake). It cleaves single-chain human factor VII to yield a two-chain molecule which is not distinguishable from the true factor VIIa.

Factor X activators have been obtained from the venom of many snake species belonging to the genus *Viperidae* and *Crotalidae*. It is also sourced from a few *Elapid* species. The best-known activator of factor X is the Russell's viper venom -X from Russell's viper. The Russell's viper venom -X is a metallo protease and share homology with C-type lectins which regulates Ca2+ -dependent activation of factor X. Russell's viper venom (RVV) that activates factor X is commercially available.

It is also engaged in a number of assays, like measurement of factor X, for differentiating between factor VII and factor X and in lupus anticoagulant (LA) assay. The clotting time of plasma using Russell's viper venom -X is known as the Stypven time and a normal Stypven time used together with a prothrombin time (PT) suggests factor VII deficiency. A prolonged Stypven time indicates factor X deficiency (40).

Snake venom proteins in the assay of Protein C

Protein C is an important component of the natural anticoagulant pathway. The inability of activated Protein C to cleave factors Va or VIIIa is known as activated protein C resistance. Protac, is derived from Southern copperhead (*Agkistrodon contortix*) snake venom (ACV). It is used in a Chromogenic assay to identify a defect affecting the protein C/protein S (PC/PS) anticoagulant system and is based on the activation of endogenous plasma protein C

Snake venom proteins used in the assay of Lupus anticoagulant (LA)

Russell Viper Venom directly activates FX, bypassing FVII of the extrinsic pathway and the contact and anti haemophilic factors of the intrinsic pathways. Therefore, DRVVT tests are more specific for LA than APTT as they are not affected by contact factor abnormalities nor by FVIII deficiency or antibodies to FVIII. Heparin levels up to 1u/ml have no effect due to the presence of a neutralizing agent in both the screen and confirm reagents (40).

Snake venom proteins used in the assay of defects in platelet plug formation

Many snake venom proteins have been identified which affect the platelet plug formation. These exert their action by either interacting with platelet integrins, membrane glycoprotein Ib (GPIb), or plasma von Willebrand factor.

a. Von Willebrand factor (vWF).

Platelet aggregating protein (Botrocetin)is found in the venoms of *Bothrops jararaca*. This protein relies on the presence of von Willebrand factor (vWF) for its effect on platelets. This property has been utilized in vWF assay.

Alboaggregin-B, a venom protein from white-lipped pit viper "*Trimeresurus* albolabris" venom is used to measure vWF receptors on the GPIb molecule (40).

b. Platelet Glycoprotein (GPIb)

GPIb-binding proteins from snake venoms origin can be divided into two groups: GPIbagonists and GPIb-antagonists.

Alboaggregins from the white lipped viper venom can be of various types A, B and C. Alboaggregin B was the first GPIb agonist to be purified. They hinder platelet agglutination by VWF. They bind to GPIb and cause platelet aggregation.

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Another protein Alboaggregin A binds GPIb and GPVI and mediates platelet aggregation.

A few other venom proteins also affect GPIb. Agglucetin from *A. acutus* agglutinates platelets via GPIb receptors. They activate surface expression of GPIIb/IIIa of intact platelets.

COAGULOPATHY IN SNAKE ENVENOMATION

The coagulopathy produced in people by snake envenomation is related to the multiple venom components affecting haemostasis. The potential clinical problems are:

- Decreased blood coagulability and an increased bleeding tendency
- Frank haemorrhage due to blood vessel wall damage
- Secondary effects of increased haemorrhage like hypovolaemic shock and secondary organ damage, such as intracranial and anterior pituitary haemorrhage or kidney injury
- Direct pathologic thrombosis and its sequelae (e.g. pulmonary thromboembolism) (41)

Haemorrhage in snake envenomations occurs due to abnormal coagulation factors, capillary endothelium or platelet function (42). *Viperid* and *Crotalid* venoms are rich in metalloproteinases (haemorrhagins), which cause local haemorrhage following intradermal or subcutaneous venom injection. This is attributed to their ability to degrade extra-cellular matrix proteins, particularly type IV collagen, which is a major component of the basement membrane. Endothelial cell adhesion to the basement membrane is disrupted, which compromises the blood vessel wall integrity. Patients

envenomed by Viperid or Crotalid snakes sometimes suffer from systemic haemorrhage with absence of evidence of coagulation abnormalities. This finding suggests that systemic haemorrhage can be due to a venom-induced platelet disorder. Venom metalloproteinases impedes platelet interaction with collagen and vWF by various mechanisms by targeting platelet receptors or their ligands. Zinc-dependent metalloproteinases from viperid snake venoms were documented to be mainly responsible for the haemorrhagic syndrome in snake bite envenomations. Many snake venoms contain procoagulant toxins that activate the coagulation cascade (50). The venom of the Brown snakes (Pseudonaja spp.) and taipans (Oxvuranus spp.) were shown to contain group C prothrombin activators. They were similar to the mammalian prothrombinase (Xa:Va) complex and activated coagulation, which leads to development of a consumptive coagulopathy. This is now referred to as a venominduced consumptive coagulopathy (VICC) and is characterized by prolonged clotting times, low fibrinogen, FV and FVIII depletion and high FDP concentrations. The consequence of VICC has often been thought to be DIC, due to the elevated D-dimer, prolonged PT, and hypofibrinogenaemia. However, a recent publication stated that other important features of DIC like the evidence of systemic micro thrombi and endorgan damage are absent in VICC. A clinical syndrome consistent with thrombotic microangiopathy has been reported in a few patients with VICC. It was suggested that the presence of thrombotic microangiopathy and VICC in these patients is the likely reason for mistakenly diagnosing DIC in such cases (43).

VENOM INDUCED CONSUMPTION COAGULOPATHY: PATHOGENESIS

The basic pathogenic mechanism involved in haemotoxic snake bites is called Venom Induced Consumption Coagulopathy (VICC). Snake venom has toxins that induce factor activation and the coagulation cascade in vivo leading to coagulopathy. These toxins are capable of producing coagulation in vitro as well and are called procoagulant toxins. The severity of VICC is directly related to the number and quantity of these activators in each snake venom which determines its potency.

Following envenomation by a haemotoxic snake bite, the common derangements seen are prolonged Prothrombin time (PT), prolonged Activated Partial Thromboplastin Time (APTT), low or undetectable fibrinogen and low or undetectable factor V, VIII and X levels. The different haemotoxic venom toxins and their effects are described below.

Prothrombin activators:

Prothrombin activators are from the serine protease family have been classified based on structure, function and cofactors required. They are divided into groups A to D.

Prothrombin activator	Action
Group A (Echis spp)	Directly activate Thrombin to Meizothrombin
Group B (Echis, Russell's viper)	Directly activate Thrombin to Meizothrombin
Group C (Elapids)	Resemble Factor Xa-Va complex and activate Prothrombin
Group D (Elapids)	Resembles Factor Xa

Factor X and V activators:

The characteristic toxins in Russell's viper venom are individual factor activators. Activation of Factor X gives rise to Prothombinase complex and leads on to positive and negative feedback loops resulting in the utilisation and consumption of Factors V and VIII.

Thrombin-like enzymes (Fibrinogenases):

These factors are responsible for direct activation and consumption of fibrinogen. They belong to the family of Zinc metalloproteinases. These enzymes lyse either the alpha chain or beta chain of Fibrinogen resulting in its depletion. The fibrin produced is lysed by the body's own mechanisms.

Haemorrhagins:

They belong to the Group A and B prothrombin activators and possess additional functions. The *Echis spp* venom contain these are proteolytic enzymes and cause damage to the integrity of blood vessel walls. The basement membrane is disrupted and suffers shear stress injury which leads to increased risk of severe and spontaneous bleeding.

Fibrinogen is the most consistently consumed factor and is the final common pathway in VICC induced by haemotoxic snake envenomation.

The risk of bleeding in VICC is due to the following causes-

- 1) Coagulation Cascade Activation
- 2) Damage to basal membrane and blood vessel wall integrity

- 3) Effects on Platelet count and function
- 4) Local toxic and enzymatic effects

VICC is commonly associated with local bleeding manifestations from the bite site/ as well as the site of cannula insertion. However, distal and systemic bleeding such as gum bleeding, gastrointestinal or genitourinary bleeding and intracerebral haemorrhage are also seen. *Echis spp* have haemorrhagins in their toxins and therefore cause the more serious bleeding manifestations (44).

As VICC often coexists with thrombocytopenia, it led to the popular belief that VICC is a form of Disseminated Intravascular Coagulation (DIC). However, VICC is now considered to be a form of Consumptive Coagulopathy which occurs due to the consumption of coagulation factors due to activation by venom toxins and depletion of fibrinogen rather than a form of DIC which occurs as a result of the activation of fibrinolytic system.

Isbister *et al*, reported in 2010 that there are significant differences between VICC and DIC. VICC specifically does not have systemic microthrombi or end-organ damage due to these thrombi. DIC is specifically mediated via the tissue factor/ VIIa pathway while initiation of VICC can occur at any point in the coagulation cascade upstream from thrombin. The time course in VICC is also very rapid in comparison to DIC and occurs within a few hours of envenomation and it also resolves without major sequelae within 24-48 hours (42).

The action of thrombin-like enzymes alone, as in *Echis spp* envenomation results in a mild hypofibrinogenemia, also called partial VICC. When the toxins contain other

factor activators which lead to the interactions of several other factors such as Factor X and V activators, there is resultant complete VICC and severe bleeding. Severe VICC is characterized by undetectable Fibrinogen levels, unrecordable Prothombin time and elevated d-Dimer levels.

DIC, on the other hand is characterized by activation of the coagulation cascade via the Tissue factor and/or factor VIIa pathway. This leads to an imbalance between the procoagulant and anticoagulant pathways resulting in unchecked activation of downstream coagulation cascade. There is also a depressed fibrinolytic system leading to impaired fibrin removal.

DIC has a much higher level of morbidity and mortality as compared to VICC. Intracranial haemorrhage is often the cause of mortality due to VICC in hospitals. However, the prognosis overall is much more favourable with VICC as compared to DIC.

THROMBOCYTOPENIA AND THROMBOTIC MICROANGIOPATHY WITH HAEMOTOXIC BITES:

In about 40-60% of patients with haemotoxic envenomation, VICC coexists with thrombocytopenia. There are numerous mechanisms that cause low platelets in snake envenomation. The proposed mechanisms include Direct venom-induced platelet destruction, immune-mediated platelet toxicity, microangiopathy and suppressed platelet production. (46)

Isbister has shown in his descriptive clinical studies of Russell's viper in Sri Lanka, that a proportion of patients have thrombocytopenia with acute kidney injury. He has referred to this subset as having VICC with associated thrombotic microangiopathy (TMA) (47). As stated therefore, not every case of VICC results in TMA. These are two distinct entities, but which are closely interrelated.

The final consensus on whether VICC is purely a consumptive coagulopathy or DIC and the relationship between VICC and microangiopathy is at present unclear. However, it is strongly stated in literature that VICC is a distinct entity. At CMC Hospital, we have observed that a spectrum of pure VICC, VICC with thrombocytopenia and VICC with thrombocytopenia and laboratory evidence of thrombotic microangiopathy all exists. We hope that the present study will provide further evidence towards clarification of this spectrum in different clinical syndromes of viperine bites which may manifest as pure haemotoxicity and haemotoxicity with acute kidney injury with or without neurotoxicity.

Due to the ongoing debate between VICC being a consumptive coagulopathy distinct from DIC and also the differences in the VICC seen following envenomation by Saw scaled viper and Russell's viper, it is important to describe the clinical and laboratory characteristics of VICC according to clinical syndrome that is manifested as well as the biting species. This study may therefore provide further details into the pathophysiology of VICC in the Indian subset in the two main haemotoxic species.

SNAKE ANTIVENOM

Antivenom is considered to be the most important treatment for snake envenoming. They are a mixture of polyclonal antibodies to toxins present in snake venoms. They are either complete immunoglobulin (IgG) molecules or a fractionated immunoglobulin such as $F(ab')_2$ or Fab. These are developed in animals, such as horse, sheep, goats and rabbits, immunized with repeated small doses of snake venom. The polyclonal nature of antivenom is of utmost importance as they results in neutralization of multiple toxins present in venom (48).

There are three major types of snake antivenom used in the world -

1) Whole IgG

These antivenom contains intact Immunoglobulin G molecules of approximately 150 kDa and are purified by ammonium sulphate or caprylic acid precipitation.

2) F(ab')₂

F(ab')₂ antivenom is developed after pepsin digestion of whole IgG.

3) Fab.

The smallest sized of all anti-venom approximately 50 kDa are produced by papain digestion of whole immunoglobulins.

All these antivenoms have different pharmacodynamic and pharmacokinetic properties, and this influences their ability to reach target tissues and duration of action. The degree and types of adverse effects also varies among them.

The World Health Organization recommends that antivenom manufacturing includes an assessment of the neutralization of myotoxic, coagulopathic, haemorrhagic, necrotizing, oedema forming and the defibrillating effects of venom by the antivenom (48). The timing of the onset of toxin effects varies. Some toxins possess rapid onset of symptoms within minutes. Pro-coagulant and anticoagulant toxins react within minutes with the clotting factors. Therefore, the coagulopathic symptoms begin immediately and the reversal of coagulopathy is less observed after giving anti-venom.

The haemorrhagic toxins also initiate their toxic effect immediately, as the target site is in the close proximity of toxins. Immediate administration of antivenom may therefore revert central toxic effects.

Following envenomation which contains neurotoxins leading to a pre-synaptic neurotoxicity administration of antivenom should be done before the patient develops symptoms.

Lack of standard predictors and standardized biological markers for early detection of on is not practical, as no rapid venom detection facilities are available in the world.

A venomous snake bite requires urgent medical attention due to the nature of the acute insult to the body. Most haemotoxic envenomations have coagulopathy of varying severity and require ASV administration to mediate and neutralize the effects of the toxin. However, ASV is expensive, and has potential adverse effects. An important bedside test that guides the decision to administer ASV is the whole blood clotting time (WBCT20) which indicates the extent of coagulopathy (49).

WBCT20 was initially advocated as supportive evidence for coagulopathy; however, it is now commonly used as a bedside diagnostic test (50).

WBCT20 is performed by leaving 5mL of the patient's blood sample undisturbed in a glass tube and assessment of clot formation at the end of 20 minutes. Failure of clot formation results in a positive test. The methodology has remained unchanged since the time of its inception in 1913 by Lee and White.

There are limited number of studies on the standardization and validation of this test. There are inadequate clinical studies on the effects of physical factors such as length, material, temperature of the tube and biological factors such as the type of snake. WBCT20 is a useful bedside test especially in resource poor settings where lab-based coagulation studies are not easily available. Although it is undoubtedly an invaluable bedside test, the reliability of the test has often been questioned (51).

Due to the lack of standardization and validity of the WBCT20, there is a need for a standardized and universally agreed bedside test. WBCT does not necessarily correlate with the clinical severity for envenomation, thereby not delivering the essential purpose of an early diagnosis of severe VICC, which can lead on to the necessary interventions. (49)

A study done in 2013 on 140 individuals with Russell's viper envenomation showed poor sensitivity of WBCT20 in detecting VICC which led to a delay in administering ASV (52). The authors therefore favoured a more clinical approach to guide treatment than WBCT20. A recent publication assessed the performance of WBCT20 in comparison to PT/INR in detection of VICC. Out of 987 patients with snake bite, 79 patients fulfilled criteria for VICC with an INR>1.4 with or without clinical bleeding. The WBCT20 was positive in 65/79 patients with a sensitivity of 82% and specificity

of 98%. (51) WBCT20 therefore shows poor sensitivity of about 80% across various studies and this might delay ASV administration due to false negative WBCT20. Hence, studies on standardizing WBCT20 are necessary for clinicians to be able to rely on this test. In situations where reliable coagulation studies are not available, there is therefore a requirement to devise a reliable, easy-to-do and cost-effective bedside test.

The properties of clotting are determined by the nature of the surface and area of contact of the blood sample as well as the inherent qualities of the sample. These features have been utilized in designing activated clotting time tests (ACT). Several techniques have been used for the same. Kaolin, Celite and glass are some of the mediators that have been used for ACT. Activation means contact of blood with enhancing factors that result in more rapid coagulation of the sample. However, on a particular device with standardized external factors, these values are reproducible (53).

The predominant factor determining the duration of ACT is anti-Factor II activity. Kaolin provides an internal activating surface, hence making the test independent of the size, nature of the material and volumes of the reagents used. Studies on Kaolin based ACT have been done in cardiological and neuro-interventional settings (54,55). **PRINCIPLE:** The ACT is a test of whole blood coagulation that gives a single parameter for interpretation. Variables like temperature, platelets, aprotinin, GP IIb/IIIa antagonists, haemodilution and various coagulopathies can alter the result (56). ACTs are indicative of inhibition of contact and common pathway (X–Xa) activation (57). The ACT tube has an activator substance that will activate factor XII through contact activation. The main activator substances used are celite (diatomaceous earth),

Characterisation of Venom Induced Consumption Coagulopathy (VICC) in patients with Haemotoxic Snake Bite and the effects of Blood Products on Coagulation Parameters.

kaolin, or glass beads, which all have large surface areas for contact. The principle of the test is to completely activate the intrinsic coagulation cascade and measure the time it takes for generation of a fibrin clot. The sample is heated to 37°C by the machine. Depending on the type of machine used, movement is created either through rotation of the sample tube, a plunger or through pressure. As a clot forms it imparts resistance onto a plunger, a free rolling bar in the sample tube or impedes flow of the blood. The end result is the time taken for the sample to form a clot. This is detected photo-optically or electromagnetically. The standard tests of coagulation (prothrombin time, activated partial thromboplastin time, thrombin time (TT), and fibrinogen level) are plasma tests and they measure plasma haemostasis. The ACT measurement uses whole blood and incorporates the importance of platelets and phospholipids in coagulation.

Kaolin based ACT are used in theatres to assess coagulation status in patients on continuous anticoagulant infusion following cardiopulmonary bypass, endovascular interventions and neurosurgical procedures. Differences between venous and arterial blood samples are not known. These values are measured in pre-calibrated machines, which lack standard reference values and validation. ACT values vary significantly across different devices; hence values must be interpreted in a device-specific manner. A modified version of the ACT has been designed in our laboratory that can be easily performed on whole blood, at the bedside and takes less time and utilizes commonly available low-cost reagents. This test has been named **VEMAC TIME- VELLORE**

MANUALLY ACTIVATED CLOTTING TIME

Principle of Vemac Time

The time it takes for whole blood drawn from a vein and placed in a container invitro to clot after the addition of activator. This test measures all the stages of intrinsic coagulation.

Equipment

Syringe & Needle.

12 x 100mm glass tube.

20 ul Sahli's pipette.

Stopwatch.

10-100ul Automated pipette & Tips

Reagents

Kaolin (80 mg/ mL)

Whole blood.

Normal saline

Procedure:

- 1. Make a clean venipuncture.
- 2. Collect 1.5 to 2mL of blood in a syringe.
- 3. Start the stopwatch as soon as the blood enters the syringe.
- 4. Add 0.5mL of blood into each of two 12x100 glass tubes.

5. Add 60uL of kaolin (80mg/ml) to both the tubes, and place the tubes in the stand so that they remain upright and undisturbed for 2 minutes.

6. After 30sec. of incubation take the first tube and gently tip it and look for clot.

7. When the first tube is clotted record the time and start tipping the second tube every

30 seconds until it too is found to be clotted. Record the time.

Results/Interpretation

The time recorded for the clotting time of the second tube is taken as the clotting time (least disturbed).

Reference range: This was established based on Clinical and Laboratory Standards Institute (CLSI) guidelines by performing the test on 120 normal controls in CMC Hospital. The reference range was 150-240secs (197.28±28.66secs).

Advantages of Vemac Time over ACT

The advantages of the Vemac Time is that it does not require a specialised equipment to perform the test. It also does not require a specified temperature to be maintained. It can be done at the bedside in any healthcare facility.

Advantages of Vemac Time over WBCT 20

The duration of the test is reduced from 20 minutes to less than 5 minutes. The test can be performed without any specialised equipment in any healthcare facility which has a provision to collect a venous blood sample.

RETROSPECTIVE DATA

1. DEMOGRAPHIC DETAILS

1.1 GENDER

A total of 280 patients from the year 2012-2017 who met the inclusion criteria of the study were evaluated. 71.07% were males (n=199) and 28.93% (n=81) were females with a male: female ratio of 2.45:1.

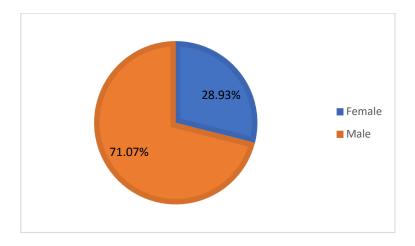


Figure 1: Gender wise distribution of the study population

1.2 AGE DISTRIBUTION

The mean age was 42.2±14.5 years. The mean age among males was 43.02±14.99 and

in females was 40.16±13.

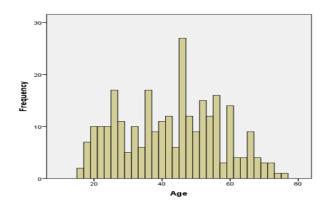


Figure 2: Age distribution of the study population

Characterisation of Venom Induced Consumption Coagulopathy (VICC) in patients with Haemotoxic Snake Bite and the effects of Blood Products on Coagulation Parameters.

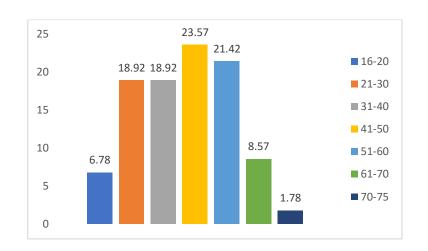


Figure 3: Distribution of patients in each decade (percentage)

1.3 AREAWISE DISTRIBUTION

The maximum number of patients who presented to our hospital were from Vellore 43.57% (n=122).

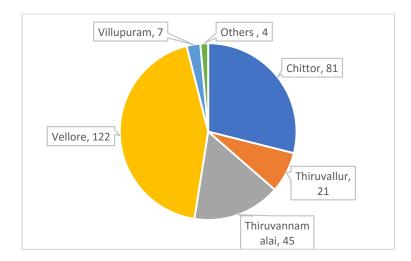


Figure 4: Area wise distribution of the study population

(Others include Cudappah, Kolar, Kanchipuram and Warangal)

Characterisation of Venom Induced Consumption Coagulopathy (VICC) in patients with Haemotoxic Snake Bite and the effects of Blood Products on Coagulation Parameters.

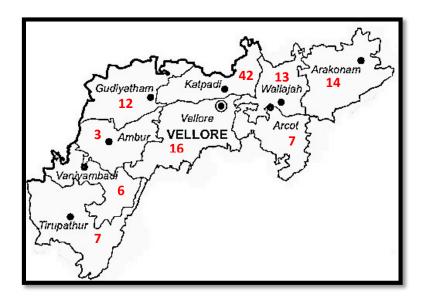


Figure 5: Taluk wise distribution of the study population in Vellore district

1.4 SEASONAL VARIATION

Two peaks were observed in the number of patients being admitted per month with a history of snake bite. The first one was in March and the second peak was seen during August.

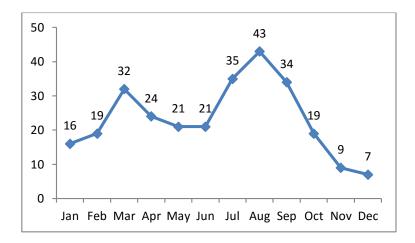


Figure 6: Monthly distribution of patients with haemotoxic snake bite

2. ENVENOMATION DETAILS

2.1 TIME OF BITE

The time of bite was registered for 238 cases. More than a third of the bites (n=88) were

between 6 pm and midnight.

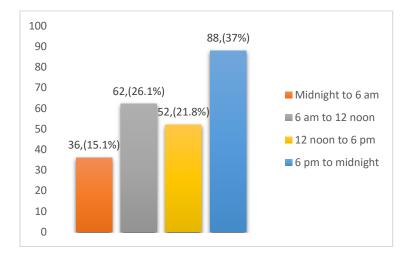


Figure 7: Population distribution according to time of bite

2.2 SITE OF BITE

Majority of bites were in the lower limbs 84.5% (n=223), followed by upper limbs with

13.3% (n=35).

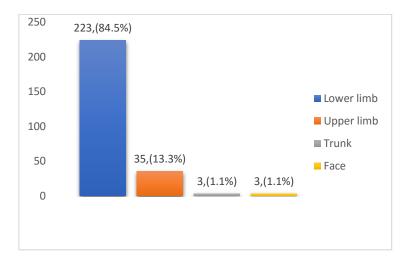


Figure 8: Distribution according to site of bite

3. PATTERN OF VARIOUS CLINICAL MANIFESTATIONS IN THE POPULATION

3.1 LOCAL MANIFESTATIONS

Local symptoms in the form of any of the following: local swelling in the absence of a tourniquet or cellulitis of the affected limb or enlarged tender lymph node draining the bitten limb or necrosis, blistering, gangrene or compartment syndrome –absent pulses were seen in 100% of the study population.

3.2 NEUROLOGICAL MANIFESTATIONS

Neurological manifestations included ptosis or ophthalmoplegia, bulbar weakness – dysphagia, difficulty in speaking, limb muscle weakness, neck holding time <5s, respiratory paralysis-reduced single breath count<10, paradoxical breathing, respiratory failure, need for mechanical ventilation. Neurotoxicity in any of these forms were seen in 60.7% (n=170)

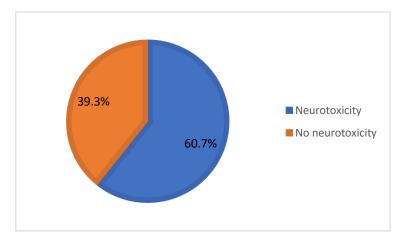
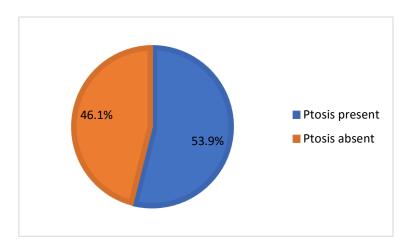


Figure 9: Distribution according to neurological manifestations

Patients with neurological manifestations who required ventilatory support formed about 20% of the study group.

3.3 PTOSIS

The initial involvement of levator palpebrae superioris in form of ptosis was seen in



53.9% (n=151) patients.

Figure 10: Distribution according to presence or absence of ptosis

3.4 RENAL INVOLVEMENT

Acute kidney injury defined by abrupt (within 48 hours), absolute increase in the serum creatinine concentration of $\geq 0.3 \text{ mg/dL}$ (26.4 micromol/L) from baseline; a percentage increase in the serum creatinine concentration of ≥ 50 percent; or oliguria of <0.5 mL/kg per hour for more than six hours was seen in 22.5% (n=63) patients.

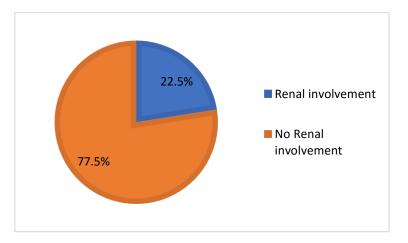


Figure 11: Distribution according to renal involvement

Out of the patients with AKI those who required haemodialysis were 49.2% (n=31).

4. ENVENOMATION SYNDROMES

The envenomation syndromes were grouped as **PURE HAEMOTOXICITY SYNDROME** and **HAEMOTOXICITY WITH RENAL/NEUROTOXIC FEATURES**. Pure haemotoxicity were seen in 32.9% (n=92) patients. Combinations of haemotoxicity with renal and/or neurological manifestations were seen in 67.1% (n=188).

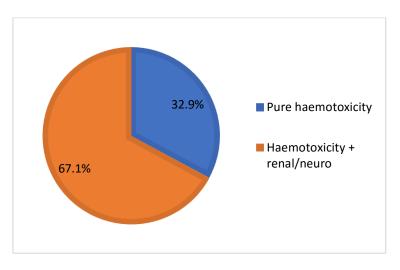


Figure 12: Distribution of envenomation syndromes in study population Haemotoxicity was considered when any of the following were present: WBCT20 was prolonged, INR above reference interval \geq 1.2 bleeding manifestations or thrombocytopenia (platelet count <1,00,000/ mm³).

5. LABORATORY PARAMETERS

AT BASELINE

5.1 WHOLE BLOOD CLOTTING TIME 20

Whole blood clotting time (WBCT 20) more than 20 minutes was considered abnormal. 96.4% (n= 270) of the study population showed an abnormal WBCT at presentation.

Characterisation of Venom Induced Consumption Coagulopathy (VICC) in patients with Haemotoxic Snake Bite and the effects of Blood Products on Coagulation Parameters.

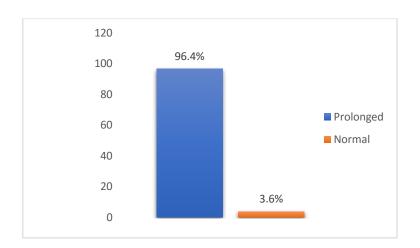


Figure 13: Whole blood clotting time (WBCT 20) at admission

5.2 HAEMOGLOBIN

Haemoglobin at baseline was done for 277 patients. The mean haemoglobin at

presentation was 13.9±2.42g%

5.3 PLATELET COUNT

Platelet count at presentation was done for 273 patients and the median platelet count was 1,78,000/cumm.

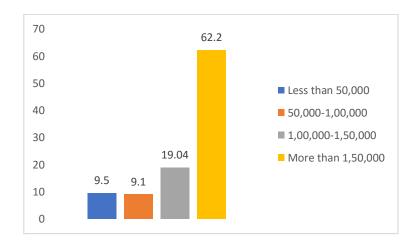


Figure 14: Distribution according to platelet count at admission

Characterisation of Venom Induced Consumption Coagulopathy (VICC) in patients with Haemotoxic Snake Bite and the effects of Blood Products on Coagulation Parameters.

5.4 INTERNATIONAL NORMALISED RATIO

International Normalised Ratio was divided into two groups. INR was considered to be abnormal if it was more than or equal to 1.2. At admission INR was done for 267 patients. An abnormal INR (\geq 1.2) was seen in 94.38% (n=252). Mean INR at presentation was 4.66±3.63

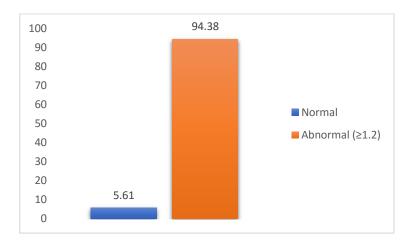


Figure 15: INR at admission

- 13 patients did not have a baseline INR as the samples were either lysed or clotted.
- Among the 267 patients with a documented baseline INR, the WBCT was prolonged in 257 (96.25%) patients and normal in 10 patients.
- Out of the 257 patients with abnormal WBCT at admission 94.55% (n=243) had a coexisting abnormal INR of more than 1.2.
- ♦ WBCT was normal in 10 patients among whom 9 had an abnormal INR.

5.5 PATTERN OF INR CHANGE OVER 24 HOURS

The PT with INR was monitored only on patients with evidence of prolonged bleeding parameters. Hence the numbers of results available for analysis shows a decreasing trend.

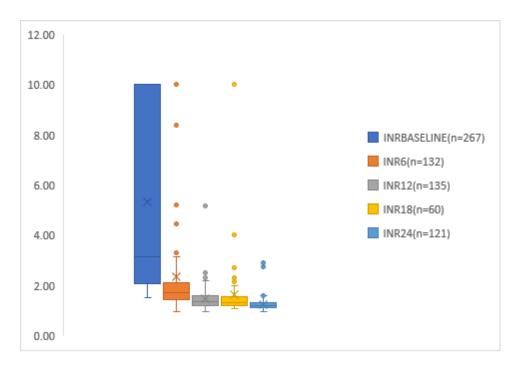


Figure 16: Change of INR over 24 hours

6. ANTI SNAKE VENOM

Anti-snake venom was administered to all the 280 patients. The mean dose of ASV received per patient was 17.43±8.4.

Reactions to ASV varied from angioedema, itching, urticaria, bronchospasm to anaphylactic shock and it was seen in 13.6% (n=38).

Characterisation of Venom Induced Consumption Coagulopathy (VICC) in patients with Haemotoxic Snake Bite and the effects of Blood Products on Coagulation Parameters.

7. TRANSFUSION REQUIREMENTS

Blood or plasma products transfusion in addition to ASV were required for 16.8%

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(n=47) patients.
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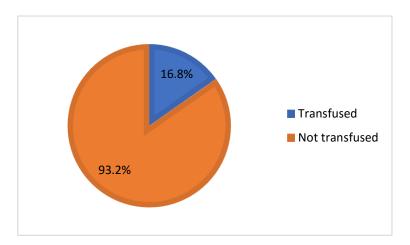
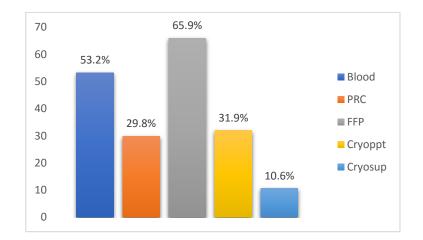
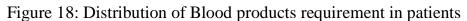


Figure 17: Distribution according to transfusion requirement





Fresh frozen plasma was required the maximum for 65.9% (n=31) patients.

7.1 COMPONENT WISE TRANSFUSION

FRESH FROZEN PLASMA

Out of the 31 patients who had received FFP transfusion, 29 (%) had an INR more than 1.5 with bleeding manifestations before the transfusion. The remaining two with an INR

less than 1.5 received FFP for haemodialysis procedure and had bleeding manifestations.

194 patients with initial INR more than 1.5 did not receive FFP transfusion since they either normalised with ASV or did not have any bleeding manifestation.

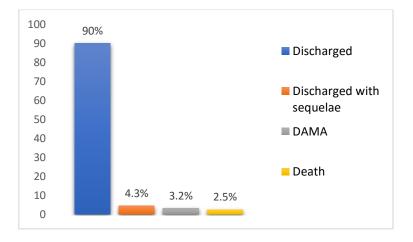
CRYOPRECIPITATE

15 patients who received cryoprecipitate had a low fibrinogen with associated bleeding manifestations. Patients who had a low fibrinogen without any bleeding manifestations were not transfused.

PLATELET RICH CONCENTRATE

14 patients who received PRC transfusion had severe thrombocytopenia with associated bleeding manifestations.

Patients who had thrombocytopenia without bleeding manifestations were not transfused.



8. OUTCOME

Figure 19: Distribution of patients according to the outcome

Most of the patients were alive at discharge, with 2.5% mortality.

PROSPECTIVE GROUP

9. DEMOGRAPHIC DETAILS OF PATIENTS BETWEEN 2017 AND 2018

9.1 GENDER

Among the patients admitted with haemotoxic snake bite in 2017 -2018 there was a male preponderance of 83.8%. The male:female ratio was 5.17:1

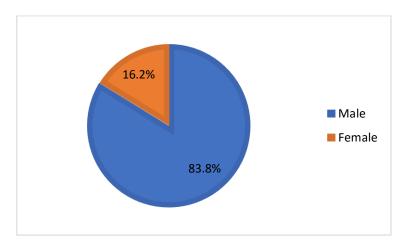


Figure 20: Gender wise distribution of the study population

9.2 AGE DISTRIBUTION

The mean age of this group was 38.11 ± 13.08 . The mean age among males was 37.54 ± 13.48 and among females was 41 ± 11.4

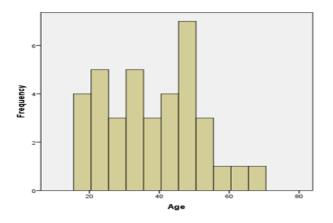
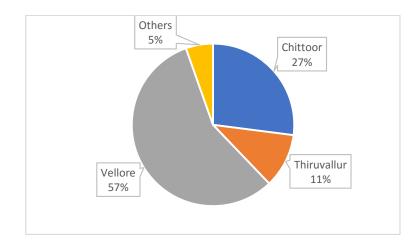
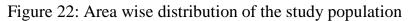


Figure 21: Age wise distribution of the study population

9.3 AREAWISE DISTRIBUTION



The maximum number of patients were from Vellore 57% (n=21).



(Others include Cudappah and Villupuram)

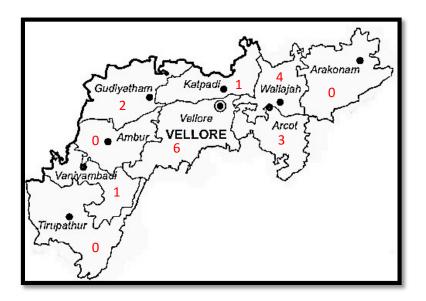
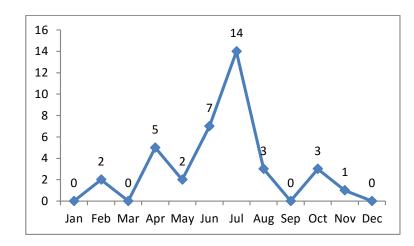
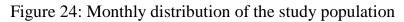


Figure 23: Taluk wise distribution of the study population in Vellore

9.4 SEASONAL VARIATION



The maximum number of patients were seen during the month of July.



10 ENVENOMATION DETAILS

10.1 TIME OF BITE

The time of snake bite was found to be maximum between 6pm to midnight and 37.8%

of the bites happened during this time.

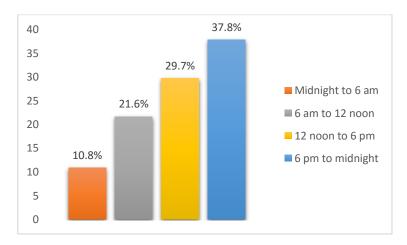


Figure 25: Distribution according to time of bite

10.2 SITE OF BITE

Lower limbs were the most common site of bite with 75.6% of patients being bitten in the lower limbs. This was followed by upper limbs with 21.6% bites. One patient also presented with a bite in the neck region.

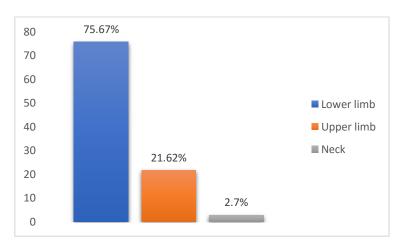


Figure 26: Distribution according to site of bite

TIME TO FIRST SYMPTOMS

The mean time taken for the first symptoms to start after snake bite was 55.19

minutes. It ranged from 5 minutes to 6 hours.

TIME TO RECEIVE FIRST AID

The median time to receive first aid was one hour. All patients received first aid

within 12 hours.

TIME TO RECEIVE FIRST ASV

The mean time taken to receive the first dose of ASV was 3.36 hours. It ranged from

30 minutes to 19 hours.

TIME TO FIRST BLEEDING SYMPTOMS

The median time to the first bleeding symptoms was 60 minutes. It ranged from 5 minutes to 12 hours.

TIME TO FIRST SYSTEMIC BLEEDING

Systemic bleeding in the form of oral cavity bleed, haematuria, hematemesis, haematochezia and malena was present in 54.1% of patients. The mean time to the first systemic bleed was 5.65 hours. It ranged from 1 hour to 24 hours.

11. PATTERN OF VARIOUS CLINICAL MANIFESTATIONS IN THE POPULATION

11.1 LOCAL MANIFESTATIONS

Local symptoms in the form of any of the following: local swelling in the absence of a tourniquet or cellulitis of the affected limb or enlarged tender lymph node draining the bitten limb or necrosis, blistering, gangrene or compartment syndrome –absent pulses were seen in 100% of the study population.

11.2 NEUROLOGICAL MANIFESTATIONS

Neurological manifestations included ptosis or ophthalmoplegia, bulbar weakness – dysphagia, difficulty in speaking, limb muscle weakness, neck holding time <5s, respiratory paralysis-Reduced single breath count<10, paradoxical breathing, respiratory failure, need for mechanical ventilation. Neurotoxicity in any of these forms were seen in 51.4% (n=19)

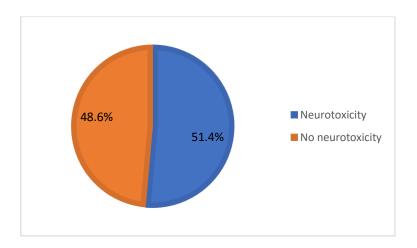


Figure 27: Distribution according to neurological manifestations

Patients with neurological manifestations who required ventilatory support were 8.1%

(n=3).

11.3 PTOSIS

The initial involvement of levator palpebrae superioris in form of ptosis was seen in 48.6% (n=18) patients.

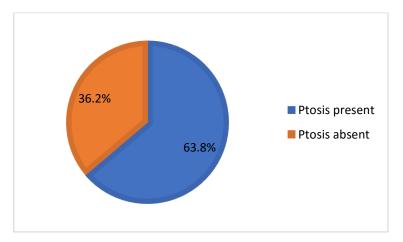
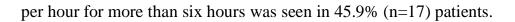


Figure 28: Distribution according to presence or absence of ptosis

11.4 RENAL INVOLVEMENT

Acute kidney injury defined by abrupt (within 48 hours), absolute increase in the serum creatinine concentration of ≥ 0.3 mg/dL (26.4 micromol/L) from baseline; a percentage

increase in the serum creatinine concentration of \geq 50 percent; or oliguria of <0.5 mL/kg



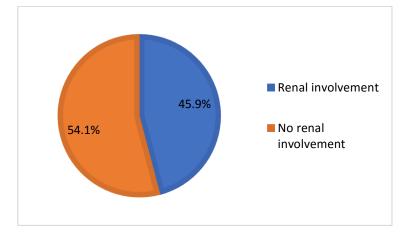


Figure 29: Distribution according to renal involvement

Patients with AKI who required haemodialysis were 16.2% (n=6).

12. ENVENOMATION SYNDROMES

The envenomation syndromes were grouped as **PURE HAEMOTOXICITY SYNDROME** and **HAEMOTOXICITY WITH RENAL/NEUROTOXIC FEATURES**. Pure haemotoxicity were seen in 24.3% (n=9) patients. Combinations of haemotoxicity with renal and/or neurological manifestations were seen in 75.7% (n=28).

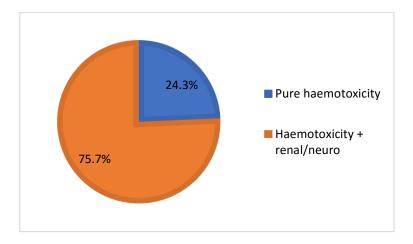


Figure 30: Distribution of envenomation syndromes in study population

Haemotoxicity was considered when any of the following were present: WBCT20 was prolonged, INR above reference interval ≥ 1.2), bleeding manifestations or thrombocytopenia (platelet count <1,00,000/ mm³).

13. LABORATORY PARAMETERS

AT BASELINE

13.1 WBCT

The Whole blood clotting time at presentation was more than 20 minutes in 81.1% (n=30).

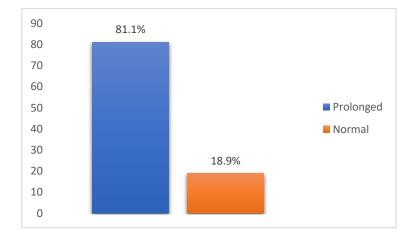


Figure 31: Distribution according to whole blood clotting time at presentation

13.2 HAEMOGLOBIN

Haemoglobin at baseline was done for 37 patients. The mean haemoglobin at

presentation was 13.78±2.46g%

13.3 PLATELET COUNT

The platelet count at admission was done for all 37 patients. 5.4% (n=2) had severe thrombocytopenia (platelet count less than 50,000/cumm). Moderate thrombocytopenia

(platelet count between 50,000/cumm and 1,00,000/cumm) was seen in 5.4% (n=2) patients. Mild thrombocytopenia (platelet count between 1,00,000/cumm and 1,50,000/cumm) was present in 21.62% (n=8). A normal platelet count of more than 1,50,000/cumm was seen in 67.56% (n=25) patients.

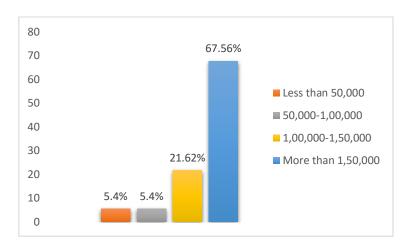


Figure 32: Distribution according to platelet count at admission

13.4 INTERNATIONAL NORMALISED RATIO

The international normalised ratio was abnormal (≥ 1.2) in 89.18% (n=33) patients.

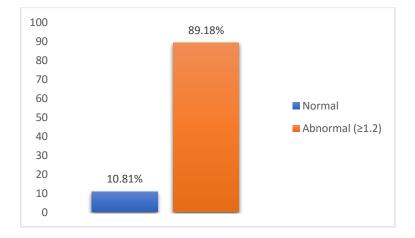
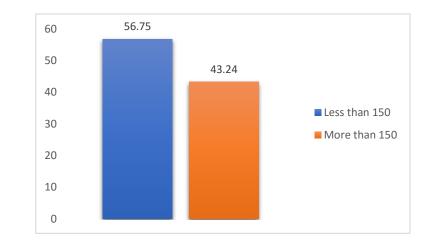


Figure 33: Distribution according to INR at admission

13.5 FIBRINOGEN



Fibrinogen at admission was abnormal (<150) for 56.75% (n=21) patients.

Figure 34: Distribution according to Fibrinogen at admission

14. ANTI SNAKE VENOM

Anti-snake venom was administered to all the 37 patients. The mean dose of ASV received from other hospital prior to reaching our centre was 8 ± 6.9 . The mean dose of ASV at our centre was 10.57 ± 5.11

18.91%(n=7) had a reaction to ASV. Reactions varied from angioedema, itching, urticaria, bronchospasm to anaphylactic shock.

15. VARIATION OF LABORATORY PARAMETERS OVER 24 HOURS

15.1 PLATELET VARIATION OVER 24 HOURS

The platelet count at admission varied from 35,000/cumm to 3,08,000/cumm.

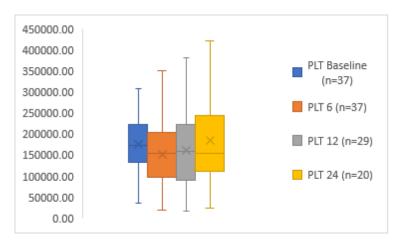


Figure 35: Variation of platelet count over 24 hours

15.2 INR VARIATION OVER 24 HOURS

The INR at presentation varied from 1.12 to 10. It ranged from 3.05 to 0.77 at 6 hours.

At 12 hours it ranged from 2.84 to 0.83. By 24 hours the range was 1.65 to 0.84

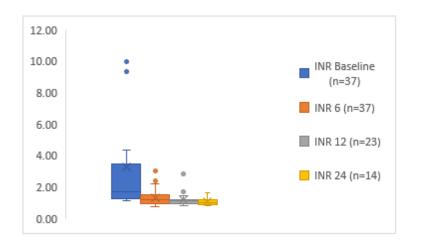


Figure 36: Variation of INR over 24 hours

15.3 FIBRINOGEN VARIATION OVER 24 HOURS

Fibrinogen at presentation ranged between a not detectable value to 455mg/dL. Those who had abnormal values were tested again at 6hours, 12hours or 24hours till they normalised.

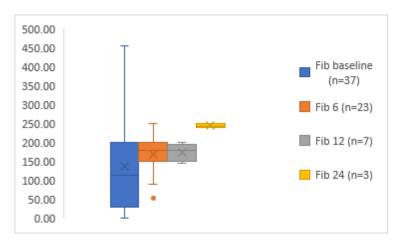


Figure 37: Variation of fibrinogen over 24 hours

16. TIME TO NORMALIZATION OF COAGULATION PARAMETERS

TIME TO NORMALISATION

The mean platelet counts for the pure haemotoxic group and the haemotoxicity +renal/neurotoxicity group have been plotted against time to show the pattern of normalisation. The pure haemotoxic group did not have thrombocytopenia at admission. Whereas the mean time to normalisation in the group with haemotoxicity +renal/neurotoxicity was 17.3±7.94 hours

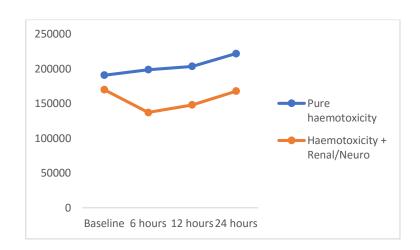


Figure 38: Time to normalization for Platelet count (Mean values)

The mean values of INR for the pure haemotoxic group and the haemotoxicity +renal/neurotoxicity group have been plotted against time to show their pattern of normalisation. The mean time to normalisation of INR in the pure haemotoxic group was 14 ± 6 hours. The mean time to normalisation among the haemotoxicity +renal/neurotoxicity group was 10.75 ± 6.61 hours.

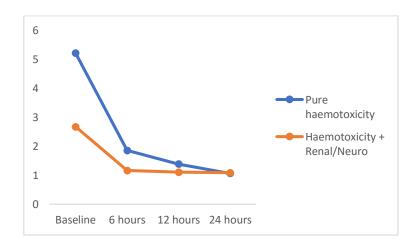


Figure 39: Time to normalization for INR (Mean values)

The mean values of fibrinogen for the pure haemotoxic group and the haemotoxicity +renal/neurotoxicity group have been plotted against time to show the pattern of normalisation. The mean time to normalisation of fibrinogen among the pure

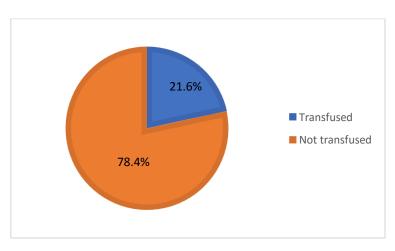
haemotoxic group was 11.25±5.9 hours. The mean time to normalisation among the



haemotoxicity +renal/neurotoxicity group was 6.5±1.7 hours.

Figure 40: Time to normalization for Fibrinogen (Mean values)

17. TRANSFUSIONS



In addition to ASV, transfusions were required for 21.6% (n=8) patients.

Figure 41: Distribution of patients requiring transfusion

Among the transfused patients, 50% (n=4) required blood and fresh frozen plasma transfusion. 50% (n=4) required fresh frozen plasma transfusion. Platelet rich

concentrate transfusion was required for 25% (n=2). Cryosupernatant was transfused for 25% (n=2) patients. Cryoprecipitate transfusion was needed for 12.5% (n=1) patient.

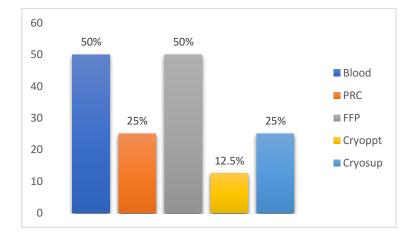


Figure 42: Distribution according to product transfused

FRESH FROZEN PLASMA

Out of the 4 patients who had received FFP transfusion, 2 had an INR more than 1.5 with bleeding manifestations before the transfusion. One patient had received FFPs from elsewhere and his INR was normal at presentation to our center. Another patient had a prolonged APTT with systemic bleed hence FFP was transfused.

22 patients with INR more than 1.5 did not receive FFP transfusion since they either normalised with ASV or did not have any bleeding manifestation.

CRYOPRECIPITATE

One patient who received cryoprecipitate had a low fibrinogen with associated bleeding manifestations. Patients who had a low fibrinogen without any bleeding manifestations were not transfused.

PLATELET RICH CONCENTRATE

Two patients who received PRC transfusion had thrombocytopenia with associated bleeding manifestations.

Patients who had thrombocytopenia without bleeding manifestations were not transfused.

18. OUTCOME

Out of the 37 patients 91.8% (n=34) were discharged in a stable condition. There was no mortality in the patients admitted.

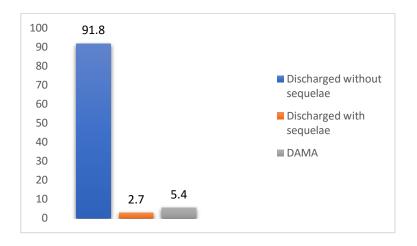


Figure 43: Distribution of patients 3according to condition at discharge

19. COMPARISON BETWEEN TWO GROUPS IS SHOWN BELOW:

Table 1: Comparison between pure haemotoxic group and haemotoxicity+

neurotoxicity/renal involvement

		PURE HAE	EMOTOXIC		HAEMOTOXICITY+			
				NEUROTOXICITY/RENAL INVOLVEMENT				
TOTAL NUMBER OF	9				28			
PATIENTS	-							
MALE: FEMALE	8:1				5:1			
AGE								
TIME TO SYMPTOMS	23.33 minutes				60.10minutes			
TIME FOR FIRST BLEEDING	36.67minutes				133 minutes			
SYSTEMIC BLEED	3 patients				17 patients			
TIME TO SYSTEMIC BLEED		2.671	hours		5.75 hours			
VENTILATOR	1 patient				4 patients			
DIALYSIS		No	one		6 patients			
MEAN ASV	15.89				20.29			
LABORATORY PARAMETERS	Baseline	6HRS	12HRS	24HRS	BL	6HRS	12HRS	24HRS
WBCT (prolonged)	8sec				22sec			
INR (mean)	5.21	1.85	1.36	1.06	2.67	1.16	1.1	1.08
APTT RATIO	2.04	1.06	0.93	0.44	1.41	1.05	0.6	0.2

81.67	139.88	169.25	242	155.64	185.6	181	245.5
190888	198888	203571	222000	170000	137250	148181	174250
20322				17610			
1			7				
0				4			
0			2				
1			3				
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All discharged			25 discharged, 1 discharged with				
			sequalae, 2 DAMA				
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20. VEMAC Time

Vemac Time standardised in our hospital was compared with PT values in prospective

group. A PT of more than 13s was considered abnormal.

	PT prolonged (>13 secs)	PT not prolonged (<13 secs)
Vemac Time prolonged	27	0
Vemac Time normal	6	4

The sensitivity of the test was found to be 81.82% and specificity was 100%.

Positive predictive value of the test was 100%.

When compared to a composite diagnosis of VICC it was found to have positive predictive value of 100%.

Table 3: Vemac Time and VICC comparison

	VICC	NO VICC
Vemac Time prolonged	27	0
Vemac Time normal	10	0

DISCUSSION:

In the present study we have attempted to characterize the effects of snake bite on the haemostatic system, transfusion requirements and effect of transfusion on haemostatic parameters.

RETROSPECTIVE DATA:

DEMOGRAPHIC DETAILS

In the retrospective study group a total of 280 cases of haemotoxic snake bite were identified. The victims of snake bite were predominantly male (72.5%, n = 200), and the male to female ratio was 3:1. Males are affected more often than females, as they constitute the working majority who are actively engaged in farming and other outdoor activities. Our findings concur with those of earlier study by Mondal et al where they revealed that majority of the patients were males (79.8%) from rural areas (85.1%). A study conducted in viper bites in a tertiary centre in South India showed a male female ratio of 1.4:1. A similar study from another southern state showed a male preponderance (58%) (58).

The mean age of retrospective group was 42.2 ± 14.5 years (range16-75 years). The mean age of the male patients was 43 (range 19-65) years, and that of female patients was 40.1 (range 25-65) years. Decade -wise distribution of male and female snake bite cases by Monteiro et.al showed that majority of snake bite victims were aged 25-55 years (n =25, 80.6%), with the peak incidence in the 3rd and 5th decades of life. In our study retrospective data showed almost similar distribution among second to fifth decade of life.

Seasonal variation in the incidence of snake bite was observed in our study with maximum number of bites occurring during August (15.3 %). Such variation was also observed in study conducted by Nagnath R et al among Maharashtra victims with maximum number of bites occurring in rainy season (83.3%) between June to October and highest number of cases during August (28.1%). Hansdak et al also reported that 51% of cases occurred during monsoon (August–October). Similar studies have shown most bites observed during the months of May to November which represents the monsoon. This poses increased risk to farmers as they harvest their crops during these seasons. Studies conducted at other centres also noticed a similar increase in the incidence of snake bites during the monsoon when compared to the drier summer. Study conducted by Monteiro et al showed the peak incidence in snake bite cases during October, followed by September (59).

ENVENOMATION DETAILS

Snake bites are more common on the lower extremities due to accidental stampede while walking or playing. In a study conducted by Kshirsagar VY et al among rural population they found that 120 (74.04%) patients had bite marks on the lower limbs similar to various studies which have shown that in 70-86% patients bite marks are present on the lower limbs (60). Most of the victims in our study were bitten mostly in the fields during 6pm to midnight (n = 88, 37%) and on their lower limbs (n = 223, 84.5%).

In a study on snake bite envenoming from Kerala, 200 cases were analysed which showed 93% were outdoor bites with 81% of bites in the lower limbs (58).

Characteristics of the 143 victims of snake bites from South-eastern Nepal showed agriculture was the dominant profession (44%) and 49% of the victims lived in a traditional hut with mud walls. Most of the snake bites occurred during the rainy season (68%), outside the house (82%), while farming (21%), doing other work (32%), or walking (32%) and mostly during the day (50%) or between 6:00 PM and midnight (40%) (61).

In a study from Maharashtra out of 38 cases of Russell's viper bites studied in one year, 28 (73%) were males. 29(76%) of these cases had bites to their lower extremities(62).

Russell's viper is very aggressive snake. Its fangs are long and sharp. It is diurnal in habit. It bites a person working in a farm, handling the debris or harvesting or walking bare foot in tall grass.

PATTERNS OF CLINICAL MANIFESTATIONS

The categorisation of syndromes was based on the WHO South East Asia Guidelines (2016). Russell's viper bite syndromes include haemotoxicity or haemotoxicity in combination with neurotoxicity and/or acute kidney injury. Pure haemotoxicity could either be due to Saw Scaled viper bites or Russell's viper. Krait bite syndrome causes neuroparalysis without local swelling and may have associated abdominal pain. Cobra bite syndrome causes neuroparalysis with local swelling. The main aim of our study was to look at the coagulation parameter abnormalities and the transfusion requirements

for patients with snake bites. Hence, we analysed only patients presenting with haemotoxic manifestations.

All the 280 patients brought to hospital had local swelling and pain at the site of bite, associated with bleeding from the site. Local signs of envenomation and systemic haemotoxic manifestations of envenomation were present in all cases. Haemotoxicity was considered when any of the following were present: WBCT20 was prolonged, INR above reference interval (>1.5), bleeding manifestations or thrombocytopenia (platelet count <1,00,000/ mm³). Study conducted from Southern India showed 40% of the victims had bleeding from the site of bite. This finding is similar to studies by Bhat RN and Reid et al. Other manifestations from the same study included generalized ecchymosis, purpura or hematomas, frank or microscopic haematuria, haemoptysis, gingival bleeding, hematemesis (63).

Study conducted from our centre which included 167 patients with systemic envenomation showed 102 patients with combinations of haemotoxicity with neurotoxicity and/or acute kidney injury (61%) (which were probably due to Russell's viper envenomation). The most common Russell's viper syndromes were haemotoxicity with neurotoxicity and haemotoxicity with neurotoxicity and acute kidney injury. There were 12 cases (7.2%) with neurotoxicity without local swelling (probable Krait bite) and 18 cases (10.8%) with neurotoxicity with local swelling (probable cobra bite). All the deaths occurred in the syndromes of Russell's viper with haemotoxicity with AKI.

Our present study showed associated neurotoxicity in 60.7% patients and 20.6% of them required ventilatory support. Ptosis was seen in 53.9% of patients. Associated renal

involvement was seen in 22.5% of patients. All symptoms including haemotoxicity, local manifestations, neurotoxicity, ptosis, renal involvement and rhabdomyolysis together was present in 8.2% of patients.

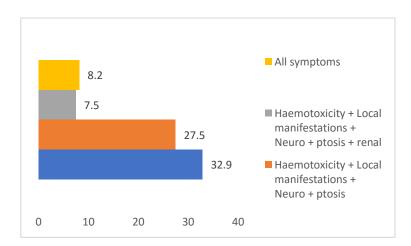


Figure 44: Symptoms wise distribution of patients

In another study conducted on 1,500 cases from a tertiary care centre in Malabar, North Kerala, India showed hemotoxic bites (61%) exceeded neurotoxic bites (34%) by a significant margin. Same study showed, haematuria as the most common (246 patients) symptom followed by bleeding gums in 218 patients. Haemoptysis was seen in 172 patients. Retinal haemorrhages were seen in 106 patients (64).

In a study of 138 patients from Western India looking into the complications of envenomation with snake bite showed haemorrhagic manifestations 123(89%). Other presenting manifestation were haematuria 38.4%, renal failure 29.7%, ptosis 7.3%, respiratory paralysis 7.3% and intracranial bleeding 2.2% in the same study. However, no cardiotoxicity was reported (65). Our study showed 3 cases with cardiac manifestations in the form of poor systolic function, suspected Kounis syndrome

characterised by acute coronary syndrome or ST-Segment elevation myocardial infarction resulting from an allergic reaction, myocarditis and one patient died of sudden cardiac death due to probable arrhythmia. Hayat and Khan also reported haemorrhagic manifestation in 95% and neurotoxicity in 5% of cases (66).

LABORATORY PARAMETERS

Snake venom contains various proteins (like prothrombin activators, thrombin like enzymes, phospholipases, factor X activating proteins etc.) which block different levels of coagulation or it may cause excessive clot formation leading to consumption coagulopathy. In most of the cases PT and APTT both are raised.

In a study conducted in a tertiary care centre by Dasaraju showed prolonged PT in 66% of the patients which was comparable with another study conducted by Monteiro et al, Ramamurthy et al, Harshavardhana et al and David et al who found deranged PT in 32.30%, 40.00%, 56.00% and 47.50% of patients respectively (59,63,67,68). In the study by Manisha et al, 75.89% of the patients showed deranged PT, which was similar to the Mahmood et al (70.00%) studies (62).

In our study PT was not considered as a parameter for analysis due to inherent variance relating to thromboplastin in the reagent and method of analysis. Instead, the INR is a more standardised parameter which offers practical advantages for monitoring was considered. We considered an INR more than or equal to 1.2 as abnormal. At presentation the majority of our patients, 94.38% of patients had an abnormal INR.

In a cohort study of patients with confirmed or suspected snake bite recruited to the Australian Snake bite Project (ASP) from January 2002 to April 2009 showed that in

178 of the 206 patients (86%), the INR was abnormal (> 1.2) on the first set of tests. In the same study by Graham et al suggests that the combination of tests for INR, APTT and CK level and serial neurological examinations is able to reliably detect envenomed patients within 12 hours (69). If there is no progression of symptoms or evidence of coagulopathy the patient can be discharged after rechecking laboratory parameters that INR is not elevated. Elevated INR indicates envenomation or impending coagulopathy.

In our study out of 273 patients for whom platelet count was done, 37.64% had thrombocytopenia at presentation. Baseline fibrinogen was available for 23 patients, among whom 6 had an undetectable fibrinogen. A study conducted by Moriarity et al looking into the role of coagulation markers in snake envenomation showed coagulation marker abnormalities in 35 (26.7%) of the 131 snake bite patients (70). Seventeen (13.8%) had an abnormal PT, 17 (13.9%) had an abnormal APTT 8 (6.2%) had thrombocytopenia, and 5 (13.2%) had abnormal fibrinogen concentrations (70).

In our study, deranged APTT ratio was seen in 39.8% of patients. Mahmood et al had similar results with 71.30% of patients having deranged APTT (59). Monteiro et al, Ramamurthy et al, Harshavardhana et al and David et al found deranged APTT in 29.00%, 40.00%, 62.00% and 32.50% of patients respectively (59,63,67).

ANTI SNAKE VENOM

In a study by Monteiro et al around 4-32 ASV vials were administered to each patient, at an average of 11.1 ASV vials per patient (59). In our study the mean dose of ASV per patient was 17.43 ± 8.4 .

In a previous study conducted in our institute the hypersensitivity reaction rate to ASV was about 8% (67). In our study it was found to be 13.6%.

TRANSFUSION

Blood products have been administered alone and in addition to antivenin in patients with snake bite-induced coagulopathies. Envenomed patients remain at risk of major haemorrhagic complications such as intracranial haemorrhage for a significant time period after antivenom treatment. This has prompted the use of blood products as replacement for clotting factors. However, there is a concern that the provision of clotting factors will worsen the coagulopathy, as more substrate will be available for the procoagulant toxins present in the venom to activate. Furthermore, there are also risks associated with the use of blood products like haemolytic reactions, nonhemolytic reactions, and infectious complications.

A recent randomized controlled trial in Australia of FFP in VICC showed that FFP (10-15 mL/kg up to maximum of 4 units, about 1000 mL) given within 4 hours of antivenom administration resulted in a more rapid recovery in the PT/INR (71). In our study 16.8% of patients required either blood and/or plasma products transfusion in addition to ASV. All the patients who received plasma products transfusion had bleeding manifestations. In a study from another tertiary care centre 20.3% of the snake bite victims were transfused with blood products (72).

The main component transfused was FFP (65.9%) followed by Red cells (53.2%), cryoprecipitate (31.9%), platelet concentrates (29.8%) and cryosupernatant (10.6%). The mean dosage of FFP was 5 ± 2.6 units and highest number of FFP required by any

patient was thirteen. The lab parameters which were usually ordered to substantiate the use of FFP were 20min WBCT, PT & INR, APTT & Fibrinogen.

Other indications for transfusion were surgical interventions like fasciotomy, debridement or haemodialysis, in the absence of overt bleeding.

Out of the 2.5% (n=7) who had expired, 2 patients had not received any transfusions. The remaining 1.78% (n= 5) patients had been transfused blood and/or plasma products. Four patients had received FFP and cryoprecipitate. Three of these patients had received platelets also.

PROSPECTIVE STUDY GROUP

In the prospective study group a total of 37 cases of haemotoxic snake bite were identified from 2017-2018. The victims of snake bite were predominantly male (83.8%, n = 31), and the male to female ratio was 5:1. In a study by Alirol et al. about snake bite in Southeast Asia, it was concluded that there was a significant 2:1 male predominance over female among victims and that lower extremities were the most prevalent sites of snake bites (10). In our study 75.67% of the patients had snake bites in the lower limbs. Maximum bites were seen in between 6pm and midnight (37.8%) which was similar to our findings in the retrospective study group.

In our prospective group study, the median time to receive first aid was one hour, with the maximum time being 12 hours. All patients received first aid within 12 hours. The previous study from our institute has shown about three fourth of the patients sought first aid in local clinics and peripheral hospitals prior to definitive treatment in the tertiary hospital (67). Most of the cases were admitted within 24 h of the bite. This reflects an increasing awareness about the need for hospital-based care for snake bites and a possible change in attitude of patients. In a study conducted by Eslamian et al mean time between snake bite occurrence and reaching first medical centre was about six hours (73).

In a study by Padhiyar et al 62.5% (40/64) patients had received ASV before they were referred to tertiary care centre, 51.6% (33/64) had received Injection Tetanus Toxoid, and 45.3% (29/64) had a tourniquet tied at or above the site of bite (72). In our study 83.78%(n=31) patients had received ASV prior to reaching our centre, reflecting the better availability of ASV in smaller healthcare facilities as a result of sustained advocacy.

The mean time taken for the first symptoms to start after snake bite was 55.19 minutes. It ranged from 5 minutes to 6 hours. Since envenomation is a function of several variables including dose of venom (in the snake bite), body mass of the patient and location of bite, our small sample size precludes us from making any significant conclusions on this parameter.

The median time to receive first aid was one hour. All patients received first aid within 12 hours. The mean time taken to receive the first dose of ASV was 3.36 hours. It ranged from 30 minutes to 19 hours. The median time to the first bleeding symptoms was 60 minutes. It ranged from 5 minutes to 12 hours. Systemic bleeding in the form of oral cavity bleed, haematuria, hematemesis, haematochezia and malena was present in 54.1% of patients. The mean time to the first systemic bleed was 5.65 hours. It ranged

from 1 hour to 24 hours. This is similar to a study by Kumar et al the mean time to reach to hospital was 12.1 ± 21.4 hours (range 1-120 hours (64).

Out of the 37 patients, 89.18% (n= 33) had an abnormal INR at presentation. Out of this 39.39%(n=13) patients normalised by 6 hours. Out of the remaining 20 patients, 14 normalised by 12 hours and five patients normalised by 24 hours. Two of the patients who did not normalise by 12 hours received FFP. One of these patients showed nephrotoxicity and underwent haemodialysis.

Out of the 9 patients who displayed features of isolated haemotoxicity syndrome, all had abnormal INR. These patients took a median time of 12 hours for normalisation with a maximum of 24hours. Fibrinogen was abnormal in 7 of these patients which normalised within a median time of 9 hours. Fresh frozen plasma transfusion was required for one patient who had a baseline INR of 3.29 and normalised only by 12 hours. The dose of FFP in our study was 10–15 mL kg⁻¹.

Out of the 28 patients with haemotoxicity and neurotoxicity/renal involvement, 25 had an abnormal INR at presentation. These patients normalised with a median time of 6 hours. However, 7 patients normalised only by 12 hours. Fibrinogen was abnormal for 32.43% (n=12) patients and the median time for normalisation was 6 hours. Plasma products were transfused for 16.2% (n=6) patients in this group. In a study done by Dempfle et al he concluded that intra venous administration of anti-snake venom resulted in normalized coagulation parameters within 48 hrs whereas in the study by Agarwal et al they saw that 51 out of 53 cases showed normalization of coagulation markers 12 hrs after administration of anti-snake venom (74). In our study it was difficult to ascertain time to normalization from time of administration since most of the patients received ASV prior to admission at our hospital.

A complete comparative analysis of two envenomation syndromes is shown in table.

Study by Isbister et al on a multicentre open-label randomized controlled trial in Australia to show utility of fresh frozen plasma for treating Venom-Induced Consumption Coagulopathy, showed that the administration of FFP within 4 hours of antivenom administration results in more rapid restoration of clotting function in the majority of patients with VICC (71). Almost three-quarters of patients receiving FFP had an INR of < 2 at 6 hours after antivenom administration, and this was also associated with more rapid complete recovery to a normal INR. In study by Harshavardhana, et al patients with coagulopathy had prolonged hospital stay and required more blood products transfusion. In his study he had 13 patients with haemoglobin less than 10 g/dl and approximately hospitalized for 22 days and they received 38 packed red cells. 24 patients had platelets less than 1 lakh and approximately hospitalized for 28 days and they received 102 platelet units. INR was more than 1.5 in 24 patients and hospitalized for 25 days and they received 136 fresh frozen plasma (63). Whole blood clotting time was prolonged more than 20 minutes in 30 patients and approximately hospitalized for 27 days and they received 488 ASV vials. In our study anti-snake venom was administered to all the 37 patients. The mean dose of ASV received from other hospital prior to reaching our centre was 8 ± 6.9 . The mean dose of ASV at our centre was 10.57±5.11.

The Vemac Time standardised at our centre was compared with WBCT 20 and Prothrombin Time to analyse its utility in detecting envenomation by haemotoxic snake bite. Using PT as the gold standard we found that Vemac Time had 100% specificity with positive predictive value of 100%.

LIMITATIONS:

- A major limitation of the present study is in the retrospective group where a retrospective chart review was done and some of the important data may be incomplete or insufficient, thus may affect statistical analysis.
- The selection bias due to a single centre data is justifiable. Referral bias explains why all of our patients were envenomated, and might have resulted in the transfer of more seriously ill patients to our facility.
- Many snake bite cases are treated at the primary healthcare centres and not referred to higher centres, leading to an underestimation of the morbidity status in studies done at tertiary healthcare centres. There may be a similar underestimation of snake bite mortality in the study.
- Vemac Time was performed at the bed side in Accident & Emergency by clinician. The precision of the test may be better when performed by laboratory staff under controlled conditions.

CONCLUSION:

RETROSPECTIVE DATA

- ***** There is a relatively higher prevalence of snake bite in males.
- ✤ There is a seasonal variability of the snake bite cases, we found bimodal distribution of the cases occur during the month of March and August
- The majority of bites were between 6 pm midnight with majority of bites in the lower limbs.
- ✤ Local symptoms in any form were seen in 100% of the study population.
- The envenomation syndromes were grouped as pure haemotoxicity syndrome and haemotoxicity with renal/neurotoxic features. Pure haemotoxicity were seen in 32.9% patients.
- * At admission INR was abnormal in 94.38% of population.
- ✤ All patients received ASV and 13.6% of patients had a reaction to ASV.
- * 16.8% patients required either blood or plasma products transfusion in addition to ASV.
- *** FFP** was the most commonly transfused blood product.
- **♦** All patients who required blood transfusion had bleeding manifestations.

PROSPECTIVE STUDY:

- ✤ Pure haemotoxicity was seen in 24.3% patients.
- ☆ The International Normalised Ratio was abnormal (≥1.2) in 89.18% patients.
- ✤ 32.43% of patients had thrombocytopenia at presentation.
- ✤ Fibrinogen at admission was abnormal (<150) for 56.75% (n=21) patients.</p>
- Venom Induced Consumption Coagulopathy was seen in 100%(n=37) patients.
- ✤ In addition to ASV, transfusions were required for 21.6% patients.
- VICC is a distinct entity that appears to have better prognosis when treated aggressively with ASV and blood components.
- The Vemac Time appears to be a sensitive and highly specific test that can be rapidly and reproducibly performed at the bedside for rapid diagnosis of envenomation.

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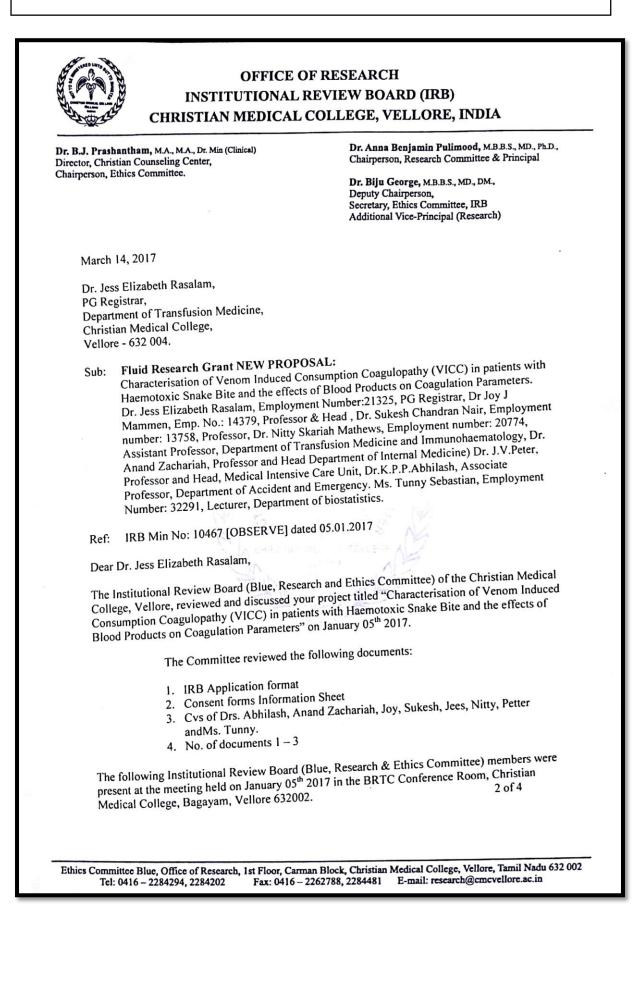
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OFFICE OF RESEARCH INSTITUTIONAL REVIEW BOARD (IRB) CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA Dr. Anna Benjamin Pulimood, M.B.B.S., MD., Ph.D., Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical) Chairperson, Research Committee & Principal Director, Christian Counseling Center, Chairperson, Ethics Committee. Dr. Biju George, MBB.S., MD., DM., Deputy Chairperson, Secretary, Ethics Committee, IRB Additional Vice-Principal (Research) March 14, 2017 Dr. Jess Elizabeth Rasalam, PG Registrar, Department of Transfusion Medicine, Christian Medical College, Vellore - 632 004. Fluid Research Grant NEW PROPOSAL: Characterisation of Venom Induced Consumption Coagulopathy (VICC) in patients with Sub: Haemotoxic Snake Bite and the effects of Blood Products on Coagulation Parameters. Dr. Jess Elizabeth Rasalam, Employment Number:21325, PG Registrar, Dr Joy J Mammen, Emp. No.: 14379, Professor & Head , Dr. Sukesh Chandran Nair, Employment number: 13758, Professor, Dr. Nitty Skariah Mathews, Employment number: 20774, Assistant Professor, Department of Transfusion Medicine and Immunohaematology, Dr. Anand Zachariah, Professor and Head Department of Internal Medicine) Dr. J.V.Peter, Professor and Head, Medical Intensive Care Unit, Dr.K.P.P.Abhilash, Associate Professor, Department of Accident and Emergency, Ms. Tunny Sebastian, Employment Number: 32291, Lecturer, Department of biostatistics. IRB Min No: 10467 [OBSERVE] dated 05.01.2017 Ref: Dear Dr. Jess Elizabeth Rasalam, I enclose the following documents:-Institutional Review Board approval 2. Agreement 1. Could you please sign the agreement and send it to Dr. Biju George, Addl. Vice Principal (Research), so that the grant money can be released. With best wishes, Dr. BIJU GEORGE Dr. Biju George MBBS...MD..DM. SECRETARY - (ETHICS COMMITTEE) Institutional Review Board. Secretary (Ethics Committee) Institutional Review Board Christian Medical College, Vellore - 632 002. l of 4 Cc: Dr. Joy Mammen, Dept. of Transfusion Medicine, CMC, Vellore Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nada 632 002 Tel: 0416 - 2284294, 2284202 Fax: 0416 - 2262788, 2284481 E-mail: research@cmcvellore.ac.in



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Dr Sneha Varkki	MBBS, DCH, DNB	Professor, Paediatrics, CMC, Vellore	Internal, Clinician
Dr. Sathish Kumar	MBBS, MD, DCH	Professor, Child Health, CMC, Vellore	Internal, Clinician

We approve the project to be conducted as presented.

Kindly provide the total number of patients enrolled in your study and the total number of withdrawals for the study entitled: "Characterisation of Venom Induced Consumption Coagulopathy (VICC) in patients with Haemotoxic Snake Bite and the effects of Blood Products on Coagulation Parameters" on a monthly basis. Please send copies of this to the Research Office (research@cmcvellore.ac.in).

Fluid Grant Allocation:

A sum of 1,00,000/- INR (Rupees One Lakh Only) will be granted for 2 years. 50,000/- INR (Rupees Fifty Thousand only) will be granted for 12 months as an 1st Installment. The rest of the 50,000/- INR (Rupees Fifty Thousand only) each will be released at the end of the first year as 2 nd Installment.

Yours sincerely,

Dr. Biju George Secretary (Ethics Committee) Institutional Review Board

Dr. BIJU GEORGE Institutional Review Board MBBS. MD.. DM. SECRETARY - (ETHICS COMMITTEE) Institutional Review Board, IRB Min No: 10473 [DIAGN@] dated 05.0 t 2017 Vettore - 632 002.

4 of 4

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002 Fax: 0416 - 2262788, 2284481 Tel: 0416 - 2284294, 2284202 E-mail: research@cmcvellore.ac.in



Figure 45: Indian Cobra (Naja naja)



Figure 46: Common krait (Bungarus caeruleus)



Figure 47: Russell's viper (Daboia russelii)



Figure 48: Saw-scaled viper (Echis carinatus)

	meets of naemotoxic sr	Consent Form	ctive observational stu	αγ
Nes	stigator: stigator: stigator:			
			Please initial each box & sig	gn at botto
1.	I confirm that I have read at the above study. I have had questions and have had the	the opportunity to con	sider the information, ask	
2.	I understand that my partic at any time, without giving rights being affected.			
3.	I understand that relevant s collected during the study r CMC Hospital. I give permis records.	may be looked at by res	ponsible individuals from	
4.	I consent to my data being	retained if I withdraw fi	rom the study	
5.	I agree to take part in the a	bove study.		
	Name of patient	Date	Signature	
	Researcher	Date	Signature	

	I. Patient Identification		
1.	Name:		
2.	Hospital No:		
3. 4.	Address: Place:		
5.	District		
6.	Age in years:		
7.	Sex: M F		
8.	Occupation:		
9.	Time of bite:		
10	. Date of Bite:		
11	. Date of admission:		
12	. Time of admission:		
	II. First aid and outside treatment		
13	Where / name of the Haspital;		
14	. Type of Hospital: PHC _ CHC _ District Govt. Hosp Priv	ate	
15	. Incision over the bite site: Yes No		
16	.Tourniquet: Yes No		
17	. If yes then the site of application :		
18	. Time duration of application of tourniquet:		
19	Number of tourniques:		
20	. Immobilization of limbs Y N		
21	Whether any other traditional treatment has been availed	Yes No	
			1
			-

22. If yes then details:	
23. Compression bandage, Y N	
24. Tetanus toxoid: Y N	
25. ASV administered outside AY N	
26. If yes, number of vials:	
III. Identification of the snake	
27.Was the dead snake brought along	
28. If yes, which snake species is it?	
29.Local reaction at site of bite: Y N	
30.Site of bite:	
31.Were fang marks visible: Y N	
32. Was there local swelling: Y N	
33. Local bleeding present: Y N	
34.Necrosis at bite site: Y N	
35. Surgical debridement: Y N	
	2

Cryoprecipitate	
Blood Product	Number of units transfused
44. Blood Transfusion Required: Y	N
43. Time to normalization of clottin administration of ASV }	ng time:hours (from, the time of
42. Other sites of bleeding:	
41. lcterus: Y N	
40. Hematuria: Y N	
39. Pallor: Y N	
38. Bruising: Y N	
37. Mouth/GIT: Y N	
If yes fill item below, sites of ble	eeding:-

Platelet Rich Concentrate		
WB/RC/LDRC		
45. Neurotoxicity: Y N		
If yes fill item below		
Drooping of eyelids: Y	N	
Loss of consciousness /altered sen	esorium: Y N	
Respiratory paralysis: Y	_ N	
Ophthalmoplegia: Y N		
Diplopia: Y N		
Needing ventilation: Y	N	
Duration of Mechanical ventilation	i:days	
Muscle weakness of limbs: Y	N	
Neostigmine administered: Y	N	
46. Anti.ucnom administration: Y	N	
Treatment before administration: Indigenous	First aid Tourniquet Immobilizat	tion
	POONAD.	mber of
vials:		
Second dose of ASV administered:	Y N Number of vials	
Maintenance dose of ASV administ	tered: Y N Number of via	ils:
47. Any evidence of organ failure -	liver/ kidney function deterioration Y	N
Dialysis required: Y N		

Recovered			-					
Discharged against i	medical adv	ine: Y	N.					
49. Cause of death: _								
49. cause of death: _								
Parameter	Baseline	6hrs	12hrs	18hrs	Day2	Day3	Day4	Day5
WBCT					-	-	-	-
MACTV								
Hb								
Platelet Count								
PT with INR								
APTT								
Fibrinogen								
Schistocytes								
Urea								
Creatinine								
CPK								
Urine analysis								
RBC/Blood								
ADAMTS 13								
D dimer								
ASV – No. of vials								
FFP- No. of units								
Cryoprecipitate -								
No. of units								
PRC - No. of units								
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TIII (CRF)	100 4240.0	100 42900	10 492.0	10 4930	100 4297.0	10 4960	100 4292.00	10220 001	200 4294.0	10 4958.0	110 4296.0	10 40090	200 4982.0	100 4081.0	10 498.0	110 45510	100 4937.0	10 4394.0	10 4397.0	10 4391.0	100 4892 00	100 4506.0	100 4250.0	10 4357.0	10 4559.0	100 42000	200 4525.0	200 4535.0	10 4201.0	200 4520 0	100 4597.0	200 4537.0	100 4336.0	10 4355.0	10 431.0
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