Original Research Article

A study of laboratory profile of snake bite patients

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Abstract

Introduction: Since ancient times, snakes have been worshipped, feared and loathed in South Asia. Snake bite is a common occupational hazard of farmers, plantation workers, construction workers, snake charmers and hunters. With urbanization and deforestation, snake bite has become an important public health problem. As the problem is so underrated that, snake bite was finally included in WHO's list of neglected tropical diseases in early 2009. **Aims and Objective:** To study the various Laboratory Profile of Snake Bite Patients. **Methodology:** After approval from institutional ethical committee, this Descriptive study carried out at Tertiary care hospital during 2 years (September 2012-September 2014) **Result:** There was no statistically significant correlation seen between type of snake bite and haemoglobin concentration ($p<0.05$). Statistically significant correlation was seen between the type of snake bite and total leukocyte count. Effect on the platelet counts was not seen in bites other than vasculotoxic bites. PT was raised in 85 (75.89%) cases and APTT in 77 (68.75%) cases. Out of 85 patients with raised PT, 19 (16.96%) had incoagulable plasma and rest had PT more than 16 seconds. Amongst the 77 patients with raised APTT, 19 (16.96%) had in coagulable plasma and remaining had APTT higher than 32seconds but not in coagulablem. **Conclusion:** Coagulopathy is the most common complication in vasculotoxic bites, which is an absolute indication of anti venom treatment. prothrombin time and activated partial thromboplastin time are early indicators and better predictors of coagulopathy, and should be used whenever possible.

Keywords: Vasculotoxic, Neurotoxic, Hemotoxic snake bite.

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INTRODUCTION

Since ancient times, snakes have been worshipped, feared and loathed in South Asia.[1]Snake bite is a common occupational hazard of farmers, plantation workers, construction workers, snake charmers and hunters. With urbanization and deforestation, snake bite has become an important public health problem. As the problem is so underrated that, snake bite was finally included in WHO's list of neglected tropical diseases in early 2009.[2]The vast majority of snake bites are accidental in nature. Homicide may rarely be committed, for instance by throwing a venomous snake on sleeping victim or slipping it under the bathroom door or through a window when a 3 person is bathing.[3] So every case of snake bite is registered as medico legal case (MLC).Snakes have wide range of habitat and prey species. All snakes are predatory carnivores, none is vegetarian. Since snakes are preyed upon by other animals they tend to be secretive and have evolved many survival strategies. Many species are mainly nocturnal (night hunters) e.g. kraits, but other species are mainly diurnal (day-time hunters). By understanding about the habits of snakes, simple precautions can be adopted to reduce the incidence of snake bites.[4] About 2500-3000species of snakes exist in the world. Most venomous snakes are found in Asia as compared to the other area of the world.[5] India has over 250 species and subspecies, out of which 50 are poisonous.[6] The families of poisonous snakes include Elapidae, Viperidae and Hydrophidae[7,8] which are responsible for neurotoxicity, vasculotoxicity and myotoxicity respectively.[9]Viperidae family includes two subfamilies: Viperinae (classic vipers) and Crotalinae (pit vipers).[8] Elapidae family includes cobras and common kraits. Hydrophidae are the sea snakes Viper bites are more common than the other venomous snake bites in human beings.[8] Viper bites are responsible for vasculotoxicity leading to bleeding tendencies and coagulation defects. These bleeding diatheses are 4 mostly caused by consumptive coagulopathy, anti coagulation, fibrinolytic or may be due to direct effect of snake venom on platelet aggregation.[10] Early detection of vasculotoxicity and prompt institution of anti venom

therapy can prevent serious complications due to snake envenoming. Presence of coagulopathy is the absolute indication for anti-venom administration, which is the specific treatment of snake bite. Coagulation parameters are also important to monitor the effect of anti snake venom and follow up of the patient. So the study is planned to study the pattern various laboratory profile changes seen in snake bite patients.

AIMS AND OBJECTIVE

To study the various Laboratory Profile of Snake Bite Patients.

MATERIAL AND METHODS

After approval from institutional ethical committee, this Descriptive study carried out at Tertiary care hospital during 2 years (September 2012-September 2014).Inclusion criteria: All the patients (irrespective of toxicity) presenting to the emergency ward with history of snake bite, who had not received ASV were included in the study.

Exclusion Criteria

Patients with unknown bite, Pregnant females, Patients with known coagulation disorder, liver disease and patients on anticoagulants. All patients admitted in the emergency ward with history of snake bite were included in the study (regardless of poisonous or non-poisonous snake bite). History of all patients as per preformed Proforma was taken along with detailed clinical examination of the patient. Identification of the species of bitten snake was done on the basis of the dead snake brought to hospital, by the description of the snake provided or identification by showing photographs of the snakes. In case where patient or relative could not see the snake, identification of species and its toxicities were defined for each case by using reference book of toxicology (Parikh's textbook of Medical Jurisprudence, Forensic Medicine and Toxicology).

RESULT

Table 1: Changes in hemoglobin levels among all snake bite patients (n=300)

Type of snakes	Anaemia status		Total
	Anaemic	Non-anaemic	
Vasculotoxic	101(90.18)	11(9.82)	112(100)
Non-vasculotoxic	160(85.11)	28(14.89)	188(100)
Total	**261(87)**	**39(13)**	**300(100)**

Figures in parentheses show percentages, X^2 = 1.60, df = 1, p = 0.21, Statistically not significant

Haemoglobin percentage was decreased in 261 patients and was within normal range in 39 patients, out of all 300 patients. Out of 112 with vasculotoxic patients, 101 (90.18%) showed anaemia and 11 (9.82%) were having haemoglobin within normal range. In 188 patients with non-vasculotoxic bites, 160 (85.11%) had anaemia and 28 (14.89%) patients were having normal haemoglobin levels. There was no statistically significant correlation seen between type of snake bite and haemoglobin concentration.

Table 2: Changes in total leukocyte count (TLC) among all snakebite patients (n=300)

Type of snake	Effect on TLC		Total
	Leukocytosis(>11,000)	TLC within normal range (4000-11000)	
Vasculotoxic	56 (50)	56 (50)	112 (100)
Non-vasculotoxic	28(14.89)	160 (85.11)	188(100)
Total	**84(28)**	**216(72)**	**300(100)**

Figures in parentheses show percentagesX^2 = 42.91, df = 1, p<0.001, statistically significant

Leukocytosis was seen in the 84 patients out of 300 snake bites. Out of 112vasculotoxic bites, 56 (50%) patients were having leukocytosis and 56 (50%) patients had TLC within normal range. Among 188 non-vasculotoxic bites only 28 (14.89%) had leukocytosis. Statistically significant correlation was seen between the type of snake bite and total leukocyte count.

Table 3: Changes in platelet counts in vasculotoxic snake bites (n=112)

Platelet count(/cmm)	Number of cases	Percentage (%)
≥150000	89	79.46
150000-100000	7	6.25
100000-50000	12	10.71
<50000	4	3.57
Total	**112**	**100**

Out of 112 cases of vasculotoxic bites, 23 (20.54%) patients showed thrombocytopenia while rest 89 (79.46%) patients had platelets counts within normal range. Among 23 (20.54%) patients with thrombocytopenia, only 4 patients had platelet counts less than 50000/cmm (severe thrombocytopenia). Effect on the platelet counts was not seen in bites other than vasculotoxic bites.

Table 4: Results of laboratory tests in vasculotoxic bites (n=112)

Interpretation of laboratory tests	Laboratory tests	
	Prothrombin time	Activated partial thromboplastin time
Not raised	27(24.11)	35(31.25)
Raised but coagulable plasma	66(58.93)	58(51.79)
Incoagulable plasma	19(16.96)	19(16.96)
Total	**112(100)**	**112(100)**

Figures in parentheses show percentages To be more precise about the type of coagulation pathway involved prothrombin time (PT) and activated partial thromboplastin time (APTT) are measured. PT was raised in 85 (75.89%) cases and APTT in 77 (68.75%) cases. Out of 85 patients with raised PT, 19 (16.96%) had incoagulable plasma and rest had PT more than 16seconds. Amongst the 77patients with raised APTT, 19 (16.96%) had in coagulable plasma and remaining had APTT higher than 32seconds but not in coagulablem.

DISCUSSION

Basis for the various laboratory changes are **Myotoxins:** Myotoxins can be generally defined as natural components of venom secretions that induce irreversible damage to skeletal muscle fibres (myonecrosis) upon injection into higher animals. In severe cases, local myonecrosis can lead to drastic sequelae such as permanent tissue loss, disability or amputation. On the other hand, widespread systemic myotoxicity (rhabdomyolysis) can lead to myoglobinuria and acute renal failure, a frequent cause of death in snake bite victims. As an example, haemorrhagic toxins that cause local blood flow impairment, ischemia, and secondary myonecrosis of slow onset, would be considered as indirect myotoxic factors.[43] Increase in total muscle Ca^{2+} concentration, lead to the hypercontraction of myofilaments, and induction of other downstream degenerative events finally resulting in cell death.[38]

Hemotoxicity Phospholipase A_2

Phospholipase A_2 (posphatidate 2-acylhydrolases, PLA_2) PLA_2 are probably the most thoroughly investigated toxins both in hemotoxic and presynaptic neurotoxic snake venoms. PLA_2 has also been classified as a presynaptic neurotoxin, identified in the venoms of *Crotalidae*, *Elapidae*, *Hydrophiidae* and *Viperidae* snakes.[10] Snake venom PLA_2 are an extremely important and diverse group of proteins affecting haemostasis. Therefore, the anticoagulant effects of these PLA_2 could be due to their competition with coagulation factors for binding to PL and not necessarily to the lipid hydrolysis.[44] PLA_2 may exacerbate haemorrhage by destabilizing the basement membranes following the hydrolysis of phospholipids.[38]

Zn^{2+}-dependent metalloproteinases[12]

Snake venom metalloproteinases (SVMPs) are major components of most Crotalid and Viperid venoms. These enzymes are the key contributors to lethal toxicity in these venoms. For efficient catalysis to occur, the catalytic domains of SVMPs require association with divalent cations such as Zn^{2+}.Other members of the SVMPs possess fibrinolytic activity, act as prothrombin activators, contain pro-apoptotic activity, serve as inactivators of blood serine proteinase inhibitors and activate factor X (a pro-coagulation protein).

Haemorrhagic SVMPs[38]

Theproteolytic degradation of capillary basement membrane proteins and the leakage of blood components from the vasculature into surrounding tissues are responsible for the haemorrhagic activity of the SVMPs. Most SVMPs can produce systemic haemorrhage in vivo. The pathophysiological spectrum induced by SVMPs also includes the formation of platelet plugs in blood vessels, the swelling and disruption of endothelial cells in capillaries, whereas other blood vessels shrivel and get disrupted. Interestingly, blood vessels containing intact intercellular junctions can still contain damaged endothelial cells and either a disorganized or lack of the basal lamina.

Fibrinolytic SVMPs[38]

A variety of pit viper venoms contain fibrinogenolytic activity. However, the level of this proteolytic activity is highly variable even within a particular genus of snakes. One of the most potent fibrinolytic enzymes is fibrolase, a P-I metalloproteinase. Fibrolase cleaves both the Aα and the Bβ-chains of fibrinogen, while having no effect on the γ-chain.

Prothrombin-activating SVMPs[12, 13]

There have been two groups of prothrombin-activating SVMPs that have been identified in snake venom: Group A and Group B, which are structurally unrelated to the human prothrombin activators - the blood coagulation serine proteinases. Group A Prothrombin Activators: These metalloproteinases efficiently activate prothrombin without the requirement of any cofactors, such as Ca^{2+} ions, phospholipids or factor Va.

Thrombin-Like Enzymes[13]

These fibrinogen-clotting enzymes are widely distributed within several pit viper genera, as well as some true vipers and the colubrid. The thrombin-like enzymes (TLEs) are single-chain serine proteinases. They act on blood plasma usually forming friable and translucent

clots, presumably due to a lack of cross-linking of fibrin by factor XIII a. **Venom proteins acting on platelets: C-type lectins:**[15]C-type lectins, in the snake venoms, affect platelets by binding to von-Willebr and Factor (vWF) or receptors such as GPIb, a2b1 and GPVI. **Disintegrins**[15] Disintegrins inhibit integrins of the b1 and b3 subfamilies including the fibrinogen receptor GPIIb/IIIa, the vitronectin receptor (avb3) and the fibronectin receptor (a5b1). **Proteinases:**[15] One group of proteinases acting directly on platelets is the thrombin like enzymes (TLEs), which may activate platelets through cleavage of PAR or binding to GPIb. **Phospholipase A_2**[15] Venom PLA_2 affects platelet functions by at least three mechanisms. One group of PLA_2 induce platelet aggregation by cleaving platelet membrane PLs releasing arachidonic acid and forming arachidonic acid metabolites such as thromboxane A2. In our study we found that here was no statistically significant correlation seen between type of snake bite and haemoglobin concentration. Statistically significant correlation was seen between the type of snake bite and total leukocyte count. Effect on the platelet counts was not seen in bites other than vasculotoxic bites. Amongst the 77patients with raised APTT, 19 (16.96%) had in coagulable plasma and remaining had APTT higher than 32 seconds but not in coagulablem

CONCLUSION

Coagulopathy is the most common complication in vasculotoxic bites, which is an absolute indication of anti venom treatment. Coagulation disturbances often develop rapidly after envenomation and may be sometimes even before the signs and symptoms of coagulopathy are seen. So it is important to spot out the coagulopathy at earliest possible point, so that appropriate treatment can be given without delay. At the peripheral centres simple bedside tests like bleeding time and 20 minute whole blood clotting test, can be used as an adequate indicator of haemotoxicity of snake bite. But the costlier tests like prothrombin time and activated partial thromboplastin time are early indicators and better predictors of coagulopathy, and should be used whenever possible.

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