ASSESSMENT OF THE LEVELS OF SOME HAEMOSTATIC PROFILES IN SNAKEBITE VICTIMS ATTENDING THE SNAKEBITE RESEARCH, TRAINING AND TREATMENT CENTRE KALTUNGO, GOMBE STATE NIGERIA

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ABSTRACT
Background: Coagulopathy is one of the major clinical issues associated with haemotoxic effect of envenomation due to snakebite. Coagulopathy in snakebite could results to decrease platelets count, prolonged Prothrombin Time (PT), International Normalized Ratio (INR), Thrombin Time (TT), Activated Prothromboplastin Time Test (APTT), and Fibrinogen level. It is also associated with increase in proteins C and S as well as D-dimer levels. This study was aim to evaluate some haemostatic profile of snakebite victims.
Methods: Two hundred (200) snakebite victims and one hundred (100) controls subjects were recruited in this study for the period of 8 months (October, 2020 to May, 2021). A total of 8m of whole blood was collected and dispensed into (2ml) of the blood was dispensed into Ethylene Diamine-Tetra Acetic acid {EDTA (2ml)} container for Platelet count as part of Full Blood Count, 4ml into a container containing 0.5ml sodium citrate for clotting profile (PT, INR, APTT, TT and fibrinogen) and another 2mlinto lithium heparin container for D-dimer assay. Haematology analyzer (McJefferson's haematology analyzer) was used for FBC and the coagulation parameters were determined using semi automated coagulation machine (Stago-Start Max).
Results: There was significant decrease in platelet count with p-value of 0.0001, significant increase in PT, INR, APTT, TT and fibrinogen levels; all with p-values of 0.0001. On the other hand, there was significant increase in protein C and S as well as D-dimer levels with p-values 0.0001). A p-value of less than 0.05 ($p<0.05$) was considered significant for all the parameters.
Conclusion: The findings from this study shows that snakebite coagulopathy greatly affects platelet count, PT, INR, APTT, TT, Fibrinogen, protein C and S as well as D-dimer levels. Hence, a major cause of coagulopathy in snakebite victims, resulting many complications and even death.
Key words: Snake, Snakebite, Envenomation, coagulopathy

INTRODUCTION
Snake is one of the most known and feared animals by man worldwide.. There are more than 20 families, about 500 genera and about 3,400 species of snakes and found almost everywhere in the world (Young *et al*, 2004; Hedges, 2008; Young, 2010; Reeder *et al*, 2015, Voelter, 2017). Venomous snakes are those that have the lethal substance called venom in saliva and can both physical (wound) and systemic harm its victims severely because their bite may be accompanied by the injection of the venom into the body.

They constitute about 25% (about 625 species) of all snake species of the world but only about 15% (about 375 species) of these can cause severe effect, including death. There are up to twenty five (25) most venomous snakes worldwide. Example of venomous snakes includes snakes of the families of Elapidae (Cobra, King Cobra and Coral snakes), Viperidae (Vipers), Atractaspidae etc. Venomous snakes are found all over the world. The Viperidae is the largest family of venomous snakes and its members can be found in Africa, Europe, Asia and America (Williams *et al*; 2004; Emerg, 2008; Syed *et al.,* 2008; Dreyer and Dreyer, 2013; AVRU, 2014).

The Snake venom - is the lethal zootoxin substance that is contained in the saliva of venomous snakes. It is the single most important venom ever known. Although all snakes have saliva, non-venomous snakes do not have venom in their saliva. It is produced and secreted from a modification of the parotid salivary glands also found in other vertebrates, usually located on each side of the head, below and behind the eye; enclosed in a muscular sheath. However, the storage site of the venom is the large glands called alveoli. It is then conveyed by a duct to the base of channeled or tubular fangs through which it's ejected during envenomation (Halliday and Kraig, 2002; Bottrall *et al*, 2010). Venom contains more than 20 different complex mixtures of compounds, mostly made up of 100s and 1000s of proteins (accounting for 90 – 95% of the venom), polypeptides, enzymes and various other substances has toxic and lethal properties. The components of snake venom vary from one snake type to the other even within the same species, season, geographical locations, age etc. The various compounds and components have different molecular weight (Bauchot, 1994; Halliday and Kraig, 2002; Oliveira *et al*, 2022). Perhaps the actual composition of snake venom is not fully elucidated.

The chemistry of the various compounds of the venom is even much more complex. It is basically made up of toxins, non-toxin proteins and enzymes. The toxic and many enzyme components have specific effects. Some of these toxins include murine LD_{50}, neurotoxins, hydrolytic enzymes like hydrolase, hydrolases, L-amino-acid oxidase, phospholipases, thrombin-like pro-coagulant and kallikrein-like serine proteases and metalloproteinases (haemorrhagins).Another component of snake venom is the Polypeptide toxins include cytotoxins, cardioxins and postsynaptic neutoxins e.g. α-bungarotoxin and α-cobratoxin. It also contains other substances like peptides, lipids, nucleosides, carbohydrates, amines, oligopeptides, phosphodiesterases, phospholipases A2 (haemplyses), hyaluronidase amino acid oxidase and proteates. Water is one of the many non-lethal substances of snake venom. Some snakes do spit out their venom, usually targeting the eyes of their victims, which can cause shock, blindness and inflammation of the eyes (Halliday and Kraig, 2002; Bottrall, 2010; Broeckhoven and du Plessis, 2017).

Snakebite is already an issue of medical emergency and major public health problem in rural Nigeria, with about 140 million (most of whom are farmers by occupation). Out of the 36 State, 12 States are said to be at particular risk because of mountainous and rocky natures. The estimated number of snakebite cases was 170/100,000 people. Most of these bites occur in very fertile areas of the country during planting and harvesting periods. This was said to be $1/5^{th}$ of all African prevalence, out of which 90% of the bites and 60% deaths were caused by E. ocellatus. The incidence is as high as 497 per 100,000 of the population per year in the Benue valley of Nigeria with 10 to 20% envenomation.

This may not even be the highest in the country with renounce places for snakebite like Kaltungo, Gombe and Zamko, Plateau State where snakebite have become a nightmare (David and Charles, 2002; Habib and Abubakar, 2011; Habib *et al*, 2013; Premiun Times, 2019).

MATERIALS AND METHODS

Study area - The Snakebite treatment, Research and Training Centre is located in Kaltungo, the Headquarters of Kaltungo Local Government Area, Gombe State, Nigeria

Research design – The study is a case-controlled study

Study population – A total of two hundred (200) victims (subjects) of haemotoxic snakebite and 100 controls were selected for the study. These consist of subjects that meet the inclusion criteria. The research took place between October, 2020 and May, 2021.

Ethical consideration - Ethical clearance was obtained from Ethical Committee of Ministry of Health Gombe, Gombe State (MOH/ADM/621/VOL. 1/221).

Sample collection and processing – A total of 8ml of whole blood was collected and dispensed into three containers as follows: - 2ml into Ethylene Diamine-Tetra Acetic acid (EDTA) container for platelet count using MacJefferson automated haematology analyzer in accordance with the manufacturer's protocol, 4ml of whole into sample container containing 0.5ml sodium citrate for collecting profile analysis using Stago (start Max) automated Clotology machine and another 2ml for D-dimer assay, using the Chroma D-dimer test kits and read by ELISA at the Federal Teaching Hospital, Gombe.

Statistical Analysis

The data obtained were analyzed using SPSS Statistic Version 20.0 software (SPSS Inc, Chicago, IL, USA). Statistics include mean, standard deviation and standard error of mean. The results obtained for FBC, PT, APTT, TT, fibrinogen, proteins C and S as well as D-dimer obtained from snakebite victims were compared with those of controls using Student's t-test. Student's t-test (Mann-Whitney Statistics/test). A p-value of less than 0.05 ($p<0.05$) was considered as significant.

RESULTS

Table 1: Mean comparison of platelet count in snakebite victims and control subjects

	Mann Whitney Statistics				
Parameters	Control (n=100)	Test (n=200)	U	Z-score	p-value
Platelet ($x10^9$/l)	362.36±164.94	188.54±87.01	2578.000	-10.479	0.0001 (s)

Statistical tool – Mann Whitney test, Values is presented as Mean±SD, (s) – significant.

Table 2: Clotting Profile in snakebite victims and control subjects

	Mann Whitney test				
Parameters	Control (n=100)	Test (n=200)	U	Z-score	p-value
PT (sec)	14.18±1.20	19.52±8.39	1842.50	-4.399	0.0001(s)
INR (sec)	1.121±0.09	1.61±0.76	1644.00	-5.147	0.0001(s)
APTT	38.24±2.78	49.03±14.25	1395.50	-5. 890	0.0001(s)
TT (sec)	16.57±2.15	30.46±15.212	1395.50	-5.890	0.0001(s)
FIB (sec)	2.63±0.64	17.25±18.62	235. 00	-10.119	0.0001(s)

Statistical tool – Mann Whitney test, Values are presented as Mean±SD, (s) – significant, PT – Prothrombin Time; INR – International Normalized Ratio, APTT – Activated Partial Thromboplastin Time Test, TT – Thrombin Time, FIB - Fibrinogen

Table 3: Protein C, Protein S and D-dimer values in snakebite victims and control subjects

	Mann Whitney test				
Parameters	Control (n=100)	Test (n=200)	U	Z-score	p-value
Protein C (unit)	104.90±21.789	53.76±16.552	679.50	-13.16	0.0001(s)
Protein S (unit)	107.48±17.431	53.71±15.562	357.50	-13. 61	0.0001(s)
D dimer (unit)	0.75±0.65	4756.60±4424.13	0.00	-14.12	0.0001(s)

Statistical tool – Mann Whitney test, Values are presented as Mean±SD, (s) – significant.

DISCUSSION

The available data of snakebite prevalence are far below reality globally for several reasons. However, it is estimated to be at least 5 million bites occurs per annum globally, worse in the Tropical and Sub-Sahara regions. Snakebite is associated with rapid and diverse mostly irreversible health complications including mortality and morbidity; due to the highly zoonotic (lethal) nature of its salivary venom no matter how small is the venom. This study reveals the severity of the burden as regards coagulopathy and the need for more urgent effects to containing the disease as captured below. It confirms that snakebite is a serious public health issue affecting the coagulation system. Statistically, $p \leq 0.05$ was considered significant in all comparison between snakebite victims and control variables using student's t-test to determine Mean, Standard Deviation and Standard Error of Mean. The relationship between mean ranks of snakebite victims and the controls shows significantly decreased in platelet, with p-value of 0.0001. This may be due to its consumption during haemorrhage by trying to form platelet plug at the site of injury, short life-span and the natural unstable nature of platelets under adverse conditions. Furthermore, several other venom components are known to inhibit or activate platelets by different mechanisms. They are also known to cause interaction with platelets and blood vessels, thereby lowering the quantity of platelets. This study agrees with some previous findings (Han *et al*, 1996; Jae, *et al*, 2003; Kalana, 2014).

A significantly increased ($p<0.0001$) levels of PT, INR, APTT, TT and FIB in the mean ranks of snakebite victims compared with control subjects was found just like other previous studies, with all their p-values as 0.0001. That is prolonged or total non-clotting clotting of these assays due to depletion (reduced or total) of blood clotting factor by the snake venom. The intrinsic, extrinsic and common path ways are all affected here (Williams *et al*, 2018; Levi, 2005; Bick, 2003).

The study also shows significant increase with p-values of 0.0001 for Protein C, S and D-dimer for snakebite victims compared to the control subjects. This is because haemorrhage stimulates the production of proteins C and S; in readiness for more clotting activity. This clotting activity does not take place because of reduced or total depletion of clotting factors. Hence, the increase in proteins C and S in the circulating blood. On the other hand, increase in D-dimer (fragments or products of fibrinolysis) is as a result of as of rapid consumption coagulopathy followed by rapid fibrinolysis. Haemorrhage is the due to the effect of haemotoxic snake venom is the manifestation of consumption coagulopathy and not neutralization coagulopathy. In summary, snake venom causes rapid coagulation and fibrinolysis; leading to high D-dimer levels in all the subjects. The study is therefore in agreement with many previous findings (Williams *et al*, 2018; Adam and Greenberg, 2009). The degree of consumption of these analytes (proteins C and S; D-dimer or Fibrinogen Degradation Products (FDP), prothrombin, fibrinogen and platelet status reflects the clinical severity of a victim, particularly as it relates to haemorrhage. This is a major clinical effect of haemotoxic snakebite (Bick, 2003; Levi, 2005).

CONCLUSION

This study showed that haemotoxic snakebite has serious effect on clotting parameters, protein C, protein S and D-dimer; leading to coagulopathy (haemorrhage) which is a major clinical effect that may lead to death or other forms of complications.

RECOMMENDATION

These parameters should be included in the management of snakebite victims.

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