ORIGINAL RESEARCH PAPER

General Medicine

ANALYSIS OF HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN SNAKEBITE

KEY WORDS: Snake bite, Hemolysis, Renal damage, Rhabdomyolysis

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ABSTRACT

Objective : To evaluate whether renal impairment is more likely in cases of Indian snakebite due to hematological and biochemical factors.. **Methodology :** Involves assessing 103 cases of snakebite (72 men and 27 women). Demographic data, outcomes of biochemical and haematological assays, including rhabdo myolysis, urinalysis, pro thrombin time, blood urea nitrogen, complete blood count, pro thrombin time, and creatinine kinase, were all included in the inquiry. The relationship between the patients' myoglobinuria, hemoglobinuria, and haematological features was investigated in relation to the likelihood of renal impairment. We also looked at the effects of venom on muscle and red blood cells (RBC). **Study results:** On evaluation, it was found that 50.5% of these patients had hemoglobinuria, 68% had red blood cell uria, 40.9% had proteinuria, 29.1% had bactriuria, 33% had anaemia, 74.8% had rhabdomyolysis, 45.6% had myoglobinuria, 12.7% had leukocytosis, 1.9% had thrombocytopenia, and 65% had coagulopathy. **Conclusion :** Rhabdomyolysis and coagulopathy are the most typical patho physiological changes seen in the majority of snakebite cases in this hospital-based analysis.

INTRODUCTION

Compared to other regions of the world, Asia is home to the majority of venomous snakes1. 21 of which have been identified as poisonous or semi-poisonous. It is estimated that up to five million snakebites, scorpion stings, and anaphylactic reactions to hymenoptera stings (bees, wasps, and ants) occur annually worldwide, according to a 1995 World Health Organization report on epidemics . The majority of snakebites occur on the feet and ankles of agricultural workers and hunters in rural areas of tropical countries in Asia, Africa, and Latin America. These bites and stings probably result in over 100.000 human deaths annually2. Envenomation is a risk to these people's jobs3,4 .Asia has the highest rate of snakebites, with an estimated 30,000 deaths annually. Africa and Latin America may each experience approximately 1,000 deaths annually. Snake venom has a very complex and heterogeneous composition, including enzymes, lethal peptides, non enzymatic proteins, metals, carbohydrates, lipids, biogenic amines, free amino acids, and direct hemolytic factors.1,6 .Some recently presented data provide an idea of the scope of the issue in some developing nations. Toxic enzymes, such as pre synaptic neurotoxins, are composed of several subunits, one of which is a phospholipase A26-8 .This composition makes it possible to see many different symptoms after envenomation. Additionally, the hemo toxins are crucial, particularly proteinases, peptidases, and phospholipases. Six to eight snake venoms contain at least 26 distinct enzymes, but no one snake venom contains them all. However, almost all venoms contain at least ten enzymes. In addition, snake venom contains enzymes that aid in digestion but are not toxic. They probably also make it easier for the venom to get in and get to the target tissue9 . Snake venom contains a number of substances that look like trypsins and break down or digest tissue proteins. The proteolytic enzymes, also known as peptide hydrolases, proteases, endo peptidases, peptidases, and proteinases, are examples of these.

The majority of hemotoxic enzymes have an anticoagulant effect in vivo, whereas their specific effects on blood coagulation proteins distinguish them from one another in vitro10-15 .The majority of venoms contain multiple enzymes, each of which has a synergistic or antagonistic effect on blood coagulation16 . A purified enzyme can simultaneously affect multiple coagulation factors. The concentration of the purified enzyme determines the degree of effect on blood coagulation. A few proteins can impact all phases of the blood coagulation. The concentration of the enzyme determines how active non purified venom is. It is demonstrated that there are individual contrasts in toxin piece and action between snakes of similar species. The purpose of this study was to determine whether hematological and biochemical parameters are associated with an increased risk of renal damage in snakebite patients. There are numerous variations based on age and location17,18 .

METHODOLOGY

In this study 103 cases were selected from GOVERNMENT GENERAL HOSPITAL VIJAYAWADA. The patients gave their informed consent. Within two hours, 5 ml of anti coagulated blood containing 1% EDTA and 5 ml of untreated blood were collected from each patient and centrifuged.

Complete blood count (CBC), electrolytes, Blood Urea Nitrogen (BUN), serum creatinine, Creatine Phospho Kinase (CPK), Prothrombin Time (PT), and partial thromboplastin time (PTT) were among the tests performed. Urinalysis was also performed.

The semi quantitative screening method was the reaction strip test for protein. Hemoglubinuria and myoglubinuria were detected with the reagent strip and chemical methods. A positive test resulted in the presence of free hemoglobin, myoglobinuria, or red blood cells (RBC) in the urine (hematuria). Finally, column chromatography was used to separate myoglobinuria from free hemoglobin. Based on light scattering, a cell counter system (Sysmex) was used to count red and other blood cells. An automated Sysmex Cell Counter was used to measure the Mean Cell Hemoglobin Concentration (MCHC), Mean Cell Hemoglobin Volume (MCV), and Mean Cell Volume (MCH). Fractional thromboplastin time (PTT) measures the thickening season of

plasma after the enactment of contact factors however without added tissue thromboplastin thus shows the general productivity of the inborn pathway. The phospholipids reagent and the kaolin suspension were mixed to equal volumes and kept in a glass tube at 37 °C in the water bath. The plasma was then mixed with 0.2 milliliters of the kaolin phospholipid solution in a new glass tube. A second stopwatch was started, the tube was kept at 370C for ten minutes, and one milliliter of pre-warmed Ca Cl2 was added. The amount of time it took for the mixture to clot was recorded .

The PT test determines the overall efficacy of the extrinsic clotting system by measuring plasma clotting time in the presence of an optical concentration of tissue extract (thromboplastin). A water bath and 0.1 ml of thromboplastin were added to a glass tube containing 0.1 ml of plasma. After that, the mixture was left to warm up for anywhere from one to three minutes. Then, 0.1 milliliters of pre-warmed Ca Cl2 were added. The end point was recorded and the contents of the tube were mixed . The presence of intact erythrocytes was confirmed by a microscopic urinalysis.

In most cases, a urine sample of a healthy person will not reveal any blood, glucose, bacteria, myoglobin, or hemoglobin. Standard methods were used to measure blood sugar, blood urea nitrogen, creatinine, na, k, and phospho creatine kinase .

STATASTICAL ANALYSIS:

Means± standard deviation are used to represent the data. The ÷ 2-test was used to determine the statistical difference between the groups. For normally distributed variables, the unpaired t test was used to compare other variables, and ANOVA was followed by Scheffe's test was used to compare group means. A significance level of less than 0.05 was considered.

Table-I:Biochemical and hematological characteristics of the snakebite patients based on risk of renal damage

Variables	Patients without risk of renal damage	Patients with risk of renal damage	P value
Age(year)	"26.8±11.5	28.8±14	0.614
Sex(Male/ Female)	11/2.	66/25	0.506
Fasting BloodSugar(mg/ dl)	108±24.1	113.9±43.3	0.63
Blood Urea Nitrogen(m g/dl)	14.2±-4.7	14.4±14.3	0.95
Creatinine(mg/ d l)	0.75±0.17	0.71±0.22	0.56
Na(meq /L)	140.8±2.6	140±2.9	0.345
K(meq/ L)	4.08±0.4	4.07±0.3	0.9
White Blood Cells (mm3)	10.7±5	119.6±1031	0.319
Red Blood Cell(mm3)	4.7±0.8	4.7±0.7	0.936
Haemoglobln(g %)	12.99±2.3	12.99±2.0	0.975
Platelets(mm3)	209.3±59.3	251.2±179.2	0.1
Partial thromboplastin time(seconds)	14.07±18.97	28.4±20.1	0.017
Prothrombin time(seconds)	5.3±6.96	10.3±7.62	0.027
Urine Protein(mg)	34.5±46	55.5±42	0.09
CK(IU/L)	181.07±61.4	306.9±102	< 0.001

Data are presented as mean±SD. Comparisons were made using student,s t test (for continuos variables) and, it was made using Chi-square test (for categorical variables). Statistically significant. P<0.05)

RESULTS

The study included 103 snakebite victims, 76 males and 27 females. Most of the patients were males somewhere in the range of 14 and 84 years old. There were 50.5 percent of these patients with hemoglobinuria, 68% with red blood cell uria, 40.9% with proteinuria, 29.1% with bactriuria, 33% with anemia, 74.8 percent with rhabdomyolysis, 45.6% with myoglobinuria, 12.7% with leukocytosis, 1.9% with thrombocytopenia, and 65 percent with coagulopathy. According to our data, snakebite affects more young people than older people, and the majority of victims are male.

Table-2:Clinical and biochemical characteristics of the snakebite patients based on gender

Variable	Sex		P value
	Male	Female	
n	76	27	
Fasting Blood Sugar(mg/ dl)	116.4±43.7	104.2±32.8	0.186
Blood Urea N itrogen(mg/dl)	23.2±3.7	17.9±2.7	0.121
Creatinine (mg/dl)	0.74±0.22	0.67±0.23	0.250
Na (meq/L)	140.02±2.9	140.4± 2.8	0.562
K (meq/ L)	4.1±0.35	4.1±0.23	0.515
Creatine Kinase (IU/L)	296.6±108.9	275.5+98.8	0.379
Risk of renaJ damage n (%)	65(72.2)	25(27.8)	0.506
Bacteriuria n (%)	21(70)	9(30)	0.635
Casturia n (%)	24(80)	6(20)	0.462
Rabdomyolysis n (%)	59(76.6)	18(23.4)	0.305
Glucosuria n (%)	27(64.3)	15(35.7)	0.109
myoglubinuria n (%)	40(85.1)	7(14.9)	0.024
Haematuria n (%)	20(60.6)	13(39.4)	0.05
Haemoglobinurian (%)	34(66.7)	17(33.3)	0.121

Data are presented as mean±SD. Comparisons were made using student,s t test (for continuos variables) and, it was made using Chi-square test (for categorical variables). Statistically significant. P<0.05)

According to their risk of renal damage, the patients were divided into two groups (Table-1). RBC, hemoglobin, hemocrit, platelet, RBCuria, anemia, and myoglobinuria showed statistically significant differences between male and female groups (P 0.05) (Table-2,3).

Table-3 :Clinical and hematological characteristics of the snake bite patients based on gender

Variable	SEX		P value
	Male	Female	
N	76	27	
White Blood Cell count(mm3)	10.7±6.3	11.02±4.3	0.805
Red Blood Cell count(mm3)	4.8±0.69	4.2±0.74	0.001
Haenloglobin(g%)	13.5±2.02	11.6± 1.7	<0.001

Haeinatocrit (%)	40.2±7.5	36.6±5.8	0.027
Platelet count(mm3)	218.02±64. 1	266.2±114.3	0.008
Mean Cell Volume(fL)	84.8±7.9	85.9±9.2	0.545
Mean Cell Haemoglobin(pg)	31.2±27.5	27.9±3.4	0.540
Partial thromboplastin time (second)	25.5±20.3	29.7±21	0.360
Prothrotnbin time (second)	9.6±7.4	9.8±8.7	0.910
Anemia n(%)	20(58.8)	14(41.2)	0.019
Leukocytosis n (%)	28(63.6)	16(36.4)	0.07
Coagulopathy (%)	49(73.1)	18(26.9)	49

Data are presented as mean±SD. Comparisons were made using student,s t test (for continuos variables) and, it was made using Chi-square test (for categorical variables). Statistically significant. P<0.05)

DISCUSSION

Snakes with venom are potentially dangerous animals. All efforts made to comprehend this animal have yielded a wealth of information. This does not mean that we have a complete understanding of the venom's properties. Because of the global prevalence of snakebite and its numerous symptoms, continued research into this topic is required. Karlsson et al revealed that snake toxin has an exceptionally perplexing heterogeneous structure[9]. Ninety percent of the dried venom is composed of proteins and polypeptides, which makes it possible to observe a wide range of symptoms following envenomation. In and of themselves, many enzymes are virtually non-toxic. The effects, which are visible after envenomation, will only become apparent when the two of them interact.

In south east asia two families of venomous snakes which include ELAPIDS (Cobras, kraits, coral snakes and sea snakes) ,VIPERS (typical, pit viper and saw scaled viper) In india most commonly encountered venoumous snakes are Indian cobra (Naja Naja),common krait (Bungarus caeruleus),Russells viper (Daboia russelli) and saw scaled viper (Echis carinatus)[15]. In addition, snake venom contains enzymes that aid in digestion but are not toxic. Myoglobin, rhabdomyolysis, and hemoglobin most likely play a significant role in acute renal failure[10,11]. Rhabdomyolysis has been reported after bites from some snakes. They probably also make it easier for the venom to get into the target tissue and be transported there13 . In some species, phospholipase A2 enzyme in poison responsible for rhabdomyolysis, results in myoglobinuria and acute tubular necrosis which causes acute renal failure . Rhabdomyolysis resulting renal failure is most commonly followed by bites of vipers, sea snakes and colubridae group [15]. Myoglobin, a pigment found in muscle cells, is similar to hemoglobin but does not function or structure in the same way. By blocking kidney nephrules, this pigment can cause indirect nephrotoxicity and renal tubular damage, leading to acute renal failure via acute tubular necrosis, as well as direct tubular toxicity from damaged muscle[19].

Our data also revealed that 74.8% of our patients had rhabdomyolysis (x2=31.5, df=1,p=0.001), which is a common complication in snakebite patients Our data also showed a positive association between rhabdomyolysis and creatinine phosphokinase (p 0.001), which is another enzyme that is released from damaged muscle cells[12,13] . Elevated creatinine phosphokinase is a clear sign that some muscle damage is present and is elevated in many snakebite patients. We found a statistically significant link between rhabdomyolysis and an increased risk of renal damage (x2=15.8, df=1, P 0.001), which resulted in myogloubinemia and myogloubinuria, both of which can lead to acute renal failure 14 .The possibility of converting prothrombin into thrombin is only known in a few snake species, the majority of which are vipers19. The majority of enzymes have an effect that is procoagulant in vitro, while they have an anticoagulant effect in vivo. This result is consistent with the findings reported by The majority of venom contains multiple enzymes, each of which has a synergistic or antagonistic effect on blood coagulation. A purified enzyme can simultaneously affect multiple coagulation factors. The concentration of the purified enzyme determines the degree of effect on blood coagulation. All phases of blood coagulation can be influenced by a number of enzymes. The concentration of the enzyme determines how active non purified venom is. It is demonstrated that there are individual contrasts in toxin arrangement and action between snakes of similar species. Numerous differences are based on age and location.

The coagulation of the blood is affected by the hemotoxic enzymes. Coagulopathy is the patho physiological change that is seen in the majority of snakebite patients enrolled in this study, with a frequency of 65%. The effects of venom are it impairs the synthesis of coagulating factor or denaturization of these factors are the causes of increased or inactivation of coagulating tests8 . Although the majority of patients have a normal platelet count, if they continue to bleed, this may indicate that a component of the venom toxin is preventing the platelets from performing their normal functions21. Musci et al indicated that reductions in platelets are seen in some snakebite patients, and this may be due to the presence of proteins that activate the platelets and destroy them21 . Hemolysis in RBC occurs due to direct or indirect poisoning effect on RBC membrane, and the level of hematocrit is reduced. Platelet dysfunction is a common concomitant finding in snakebite patients with coagulopathy. Phospholipid enzyme A2, which is found in every snake's venom and a specific factor in some snakes, is what causes hemolysis. Phospholipids A2 have a direct impact on the cell membrane or the production of plasma lysolocithine24 . Three-quarters (33%) of these patients had anemia. The effect of venom on the liver causes an increase in or inactivation of the coagulating test, which then results in impaired synthesis of the coagulating factor or denaturation of these factors. Most of the coagulopathy changes are associated with Russells viper followed by saw scaled viper .

An increase in PT and PTT was observed in this study (P=0.027), and it may be due to impairment of coagulating factors25-29 . Before beginning antibiotic treatment for a snake bite, suitable microbiological samples should be obtained for culture. There are some specific circumstances in which antibiotic therapy should be considered. In our study, bactriuria occurred 29.1% of the time.

According to the findings of this paper, venomous snakes are potentially dangerous animals and humans are highly susceptible to their venom as a result of a freak accident caused by Mother Nature. The most frequent pathophysiological changes that are observed in the majority of snakebite patients who are admitted in government general hospital are rhabdomyolysis and coagulopathy.

According to our findings, rhabdomyolysis and coagulopathy probably play a significant role in determining the likelihood of renal damage in snakebite victims.

Conclusion

The most frequent patho physiological alterations observed in the majority of snakebite cases in this hospital-based investigation are rhabdomyolysis and coagulopathy.

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