Original Article

EVALUATION OF HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN SNAKEBITE PATIENTS REFERRED TO RAZI HOSPITAL, AHWAZ, IRAN

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ABSTRACT

Objective: To investigate whether hematological and biochemical parameters are associated with increased risk of renal damage in Iranian snakebite patients.

Methodology: In this study 103 snkebite patients (72 males, 27 females) were evaluated. Demographic data, biochemical and hematological tests including Serum creatinine, Creatine Kinase(CK), blood urea nitrogen (BUN), complete blood cell (CBC), prothrombin time(PT), partial thromboplastin time (PTT), urinalysis and rhabdomyolysis were investigated. Hemoglubinuria, myoglubinuria and hematological parameters of the patients in relation to the risk of the renal damage were studied. We also studied the effects of venom on the red blood cell (RBC) and Muscle cells.

Results: Among these patients 50.5% were found to have hemoglubinuria, 68% had red blood cell uria, 40.9% had proteinuria, 29.1% bactriuria, 33% had anemia, 74.8% rhabdomyolysis, 45.6% had myoglubinuria, 12.7% had leukocytosis, 1.9% thrombocytopenia and 65% had coagulopathy.

Conclusion: Rhabdomyolysis and coagulopathy were the most common pathophysiological changes seen in most snakebite patients in this hospital based study.

KEY WORDS: Hemolysis, Snakebite, Rhabdomyolysis, Renal damage.

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INTRODUCTION

Most venomous snakes are found as Asia as compared to other areas of the world.[1] Various species are found in Iran and 21 of them have been recognized as poisonous and semi-poisonous. In a recent report on epidemics published by the World Health Organization (1995), it is estimated that up to five million snakebites, scorpion stings and anaphylactic reactions to hymenoptera stings (bees, wasps and ants) occur worldwide, each year. These bites and stings probably cause more than 100.000 human deaths in the world each year.[2] Most snakebites occur on the feet and ankles of agricultural workers and hunters in rural areas of tropical countries of Asia, Africa and Latin America. For these people,

envenomation is an occupational hazard.[3,4] The highest incidence of snakebites occurs in Asian countries, with an estimated 30.000 annual deaths. For Africa and Latin America about 1.000 deaths may occur in each area annually. Some data recently presented, give an idea of the magnitude of the problem in some developing countries.[3,5]

Snake venom has a very complex heterogeneous composition, containing enzymes, lethal peptides, nonenzymatic proteins, metals, carbohydrates, lipids, biogenic amines, free amino acids and direct hemolytic factors. This composition makes it possible to see many different symptoms after envenomation.[1,6]

Toxic enzymes are e.g. the presynaptic neurotoxins is composed of several subunits, one of which is a phospholipase A2. Furthermore, the hemotoxins are important, especially proteinases, peptidases and phospholipases.[6-8]

Snake venoms contain at least 26 different enzymes although no single venom has all of these. However, at least 10 enzymes are found in almost all venoms. There are also enzymes in snake venom that are not toxic but support the digestion. They probably also facilitate the entering of the venom and the transport to the target tissue.[9]

Snake venoms contain a variety of trypsin-like substances, which break down or digest tissue proteins. These are referred to as the proteolytic enzymes or as peptide hydrolases, proteases, endopeptidases, peptidases and proteinases.

The hemotoxic enzymes are classified, based on their specific effects on the blood coagulation proteins.[10-15] Most enzymes have in vitro a procoagulant effect, while in vivo there is an anticoagulant effect. Most venoms contain more than one enzyme, which effect on the blood coagulation is synergistic or antagonistic.[16] A purified enzyme can influence several coagulation factors simultaneously. The level of influence on the blood coagulation depends on the concentration of the purified enzyme. Several enzymes are able to influence all stages of the blood coagulation. The activity of nonpurified venom depends on the enzyme concentration. It is proven that there are individual differences in venom composition and activity between snakes of the same species. Many differences are based on age and geographic location.[17,18] The objective of this study was to investigate whether hematological and biochemical parameters are associated with increased risk of renal damage in Iranian snakebite patients.

METHODOLOGY

In this study 103 cases were enrolled from Razi Hospital, Ahwaz University of Medical Sciences, Ahwaz, Iran. Informed consent was obtained from the patients. 5ml of 1% EDTA anticoagulated blood sample and 5 ml of blood without anticoagulant were obtained from each patient and centrifuged within two hours.

The investigations done included complete blood count(CBC), electrolytes, Blood Urea Nitrogen(BUN), serum Creatinine, Creatine Phospho Kinase (CPK), Prothrombin Time (PT), partial thromboplastin time (PTT) and urinalysis.

Reagent strip test for protein was semiquantitative screening procedure. The reagent strip and chemical methods were used for hemoglubinuria and myoglubinuria. A positive test indicated the presence of red blood cells (RBC) in the urine (hematuria), free hemoglobin/ or myoglobinuria. Finally, free hemoglobin/ or myoglobinuria were separated by column chromatography. Red cells and other blood cells were counted in cell counter system (Sysmex) based on light scattering. Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH) and Mean Cell Haemoglobin Concentration (MCHC) were measured by automated system (Sysmex Cell Counter). Partial thromboplastin time (PTT) measures the clotting time of plasma after the activation of contact factors but without added tissue thromboplastin and so indicates the overall efficiency of the intrinsic pathway. Equal volumes of the phospholipids reagent and the kaolin suspension were mixed and kept in a glass tube in the waterbath at 37^{o}C. Then ‘0.1ml of plasma was placed into a new glass

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)	[a]26.8±11.5	[a]28.8±14	0.614
Sex(Male/Female)	11/2	65/25	0.506
Fasting Blood Sugar(mg/dl)	108±24.1	113.9±43.3	0.63
Blood Urea Nitrogen(mg/dl)	14.2±4.7	14.4-14.3	0.95
Creatinine(mg/dl)	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 Cell(mm^3)	10.7±5	119.6±1031	0.319
Red Blood Cell(mm^3)	4.7±0.8	4.7±0.7	0.936
Haemoglobin(mg%)	12.99±2.3	12.99±2.0	0.975
Platelet(mm3)	209.3±59.3	251.2±179.2	0.1
partial thromboplastin time (second)	14.07±18.97	28.4±20.1	0.017
prothrombin time (second)	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

tube and 0.2ml of the kaolin phspholipid solution was added and mixed. The tube was kept at 37^0C for 10 minutes and o.1ml of prewarmed Ca Cl_2 and a second stopwatch was started. The time taken was recorded for the mixture to clot (Ortho, USA Kits).

The PT test measures the clotting time of plasma in the presence of an optical concentration of tissue extract (thromboplastin) and indicates the overall efficiency of the extrinsic clotting system. 0.1ml of plasma was put into a glass tube and placed in a water bath and added 0.1 ml of thromboplastin. Then the mixture was allowed to become warm for one to three minutes. Then 0.1 ml of prewarmed Ca Cl_2 was added. Contents of the tube were mixed and the end point recorded (Ortho, USA Kits). A microscopic urinalysis was performed to confirm the presence of intact erythrocytes.

Healthy individuals usually do not have detectable blood, glucose, bacteria, myoglobin and hemoglobin in their urine specimen. Fasting Blood sugar, Blood Urea Nitrogen, Creatinine Na, K and phospho creatine kinase were measured by standard methods (Pars AZmon Kits, Cobas Autoanalyser).

Statistical Analysis: The data are expressed as means ± SD. All statistical analyses were performed using the Statistical Package for Social Science program (SPSS for Windows, version 15). The statistical difference among the groups was assessed by the ÷2-test. Other variables were compared using unpaired t test for normally distributed variables and ANOVA followed by Scheffe's test was used to compare the group means. P values less than 0.05 were considered significant.

RESULTS

One hundred three snakebite patients (76 males and 27 females) were studied. Most victims were males between 14 and 84 years of age. Among these patients 50.5% were found to be hemoglobinuria, 68% Red blood Cell uria, 40.9% had proteinuria, 29.1% bactriuria, 33% anemia, 74.8% rhabdomyolysis, 45.6% myoglobinuria, 12.7% leukocytosis, 1.9% thrombocytopenia and 65% had coagulopathy. Our data indicated that snakebite in young population is more than elderly and most victims are male. The patients were classified into two groups in accordance with risk of the renal damage

Table-II: 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 Nitrogen(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 renal 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
Haemoglobinuria n(%)	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

(Table-I). There were statistically significant differences between male and female groups in RBC, Haemoglobin, Haemathocrit, Platelet, RBCuria, Anemia and myoglobinuria (P<0.05) (Table-II, III).

DISCUSSION

Venomous snakes are potentially dangerous animals. All efforts made in understanding this animal, have given a lot of information. This however, does not mean that we now fully understand the characteristicss of the venom. Because of the wide spread incidence of snakebite around the world and its many symptoms, continuing attention to this subject is needed. Karlsson et al reported that snake venom has a very complex heterogeneous composition. This composition makes it possible to see many different symptoms after envenomation.[9] Ninety percent of the dried venom consists of proteins and polypeptides. Many enzymes themselves are hardly or non-toxic at all. Only when they interact will they show the effects, which are seen after envenomation.

There are also enzymes in snake venom that are not toxic but support the digestion. They probably also facilitate the entering of the venom and the transport to the target tissue.[13] Myoglobin, rhabdomyolysis and haemoglobin most likely have a significant role in the acute renal failure.[10,11] Rhabdomyolysis has been reported after bites by some of snakes.Chang et al showed that Mulga snakes (*Pseudechis cf. australis*) and the small-eyed snake (*Micropechis ikaheka*) have specific myotoxins that cause rhabdomyolysis. In other species, phospholipase A2 toxins have been long associated with rhabdomyolysis, leading to varying degrees of myoglobinuria, tubular necrosis of the kidneys and acute renal failure. Rhabdomyolysis has been reported after bites by Papuan taipans (*Oxyuranus scutellatus canni*), and should be expected after bites by Papuan blacksnakes (*Pseudechis papuanus*). Muscle cells contain a pigment called myoglobin that is similar to haemoglobin, although not the same functionally or structurally. This pigment is released from damaged muscle in large quantities and can cause indirect nephrotoxicity and renal tubular damage by blocking kidney nephrules, leading to acute renal failure via acute tubular necrosis, as well having direct tubular toxicity. Acute renal failure is a common complication in snakebite patients.[19] Our data has also

Table-III: Clinical and hematological characteristics of the snakebite patients based on gender

Variable	*Sex*		*P value*
	Male	*Female*	
n	76	27	-
White Blood Cell count(mm^3)	10.7±6.3	11.02±4.3	0.805
Red Blood Cell count(mm^3)	4.8±0.69	4.2±0.74	0.001
Haemoglobin(gr/%)	13.5±2.02	11.6±1.7	<0.001
Haematocrit (%)	40.2±7.5	36.6±5.8	0.027
Platelet count(mm^3)	218.02±64.1	266.2±114.3	0.008
MeanCell 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
prothrombin time (second)	9.6±7.4	9.8±8.7	0.910
Anemia n(%)	20(58.8)	14(41.2)	0.019
Leukocytosis(%)	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 continues variables) and, it was made using Chi-square test (for categorical variables). Statistically significant;*P<0.05

shown that 74.8% of our patients had rhabdomyolysis (x2=31.5, df=1, p=< 0.001). Elevated creatinine phosphokinase (another enzyme liberated from inside damaged muscle cells) is elevated in many snakebite patients and is a clear indication that some muscle damage is present.[12,13] Our data also showed a positive association between rhabdomyolysis and creatinine phosphokinase (p <0.001). Rhabdomyolysis caused myoglubinaemia and myoglubinuria, resulting in kidney damage that leads to a degree of acute renal failure.[14] We indicated a statistically significant difference between rhabdomyolysis and increased risk of renal damage(x2=15.8, df=1, P< 0.001). This result corresponds with reports from Reid.[12] and maria.[20] The possibility to change prothrombin into thrombin is only known in a few snake species, most of them Australian elapids, two Afro-Asian Uiperidae and Echis carinatus, Echis Coloratus and two African Colubridae.[19] Most enzymes have in vitro a procoagulant effect, while in vivo there is an anticoagulant effect. Most venom contains more than one enzyme, their effect on the blood coagulation is synergistic or antagonistic. A purified enzyme can influence several coagulation factors simultaneously. The level of influence on the blood coagulation depends on the concentration of the purified enzyme. Several enzymes are able to influence all stages of the blood coagulation. The activity of nonpurified venom depends on the enzyme concentration. It is proven that there are individual differences in venom composition and activity between snakes of the same species. Many differences are based on age and geographic location.[21-23]

Our study has indicated that Echis Carinatus, Vipera Lebetine, Carastes Cerastes in Ahwaz, Iran change coagulation tests. The hematoxic enzymes have effects on the blood coagulation. Increased or inactivation of coagulating tests are due to the effect of venom on live and impaired the synthesis of coagulating factor or denaturization of these factors.[8] Coagulopathy is the pathophysiological changes seen in most snakebite patients enrolled in this study with frequency of 65%. Most patients have a normal platelet count, but if they continue to bleed, this may suggest that some component of the venom toxin is suppressing the normal function of the platelets. Platelet dysfunction is a common concomitant finding in snakebite patients with coagulopathy.[21] Musci et al indicated and reduction of platelets are seen in some of them and it may be due to the presence of proteins that active the platelets and

destroyed them.[21] Hemolysis in RBC occure due to direct or indirect poisonous effect on RBC membrane and level of hematocrit is reduced. Only in one case of increased hematocrit was found in this study and it may be laboratory fault or due to hemolysis and obligatory blood formation. Hemolysis occur due to the action of phospholipids enzyme A2, which is present in all snakes venome and specific factor present in some snakes. Phospholipids A2 directly effect on the cell membrane or producing plasma lysolocithine.[24] Among these patients 33% were found to be anemia. Increase or inactivation of coagulating test are due to the effect of venom on liver and finally impaired the synthesis of coagulating factor or denaturization of these factors. Increase in PT and PTT were observed in this study (P=0.027) and it may be due to impairment in coagulating factors.[25-29] Amozegari et al have also indicated that Vipera Leptina venum (Ahwaz, Iran) inactives coagulation factors.[30] The typical snake mouth is not different than a human. So, they tend to induce microbial contamination into bites. There are some special cicumstances in which antibiotic therapy should be considered in snake bite.[3] Therefore, suitable microbiological samples should be obtained for culture before antibiotic therapy. Frequency of the bactriuria in our study was 29.1%.

A number of statements can be made and conclusions drawn from the findings of this paper that venomous snakes are potentially dangerous animals and because of a freak mishap of Mother Nature, man has a high susceptibility to their venom. The rhabdomyolysis and coagulopathy are the most common pathophysiological changes seen in most snakebite patients referred to hospitals in Ahwaz, Iran. Our results suggest that rhabdomyolysis and coagulopathy most likely have an important role in determining the risk of renal damage in snakebite patients.

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