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Research Article

Blood Hemoglobin, Lactate Dehydrogenase And Total Creatine Kinase Combinely As Markers Of Hemolysis And Rhabdomyolysis Associated With Snake Bite

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ABSTRACT
Snakebite is a serious and important problem in tropical and subtropical countries including india. The most common symptoms of snakebites are overwhelming fear, panic, emotional instability, nausea , vomiting, diarrhea, vertigo, fainting, tachycardia, cold, clammy skin etc. Death may occurs if sudden treatment never get. Most commonly the toxins from snake venom affect blood cells, muscles and renal tissue thus causes hemolysis, rhabdomyolysis and renal failure respectively. In this topic we had estimated blood hemoglobin, serum creatinine, serum AST , serum ALT, serum LDH and serum creatine kinase activity. Blood hemoglobin found significantly lowered along with increase in LDH as a result of hemolysis while serum creatinine level found elevated due to acute renal failure. Creatine kinase, AST, ALT and lactate dehydrogenase activity in serum were found significantly higher in patients with snake bite as a result of rhabdomyolysis. However, AST and ALT are present in all body cells and hence are non-specific. Thus, increase in serum creatine kinase activity can bestly utilized to access extent of rhabdomyolysis. On other hand , increase in serum lactate dehydrogenase activity can bestly utilized to access extent of rhabdomyolysis as well as hemolysis. Thus, serum LDH and serum creatine kinase activity can be combinely utilized along with blood hemoglobin level in the diagnosis and prognosis of snake bitten cases and to access extent of hemolysis, renal failure and rhabdomyolysis.

Keywords: Snake venom, creatinine, creatine kinase , hemoglobin, lactate dehydrogenase.

INTRODUCTION

Snakebite is a serious and important problem in tropical and subtropical countries including India. It has been estimated that 50,00,000 snakebite cases occur every year, resulting in about 1,00,000 deaths annually worldwide. On average, nearly 20,00,000 persons fall prey to snakebite per year in India, resulting in 35,000 to 50,000 deaths. In developing countries, snakebite is an occupational hazard for rice field workers, rubber plantation workers, herders and hunters; whereas in industrialized countries, snakes are increasingly popular as pets and most bites are inflicted when snakes are mishandled or attacked.(1) In India, there are 216 species of snakes but the most important species are Cobras (Naja naja, N. oxiana, N. kaouthia), Common Krait (Bungarus Caeruleus), Russell's Viper (Daboia russelii) and E. Carintus.(2)

Snakes are elongated, legless, carnivorous reptiles of the suborder serpents that can be distinguished from legless lizards by their lack of eyelids and external ears. Like all squamates, snakes are ecto-thermic, amniote vertebrates covered in overlapping scales. Many species of snake have skulls with many more joints than their lizard ancestors, enabling them to swallow prey much larger than their heads with their highly mobile jaws. To accommodate their narrow bodies, in snakes paired organs (such as kidneys) appear one in front of the other instead of side by side and most have only one functional lung. Some species retain a pelvic girdle with a pair of vestigial claws on either side of the cloaca.(3)

There are different types of snakes. Out of them, some produce venom and are poisonous while those not producing venom are non-poisonous.(4,5) Snake venom is one kind of special liquid produced by the poison gland of the poisonous snake. The toxicity, pharmacological and toxicological roles of different snake venom are different. It is a complex mixture containing peptides, polypeptides, enzymes, glycoproteins and other substances, capable of several pharmacological activities. In addition, snake venoms contain inorganic substances including metals like sodium, calcium, magnesium, zinc and small amounts of Iron. Some snake venom, contains glycoprotein, lipids and biogenic amines while other venom contain free amino acids.(6,7,8,9)

The venom has neurotoxic, myotoxic, nephrotoxic and hematotoxic activities. In neuromuscular junctions, the venom leads to a powerful presynaptic inhibition of acetylcholine release, which is responsible for the neuromuscular blockade and progressive flaccid paralysis of variable degrees. Eyelid ptosis, blurred and/or double vision, ophthalmoplegia and facial muscle paralysis are common manifestations of venom neurotoxicity. The myotoxic activity of the venom produces severe skeletal and cardiac muscle injury leading to myalgia and rhabdomyolysis with the subsequent release of myoglobin

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Table:-1:: Concentration of blood hemoglobin and serum creatinine in controls and snake bitten patients (Mean ± SD)

Concentration (Mean ± SD)	Blood Hemoglobin (Gm/dl)	Serum Creatinine (mg/dl)
Controls (n = 30)	13.95 ± 1.92	0.85 ± 0.18
Snake bitten patients (n = 30)	7.32 ± 1.38	2.18 ± 0.67
'p' value when compared with control	$p < 0.01$	$p < 0.01$

from damaged skeletal muscle into serum and urine. Rhabdomyolysis is associated with release of myoglobin from muscle to the blood leads to myoglobinemia. This further leads to myoglobinuria. This can result in damage to the kidneys (nephrotoxicity) as a result of myoglobin accumulation in the renal tubules. This is coupled with hypotension and may be lead to acute renal failure. Acute renal failure is the main cause of death among patients surviving to the early effects of snakebites.[10,11] Hematotoxins produces hemolysis. Hemolysis occurs due to direct or indirect poisonous effect on RBC membrane and level of hematocrit is reduced. Hemolysis occur due to the action of an enzyme phospholipids A_2, which is present in all snakes venom. Phospholipids A_2 directly effect on the cell membrane and degrade lecithin to lysolocithine.[12,13,14] Tissue damage at the site of the bite has been reported to be minimal or absent. Spontaneous bleeding has only been rarely observed in human patients, despite the presence of blood incoagulability in some cases.

There is no detailed study on rhabdomyolysis that occurs in snake bite. Further, investigation of snake bite is done according to symptoms, marks at injured site and answers by patient after enquiry. Many times patient is unconcious and hence severity of snake poisoning must be know for prompt treatment. Thus, the purpose of this study was to detect and correlate extent of hemolysis, rhabdomyolysis and acute renal failure occurring in snake bite cases.

MATERIALS AND METHOD

Subjects: The study was carried out at Department of Biochemistry, V. G. Shivdare College of Biotechnology, Solapur during the period December 2010 to February 2012. We have included total 30 patients with snake bite in our study. 30 healthy subjects were selected as controls.

Collection of samples: Blood samples of snake bitten patients were collected from ICU of various multi-speciality hospitals and Shri. Chhatrapati Shivaji Maharaj General Hospital, Solapur at the time of admission and before treatment. 30 samples of controls were collected from Damani Blood Bank, Solapur. Patients with other types of bite such as dog bite and scorpion bite were excluded from the study group. Individuals with any disease/s were excluded from the control group. Samples were collected in two forms as follows.

2 ml of whole blood was collected in heparin bulb which was directly used for estimation of hemoglobin.

Total 5 ml of venous blood were collected in a plain bulb. The obtained blood were centrifuged at 3000 rpm for 10 minutes to separate serum and cells. The serum was used to estimate concentration of creatinine and activity of creatine kinase.

By using above samples we estimated, blood hemoglobin, serum creatinine and serum activities of enzymes that includes aspartate transaminase (AST) , alanine transaminase (ALT), lactate dehydrogenase (LDH) and total creatine kinase (CK).

Hemoglobin estimation: The hemoglobin was estimated by Modified Drabkins method. The colour formed for test was directly read against blank without using standard. In test tube, 5 ml of Drabkins reagent was taken and to this 20 µl of whole blood was added. In blank tube, 5 ml of Drabkins reagent was taken and to this 20 µl of distilled water was added. The content in the tube were mixed and kept at room temperature for 10 minutes. The colour formed was read against blank at 540 nm. Hemoglobin concentration in Gm/dl is determined by multiplying absorbance of test tube with factor 36.62. [15]

Creatinine estimation: The creatinine was estimated by Jaffes method. First protein free filtrate was prepared. For the purpose of which, 1 ml of serum, 7 ml of distilled water, 1 ml of 10 % sodium tungstate and 1 ml of 2/3 N sulphuric acid were mixed. Centrifugation was carried out and supernatent was used. To 5 ml of filtrate obtained above, 2.5 ml alkaline picric acid was added and kept at room temperature for 10 minutes. The colour formed was compared with the standard tube at 540 nm. [16]

Creatine kinase activity: The creatine kinase activity was assayed by Nelsons method. 1.0 ml of reconstituted reagent was pipette out in appropriate tube and pre-warmed for 5 minute at 37^0C. Semi-autoanalyzer was set to zero at 340 nm with distilled water. 25 µl of serum was transferred to reagent, mixed and incubated at 37^0C for two minutes. After two minutes, read and record the absorbance. Tube returned to 37^0C. Readings were repeated every minute for the next two minutes. The average absorbance difference per minute (ΔAbs/min) were calculated. The ΔAbs/min were multiplied by the factor 6592 to get creatine kinase activity in U/L. [17]

Lactate Dehydrogenase (LDH): 100 µl of serum was added to 1.0 ml buffered substrate. Then 100 µl NADH was added. After incubation of 15 minutes 1.0 ml DNPH added. After 20 minutes 10 ml 0.4 N NaOH was added. In control tube 1.0 ml each of buffered substrate and DNPH, 200 µl of buffer was taken and after 20 minutes 10 ml 0.4 N NaOH was added. The liberated pyruvate was read at 540 nm.[18]

SGOT and SGPT: Reitmann and Frankel method was used. 100 µl serum was added to 500 µl of buffered substrate and incubated for one hrs (half hrs for SGPT). After one hrs (half hrs for SGPT) 500 µl of DNPH was added. After 10 minutes 5.0 ml of 0.4 N NaOH was

Table:-2:: Activities of enzymes in controls and snake bitten patients (Mean ± SD)

Serum enzyme activity (Mean ± SD)	Creatine Kinase (U/L)	Lactate Dehydrogenase (IU/L)	Aspartate Transaminase (IU/L)	Alanine Transaminase (IU/L)
Controls (n = 30)	66.15 ± 12.73	160 ± 12.1	24 ± 5.69	26.47 ± 2.29
Snake bitten patients (n = 30)	162.51 ± 50.23	225.8 ± 31.33	91.23 ± 13.7	77.28 ± 7.53
'p' value when compared with control	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$

added. Colour formed in 20 minutes and read at 540 nm.[19]

RESULT AND DISCUSSION

In this topic we estimated, blood hemoglobin, serum creatinine and serum activities of enzymes aspartate transaminase (AST) , alanine transaminase (ALT), lactate dehydrogenase (LDH) and total creatine kinase (CK) in patients of snake bite. According to our estimation, hemoglobin concentration in controls was 13.95 ± 1.92 Gm/dl. Decrease in hemoglobin concentration in snake bite patients was found to a concentration of 7.32 ± 1.38 Gm/dl. **(Table-I)** It was nearly half of the normals. The decrease in blood hemoglobin is observed due to hemolysis which causes dilution of the blood. Serum activities of enzymes aspartate transaminase (AST) , alanine transaminase (ALT), lactate dehydrogenase (LDH) and total creatine kinase (CK) was found to be specifically elevated in patients of snake bite. The increase in serum activities of above said enzymes is due to secretion of the enzyme in the serum as a result of muscular damage i.e. rhabdomyolysis produced by toxins from venom. Aspartate transaminase and creatine kinase found elevated due to cardiac as well as skeletal muscle damage. Alanine transaminase found elevated due to skeletal muscle damage. While lactate dehydrogenase increases due to hemolysis and cardiac as well as skeletal muscle damage.

Our estimation tolds increase in serum creatine kinase activity in snake bitten patients which found us as 162.51 ± 50.23 U/L. We found serum creatine kinase activity in controls as 66.15 ± 12.73 U/L which was nearly 2.5 times lower to that of snake bitten patients.(Table-II) Serum lactate dehydrogenase activity in snake bitten patients was found us as 225.8 ± 31.33 IU/L. We found serum lactate dehydrogenase activity in controls as 160 ± 12.1 IU/L which was nearly 1.4 times lower to that of snake bitten patients.(Table-II) Serum aspartate transaminase (AST) activity in snake bitten patients was found us as 91.23 ± 13.7 IU/L and in controls it was 24 ± 5.69 IU/L which was nearly 3.8 times lower to that of snake bitten patients. (Table-II) Serum alanine transaminase (ALT) activity in snake bitten patients was found us as 77.28 ± 7.53 IU/L and in controls it 26.47 ± 2.29 IU/L which was nearly 2.9 times lower to that of snake bitten patients.(Table-II) Increase in serum creatinine found due to damage to kidney by toxins from venom that causes acute renal failure and thus decreased excretion of creatinine from urine. Creatinine concentration in controls was 0.85 ± 0.18 mg/dl. The creatinine concentration was found to be increased to 2.18 ± 0.67 mg/dl in snake bitten patients. It was nearly 2.6 times more than the normal victims. (Table-I)

CONCLUSION

From our study, we found decrease in blood hemoglobin, increase in serum creatinine level and increase in serum activities of creatine kinase, AST, ALT and LDH which are proportionally associated with severity of snake bite. Decrease in blood hemoglobin level and increased serum LDH can be used to access severity of anemic conditions generated in snake bitten patients due to hemolysis. Increased serum LDH tells extent of hemolysis and hence activity of hemotoxins. On other hand, increase in serum creatinine level is used to access acute renal failure. However, in general acute renal failure in snake bitten patients occurs due to damage to the kidneys as a result of myoglobin accumulation in the renal tubules. Myoglobin is released in serum from muscle due to rhabdomyolysis. Thus, acute renal failure is proportionally associated with rhabdomyolysis. Due to rhabdomyolysis creatine kinase, AST, ALT and LDH from muscles enters in serum and hence their serum activities increases. However, AST and ALT are present in all body cells and hence are non-specific. Thus, increase in serum creatine kinase activity can bestly utilized to access extent of rhabdomyolysis. On other hand , increase in serum lactate dehydrogenase activity can bestly utilized to access extent of rhabdomyolysis as well as hemolysis. Thus, serum LDH and serum creatine kinase activity can be combinely utilized along with blood hemoglobin level in the diagnosis and prognosis of snake bitten cases and to access extent of hemolysis, renal failure and rhabdomyolysis.

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