Case discussion:
A 28 year old farmer presented to the Emergency Department of a hospital with history of snakebite the previous day while watering his fields. Following the bite, he had developed local swelling in the left foot and continuous bleeding from the bite site. He also developed drooping of his eyelids and brown urine. He was taken to a local hospital and was managed with 26 vials of ASV and supportive care and was then referred following development of reduced urine output, worsening dyspnoea and ptosis.

On arrival, he was conscious and oriented, with a regular pulse rate of 96/min, blood pressure of 130/80 mm Hg and respiratory rate of 24 per minute. Left foot examination revealed cellulitis. His cardiovascular examination was normal, with no elevation of jugular venous pressure (JVP) or other signs of heart failure. However examination of the respiratory system revealed bilateral basal crepitations suggestive of pulmonary oedema. Examination of central nervous system was unremarkable except for bilateral ptosis and abdominal examination revealed no abnormalities.

What is the clinical syndrome and what is the snake most likely implicated?

SNAKE BITES CAUSING RENAL INJURY
This young man presented with a syndrome of local snake envenomation with bleeding manifestation, mild neurological involvement and an acute kidney injury. The most probable snake involved is a Russel’s Viper which produces venom which is primarily hemotoxic, can cause neurotoxic symptoms and is most notorious for causing Acute Kidney Injury (AKI).

A wide variety of snakes are associated with acute kidney Injury following a bite (Table 1). In India, the Russel’s Viper and the Saw scaled Viper are the commonly implicated snakes. The myotoxic sea snakes also commonly cause AKI in the coastal areas of Kerala. The incidence of AKI following viper bite in India as reported from various studies ranges from 13-32%. According to CMC data from Jan – Oct 2014 from the ongoing national Snake bite study, 36 out of 75 recruited patients developed rhabdomyolysis, an important pathogenic mechanism for AKI. Twenty six developed AKI and 14 required Dialysis. There was a 100% association of AKI with haematotoxicity, with 16 of the 26 patients having features of classical Russel’s Viper envenomation and the remaining 10 developing classical Saw scaled viper envenomation syndrome (unpublished).

Pathogenesis of AKI after snake bite
Why did the patient develop AKI following Russel’s Viper Bite?
A number of factors could have contributed to nephrotoxicity leading to AKI viz. bleeding, transient hypotension, intravascular haemolysis, pigment induced tubular injury, disseminated intravascular coagulation, microangiopathic haemolytic anaemia, haematuria and direct nephrotoxicity of the venom

The pathogenesis of renal injury in snakebite is complex involving both the direct action of venom on the kidney and the inflammatory effects due to the release of various endogenous cytokines and vasoactive mediators leading to hemodynamic changes and triggering of immune response. Snake venom poisoning shares the same inflammatory process as infection or sepsis with the roles of cytokines, mediators, complement activation, reactive oxygen species and immunologic reaction (Figure 1). (Pathogenesis contd. on next page)
Some of the features in the pathogenesis are:

- **Hemodynamic alterations**
  In various animal and human studies, Russell’s viper envenomation has been found to decrease the cardiac output and systemic and renal vascular resistance, leading to hypotension, decreased renal blood flow and Glomerular Filtration Rate (GFR). All these cytokine mediated hemodynamic alterations lead to renal ischemia and pre-renal failure. It is also observed that the duration of hypotension was directly related to the development of AKI.

- **Direct nephrotoxicity**
  Direct nephrotoxicity of venom explains renal failure after snake bites, without hypotension, haemorrhage, intravascular haemolysis and rhabdomyolysis.
  - Phospholipase A2 can cause membrane injury and tubular necrosis.
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- Metalloprotease can cause proteolysis of the extracellular matrix and disrupts cell-matrix and cellular adhesion.\(^1\)

Besides the direct injurious effects of the venom, indirect injury can be caused by cytokines and inflammatory mediators induced by both metalloproteases and phospholipase A2 without haemodynamic changes.

- **Immunologic mechanism**
  Immune complex deposition and immune mediated injury similar to post streptococcal glomerulonephritis (PSGN) can cause glomerulonephritis and nephrotoxicity.\(^2\)

- **Pigment related injury**
  Intravascular hemolysis leading to hemoglobinuria and rhabdomyolysis leading to myoglobinuria can result in direct pigment related injury.\(^2\)

- **Disseminated Intravascular Coagulation**
  The presence of fibrin thrombi in renal microvasculature and in the glomerular capillaries, together with the finding of microangiopathic haemolytic anaemia and thrombocytopenia in subjects with cortical necrosis strongly suggest that DIC plays an important role in the pathogenesis of snake bite induced renal changes.\(^3\)

**Spectrum of Renal Pathology**

Snake venom can cause injury to virtually any part of the kidney – glomerulus, tubules, interstitium or the vasculature. Acute tubular necrosis and cortical necrosis are the most significant histopathological changes associated with snake bite in India.\(^4\) The pathological features are often found to correlate with disease severity and outcome, cortical necrosis being associated with a fatal outcome in 80% of cases.\(^3\) The commonly found pathological features are given in Table 2.\(^1\)

**DETECTION OF RENAL INJURY FOLLOWING SNAKE BITE**

What clinical features led to the suspicion of AKI in the patient?

The patient developed haematuria and later progressive oliguria. He also had bilateral crepitations which was suggestive of pulmonary oedema from fluid overload, a component of clinical uraemia syndrome.

The onset of renal failure may be a few hours to several hours after the bite.\(^6\) The following clinical features may manifest.\(^6\)

a) **Dwindling or no urine output:** Oliguria or anuria, if present, is a clear indicator of renal involvement. However not all patients with kidney involvement will develop anuria. Of the 26 patients with renal failure from the aforementioned national snake bite study, only 12(46%) had anuria/oliguria, whereas urine output remained normal in the remaining.

b) **Renal Angle Pain/Tenderness**

Some patients complain of pain in the renal angle preceding oliguria, which may be a useful clue to impending renal failure.\(^1,5\)

c) **Hematuria**

d) **Clinical “Uremia Syndrome”**

This syndrome is due to systemic toxicity of accumulated nitrogen containing waste products manifesting as nausea, vomiting, hiccups, fetor, drowsiness, confusion, coma, flapping tremor, muscle twitching, convulsions, pericardial friction rub and signs of fluid overload.

Viper victims often survive the initial bite only to succumb later to renal failure. The patient should therefore be closely monitored for the following

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**TABLE 2- SPECTRUM OF RENAL PATHOLOGY IN SNAKE BITE**

<table>
<thead>
<tr>
<th>Renal Pathology</th>
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<tbody>
<tr>
<td>Mesangiolyisis</td>
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<tr>
<td>Mesangial proliferative glomerulonephritis</td>
</tr>
<tr>
<td>Diffuse proliferative glomerulonephritis</td>
</tr>
<tr>
<td>Extracapillary proliferative glomerulonephritis</td>
</tr>
<tr>
<td>Vasculitis</td>
</tr>
<tr>
<td>Tubular necrosis</td>
</tr>
<tr>
<td>Acute diffuse interstitial nephritis</td>
</tr>
<tr>
<td>Cortical necrosis</td>
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</tbody>
</table>

Snakebite nephropathy (Review Article), Visith Sitpria; Nephrology 2006
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parameters to detect renal failure early, especially when the snake involved is a Viper.

- Pulse rate
- Blood pressure (in supine and sitting positions to detect postural hypotension)
- Respiratory rate
- Temperature
- Jugular venous pressure
- Auscultation of lung bases for crepitations
- Urine colour and output

INVESTIGATIONS

A complete haemogram of the patient being discussed was relevant only for leucocytosis. Coagulation profile showed deranged clotting parameters. Urea and Creatinine (106 mg% and 4.4 mg%) were above the normal limits, substantiating the clinical diagnosis of AKI. His total serum bilirubin and transaminases were also elevated, so was CPK (8507 IU/L). Electrolyte analysis revealed potassium of 5.5 mEq/L. Urine analysis revealed haematuria and proteinuria. Chest X-Ray showing bilateral inferior zone infiltrates was confirmatory of pulmonary oedema. ABG showed severe metabolic acidosis with (pH of 7.19 and bicarbonate of 11). ECG showed sinus tachycardia with no evidence of hyperkalaemia. Haemoglobin, Platelet count, WBC count, Serum electrolytes, Creatinine, Urea and calcium were monitored at frequent intervals. His haemoglobin levels showed a steady decline and there was a progressive rise of serum Creatinine and Urea.

Laboratory investigations have an important role in detecting AKI, assessing severity, predicting the prognosis and guiding the management.

- Complete Hemogram
  There may be anemia due to haemolysis (both toxin induced and microangiopathic), and local bleeding. Platelet count may be decreased in viper bite due to microangiopathy mediated mechanisms. Early neutrophilic leukocytosis is seen in systemic envenoming from any species.

Fragmented RBCs (‘helmet cell’, schistocytes) in blood smear are seen in microangiopathic haemolysis.

- Coagulation profile
  20 minute whole blood clotting test, if deranged is an important predictor of viper envenomation and also has been found to be a risk factor for development of AKI. The serum may appear pink or brownish if there is gross haemoglobinaemia or myoglobininaemia. A PT/aPTT should be done when available.

- Electrolytes
  Early hyperkalemia may be seen following extensive rhabdomyolysis. Bicarbonate will be low in metabolic acidosis.

- Arterial Blood Gas Analysis
  Impaired acid secretion and bicarbonate metabolism leads to metabolic acidosis, which may need prompt correction.

- Electrocardiogram
  In the absence of electrolyte analysis, ECG can provide evidence of hyperkalemia.

- Liver Function Tests
  Haemolysis results in unconjugated hyperbilirubinemia. Aminotransferases and creatine kinase are elevated if there is severe local damage or, particularly generalized muscle damage.

- Renal Function Tests
  Creatinine, urea or blood urea nitrogen levels are raised in the renal failure.

- Urine analysis
  Dipsticks for blood/haemoglobin/myoglobin are available. Microscopy reveals microscopic hematuria. Red cell casts indicate glomerular bleeding. Massive proteinuria is an early sign of the generalized increase in capillary permeability in Russell’s viper envenoming.

- Calcium and Phosphorus
  Low GFR can lead to hyperphosphataemia and hypocalcaemia may be present especially with extensive muscle damage and with bicarbonate therapy.

  Serum potassium, urea,
creatinine and, if possible, pH, bicarbonate, calcium and phosphate should be monitored frequently\(^5\).

### Predictors of renal failure

In a study from Maharashtra the following were found to independent risk factors for development of AKI following snake bites\(^7\):

- Long bite-to-hospital time
- Hypotension
- Albuminuria
- Deranged Bleeding time
- Deranged Prothrombin time
- Low Hemoglobin
- High Total bilirubin

Cellulitis, regional lymphadenopathy\(^8\), intravascular hemolysis, bite to needle time more than 2 hours \(^8\), black or brown urine \(^9\), 20 minute whole blood clotting time > 20 minutes, and longer bite to hospital time \(^9\) were also associated with higher incidence of AKI in other studies. Children were also found to be more likely to develop AKI as compared to adults\(^1\).

### MANAGEMENT

**What would be the appropriate line of management for the patient?**

The patient received\(^10\) more vials of ASV in and careful IV fluid therapy. However he developed rapid shallow breathing, and required intubation and invasive ventilation in view of impending respiratory failure. His electrolyte imbalances were promptly corrected. His creatinine kept rising requiring dialysis. He was also started on Piperacillin-Tazobactam for his left foot cellulitis.

As in other forms of AKI, 3 distinct phases may be seen in Snake bite induced AKI\(^6\)

1) **Oliguric Phase:** Most, but not all, patients with acute renal failure are oliguric, defined as a urine output of less than 400 ml/day or less than 20 ml/hour

2) **Diuretic phase of kidney injury:** This is as important and as life-threatening as the oliguric phase. Urine output increases to 5-10 litres/24 hours following the period of anuria. The patient may become polyuric and volume depleted

3) **Renal recovery phase:** The diuretic phase may last for months after Russell’s viper bite. Hypopituitarism may often complicate this phase

   The anti-venom therapy has to be initiated as early as possible as in any other case of snake bite. Other principles of management for hematotoxicity and neurotoxicity need to continue concomitantly. The patient may require care in ICU.

**Management of Oliguric Phase\(^6\)**

1. **IV access** must be established, a urinary catheter must be inserted.

2. **A fluid challenge** is given with isotonic saline (up to 2 litres over 1 hour in an adult) Give Fluid Challenge. A jugular venous pressure (8-10 cm above sternal angle at 45’ inclination) or CVP can guide fluid therapy. The patient must be closely observed while this is being done. The fluid challenge must be stopped immediately if pulmonary oedema develops. In someone who is obviously volume-depleted, resuscitation should start immediately.

3. If fluid challenge does not improve a dose of **Furosemide** 100 mg slow IV (4-5 mg per minute) followed by a second dose of 200 mg IV if necessary may be tried to bring the urine output to >40ml/hour.

4. If the above interventions do not increase the urine output, the patient is started on **conservative management**.

   - No further diuretics should be given and fluid intake should be restricted to a total of the previous day’s output plus “insensible losses” (500-1000 ml/day).
   - The diet should be bland, high on calories (1700/day), low in protein (less than 40g/day), low in potassium and low in salt
   - Infections should be prevented to prevent worsening of renal function or treated promptly with non-nephrotoxic antibiotics

5. **Hyperkalemia** should be promptly diagnosed based on serum potassium values and ECG changes, and managed by administering Insulin-Dextrose, Salbutamol Nebulization and IV Calcium Gluconate according to the severity.
6. If the patient is hypotensive and profoundly acidotic (deep sighing “Kussmaul” respirations, has very low plasma bicarbonate concentration or very low pH, then sodium bicarbonate should be given, after calculating the bicarbonate deficit. Usually 2-3 ampoules (40 ml of 8.4% sodium bicarbonate equivalent to 1 mmol/ml) in 5% dextrose water or half of the calculated deficit can be replaced in 3-4 hours. Intravenous bicarbonate may precipitate profound hypocalcaemia and seizures, especially in patients with rhabdomyolysis. Volume expansion by sodium bicarbonate can cause fluid overload. Therefore, if there is no clinical improvement dialysis is required.

In patients with Haemoglobinuria/myoglobinuria, renal damage can be prevented by prompt fluid replenishment, maintaining saline diuresis, timely potassium correction and management of acidosis. A single infusion of mannitol (200 ml of 20% solution over 20 minutes) may be tried, but is of no proven benefit.

7. **Indications for Dialysis** include
   (a) Clinical uremia
   (b) Fluid overload
   (c) Blood biochemistry - one or more of the following (not responding to medical therapy)
      - Creatinine >4 mg/dl (500 μmol/l)
      - Urea >130 mg/dl (27 mmol/l)
      - Potassium >7 mmol/l (or hyperkalemic ECG changes)
      - Symptomatic acidosis

**Management of Diuretic Phase**

The patient may become polyuric and volume depleted so that salt and water must be carefully replaced. Hypokalaemia may develop, in which case a diet rich in potassium (fruit and fruit juices) is recommended.

**Management of Renal Recovery Phase**

Fluid and electrolyte replacement may be needed in these patients. Though most patients improve without any sequelae, a small proportion of those with significant cortical necrosis may go on to require renal replacement therapy.

**Case discussion continued…..**

The patient’s respiratory muscle weakness gradually improved after a day of dialysis and invasive ventilation allowing him to be extubated the next day. He required corrections for hyperkalaemia and hypocalcaemia intermittently. He was moved out of ICU and continued to require alternate day dialysis for about 3 weeks, after which his urine output gradually improved and renal function normalised. His electrolytes and haemogram continued to remain steady and he was discharged in a stable condition.

**TAKE HOME MESSAGE**

- Acute Kidney injury is not rare in patients with snake bite
- AKI is almost exclusively associated with haemotoxic viper bite is most parts of India.
- Early identification, prevention by effective fluid and metabolic management, effective infection management and early referral for dialysis can save lives.

**REFERENCES**

Use of Anti-snake venom in India – Practical Issues

Anand Zachariah, Professor and Head, Department of Medicine, Christian Medical College, Vellore.

Introduction

Is Indian anti-snake venom an optimal antidote?

Snake bites result in about 50,000 deaths per year and anti-snake venom (ASV) is the cornerstone of management of snake-bite envenomation. Anti-snake venom was discovered in 1896 by Dr. A. Calmette. It contains immunoglobulins (enzyme refined fragments of IgG) obtained by collecting serum or plasma from horses or sheep that have been immunized with the venom of different snake species. This was the technique that Dr. Calmette used for preparation of ASV, and is still being followed today with some changes in processing and purification.

In India there are certain issues that need to be addressed with respect to anti-snake venom. The issues concern variations in efficacy, cost, allergic reactions and dosing which make it a less than optimal antidote in a case of snake bite envenomation. The aim of this review is to discuss these issues and the possible ways to address some of the problems inherent in using the ASV preparation available in India.

Variability in the efficacy of ASV

The ASV available in India is a polyvalent ASV that has efficacy against the venom of 4 snake species also known as the ‘big four’ (Indian cobra, krait, saw-scaled viper and Russel’s viper). However, there appears to be variability in the efficacy of ASV across the different snake species and even within the same species. In a study done in Vellore (CMC and Government Medical College, Vellore), ASV was found to be more effective in Saw Scaled Viper and Indian Cobra bites (unpublished data). ASV was relatively less effective in Russel’s Viper bite (increased death rate, transfusion and dialysis) and Common Krait bite (increased death rate and mechanical ventilation).

In our experience we also found a batch to batch variability in the efficacy of ASV. There was an increase in the death rate, in our experience when that certain batch of ASV was used. This was resolved when a particular batch of ASV was discontinued and a new batch was used. Subsequent ELISA analysis of these batches showed that the titre of specific antibodies varies between different batches of ASV, and titres were low in the batches associated with a higher mortality (unpublished data).

Reasons for variability of efficacy of ASV

There are many reasons for the observed variability of ASV efficacy and these are related to the source, method of production and action of the antivenom.
Source: About 85% of venom extracted for antivenom production comes from one source – The Irula Co-operative Society near Mamallapuram, Tamilnadu. It is known that venom composition varies even within the same species in different parts of the country. Therefore ASV produced from one source may show geographical variability and not have the same efficacy all over the country.

2. Production of ASV

There are quality control issues related to venom extraction and production. The quality of venom varies according to the age of the snake and season. Also, if the venom is not frozen immediately, there may be inactivation of the enzymes within.

After extraction, the venom is sent to pharmaceutical companies. Here, the venom is injected into horses and blood is then collected periodically from the immunized animals. Serum is extracted from the blood, antibodies are precipitated, then purified using an enzyme digestion method and finally sterilized before the final product is made available either in a liquid or lyophilized form. Variability in any of the steps in production can result in variability in efficacy. The immune response to venom in the horse varies from individual to individual. Quality control issues in the final purification and production of ASV also influence the efficacy of the product.

3. Low title of neutralizing antibody

Studies show that the bulk of antibodies in anti-snake venom are not against snake proteins. This means that the purification procedures are unable to separate specific antibodies against venom proteins and non-specific antibodies. Only 20% of ASV has specific antibodies against venom protein. Of these only 5% antibodies have capacity to specifically neutralize the toxic effect of venom proteins (venom-neutralising antibody).

4. Complexity of venom

The venom from any species is complex and may comprises of around 25 enzymes and polypeptides which vary from snake to snake. Many of the toxic venom proteins are of low molecular weight and many not be immunogenic. Therefore ASV produced may not contain antibodies against all the individual toxic venom proteins. Venom protein variability within a species only compounds this problem. Therefore it not clear that each venom sample that is used for horse immunization has similar composition. Therefore the resultant ASV that is purified, may vary in its antibody composition.

5. Tissue bound venom proteins

Krait venom travels to the neuro-synaptic junction and binds with the pre-synaptic terminal. The ASV given does not neutralize tissue bound venom protein and is therefore relatively ineffective in Krait bite envenomation. This may true of Russell’s viper envenomation where muscle injury and kidney injury have set in even before the patient presents to the hospital and cannot be effectively neutralized by ASV. Although ASV may bind circulating venom proteins, it cannot reverse the action of tissue bound proteins and their consequences.

6. Inter species and intra species variability

The polyvalent ASV produced in India is specifically made to neutralize venom from four species (known as the ‘big four’ – Indian Cobra, Common Krait, Russel’s Viper and Saw Scaled Viper). The ASV may be ineffective against other species of snakes which are known to bite humans even if they belong to the Elapidae or Viperidae families. Antibodies against the venom of one species is sometimes known to have a cross
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reactivity against venom of a closely related species.

The need for improved antivenom

The figure given below is a timeline demonstrating the evolution of ASV over the years. Despite improvements in purification, stability and efficacy, the basic method of production of ASV however has not changed over the last 120 years (antibodies are still derived from serum of immunized horses). As a result ASV continues to be associated with a very high rate of anaphylactic reaction. This, along with geographic variations in the venom constituents and variability in efficacy due to quality control issues makes ASV a less than optimal antidote for management of snake bite envenomation in India.

Some suggestions for an improved antivenom are as follows.

1. Venom from different geographical sources: This will improve the efficacy of ASV across India as antibodies will be developed against a greater variety of venom proteins.

2. Identification of the specific neutralising antibodies for venom proteins: This may result in a more efficient ASV and with a lower rate of anaphylaxis.

3. Improvements in the production process

4. Tests to assess quality of ASV

5. Monoclonal technology to develop a cocktail antivenom

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