Snakebite nephropathy

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SUMMARY: There is a broad clinical spectrum of renal involvement in snakebite. Besides the local and systemic symptoms, clinical renal manifestations vary from mild proteinuria, haematuria, pigmenturia to acute renal failure. Bites by haemotoxic snakes and myotoxic snakes are the common causes of renal involvement especially acute renal failure. Therefore, renal failure is often associated with haemorrhagic diathesis, intravascular haemolysis and rhabdomyolysis. Renal pathological changes include mesangiolysis, glomerulonephritis, vasculitis, tubular necrosis, interstitial nephritis and cortical necrosis. Tubular necrosis is an important pathological counterpart of acute renal failure. Haemodynamic alterations induced by cytokines and vasoactive mediators leading to renal ischaemia are important in the pathogenesis of acute renal failure. Haemolysis, intravascular coagulation and rhabdomyolysis are important contributing factors. Direct nephrotoxicity can be induced by the venom through metalloproteases and phospholipase A2. Immunologic mechanism plays a minor role in the pathogenesis of the renal lesion.

KEY WORDS: glomerulonephritis, haemodynamics, metalloproteases, phospholipase A2, renal failure, snakebite.

Clinical toxicology from natural toxins is an important health problem in the tropics. With biological diversities and a favourable environment, tropical areas are rich in animals, plants, microbes and their toxins. Snake bites, animal bites and stings are common occurrences, and snake bites are the highest among animal toxin poisoning. Snake venoms can cause cellular injury through enzymes, polypeptide toxins, cytokines and mediators. Important venom enzymes consist of proteases, hydrolases, hyaluroxidase, oxidases, phospholipases and esterases. Among these enzymes, phospholipases A2 and proteases (especially metalloproteases), contribute significantly to tissue injury. Cytokines and vasoactive mediators are responsible for inflammatory changes and haemodynamic alterations that can ultimately lead to cellular injury.1–6

The clinical symptoms in snakebite vary from local pain, swelling and necrosis at the site of the bite to systemic involvement, including hypotension, haemorrhage, conjunctival oedema, chemosis, central nervous system symptoms, myalgia, paralysis, abdominal pain and renal failure.2 A highly vascularized organ, the kidney is prone to venom toxicity. Renal involvement in snakebite varies widely.7–10 Acute renal failure is frequently described and is life threatening. Haematuria and proteinuria are common. There is a broad spectrum of renal pathological changes.11–14 All renal structures are involved. A review of snakebite nephropathy is necessary due to the proliferation of clinical and scientific data on the topic.

CLINICAL RENAL MANIFESTATIONS

Proteinuria, haematuria and acute renal failure are among the common clinical renal manifestations in snakebite.

Proteinuria

Proteinuria may be observed following snakebite. Proteinuria has been noted in rats following intrarenal injection of cobra venom.15 The incidence of proteinuria in snakebite is variable depending on the kind of snakes involved and there is also geographical variation. In a series of 400 tropical snakebite patients proteinuria was observed in 4%.16 The magnitude of proteinuria is less than 500 mg/24 h, and this is usually a transient finding which completely resolves when the patient recovers. However, significant proteinuria over 1 g/24 h has been observed in 50% of Russell's viper bite patients in Myanmar, suggesting that geographical variation can have an effect on the venom composition of the snake of the same species.17 Despite heavy proteinuria the effect is temporary and completely...
resolves during the recovery phase. Nephrotic syndrome in snakebite has been reported, but the cause and effect relationship was not substantiated.

Haematuria

Haematuria is often seen in the patient bitten by haemotoxic snakes, either viperid or crotalid snakes due to haemorrhagic diatheses, and this can be either microscopic or gross haematuria depending upon the severity of envenomation. The incidence can be as high as 35%. Although not common, nephritic syndrome has been described in viper bite with pathological changes of diffuse proliferative and crescentic glomerulonephritis. There is some diminution of renal function. The clinical outcome is usually favourable; however, with associated tubular necrosis, renal failure can be severe.

Pigmenturia

Intravascular haemolysis is common in viperid and crotalid snake bites. Haemoglobinuria is therefore frequently observed in these haemotoxic snake bites. Haemolysis is preceded by erythrocyte swelling with the rise in haematocrit. Chelation of calcium by citrate decreases erythrocyte swelling and raises the threshold for intravascular haemolysis. Rhabdomyolysis induced by phospholipase A₂ in myotoxic snake envenoming results in myoglobinuria. Both haemoglobinuria and myoglobinuria are important in the pathogenesis of acute renal failure in snakebite.

Acute renal failure

Snakes that cause renal failure are either myotoxic or haemotoxic snakes causing rhabdomyolysis, intravascular haemolysis, disseminated intravascular coagulation (DIC) or haemorrhage. In tropical Asia acute renal failure (ARF) in snakebite constitutes 1.2% of total acute renal failure in Thailand, 3% in India, and as high as 70% in Myanmar. Children are more prone to develop renal failure than adults. Table 1 lists the snakes with myotoxicity and haemotoxicity that can cause acute renal failure. The incidence of acute renal failure caused by these snakes varies from 5% to 29% depending on the species of snake and the severity of envenoming. The onset of renal failure is a few hours to several hours after the bite. Renal angle tenderness may be observed. In a series of 123 patients bitten by Russell's viper, renal angle tenderness was noted in 39% and hypotension noted in 35%. Renal failure may not be associated with hypotension, however, transient hypertension has been observed. In viper bite renal failure may accompany intravascular haemolysis or intravascular coagulation. Haemoglobinuria and haematuria are observed. Haemolytic uraemic syndrome has been reported following haemotoxic snake envenomation. In myotoxic snakebite renal failure is associated with muscular pain, weakness, myoglobinuria and high serum creatine phosphokinase due to rhabdomyolysis. In both instances hyperkalaemia may be of an alarming degree. Renal failure is catabolic with rapid rises in blood urea nitrogen and serum creatinine. Hyperuricaemia may be present. Nonoliguric renal failure is not uncommon. Renal failure averages 2–3 weeks in duration. Prolonged renal failure with oligo-anuria is observed in the patient with cortical necrosis or acute tubular necrosis associated with either interstitial nephritis or extracapillary glomerulonephritis. Acute glomerulonephritis in viper bite can cause mild renal failure. The patient usually completely recovers except for those with cortical necrosis. Mortality in snakebite acute renal failure range from 1% to 20%. Bad prognosis is observed in the elderly and those with cortical necrosis or severe haemorrhagic complications.

MANAGEMENT

Specific antivenom treatment is important and this is usually given before renal failure sets in. Monovalent antivenoms are preferred. Phasmapheresis and blood exchange have been used in snake envenomation where antivenom was unavailable. However, this is not practical since it has to be performed very early before the venom fixes to the tissue. Either peritoneal dialysis or haemodialysis is life saving.

Table 1 Snakes with nephrotoxicity

<table>
<thead>
<tr>
<th>Haemotoxic snakes</th>
<th>Myotoxic snakes</th>
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<tbody>
<tr>
<td>Russell's viper (Daboia russelli siamensis)</td>
<td>Sea snakes (Family Hydrophidae)</td>
</tr>
<tr>
<td>Saw-scaled viper (Echis carinatus)</td>
<td>Mulga snake (Pseudechis australis)</td>
</tr>
<tr>
<td>Lance-headed viper (Genus Bothrops)</td>
<td>Rough-scaled snake (Tropidechis carinatus)</td>
</tr>
<tr>
<td>Puff Adder (Bitis arietans)</td>
<td>Taipan (Oxyuranus scutellatus)</td>
</tr>
<tr>
<td>Pit viper (Family Crotalidae)</td>
<td>Tiger snake (Notechis scutatus)</td>
</tr>
<tr>
<td>Rattlesnake (Genus Crotalus)</td>
<td>Small-eyed snake (Cryptophis nigrescens)</td>
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<tr>
<td>Tiger snake (Notechis scutatus)</td>
<td></td>
</tr>
<tr>
<td>Brown snake (Genus Pseudonaja)</td>
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<tr>
<td>Taipan (Oxyuranus scutellatus)</td>
<td></td>
</tr>
<tr>
<td>Mocassin (Agliprostom piscivorus)</td>
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<tr>
<td>Boomslang (Dispholidus typus)</td>
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Dialysis should be performed early and frequently. Early and frequent dialyses are important for the patient survival. Haemodialysis improves muscular symptoms in snakebite, and suggests that dialysis corrects hyperkalaemia and removes some postsynaptic neurotoxin with small molecular weight.\textsuperscript{27} Renal failure should be prevented. Prompt treatment with specific antivenom is required. Maintenance of good urine flow is important. Alkalization of urine by sodium bicarbonate helps in the prevention of acute renal failure in the patients with myoglobinuria or haemoglobinuria provided that this is done early when dark urine is observed or the snake is known to be myotoxic or haemotoxic. In animal studies alkalization of urine prior to envenomation prevents pathological changes and impairment of renal function.\textsuperscript{28,29} In established acute renal failure, administration of sodium bicarbonate and mannitol can be dangerous and should be avoided due to fluid overload and hyperosmolality. Dopamine and furosemide attenuated impaired renal function in animal model of Russell’s viper envenomation at the early stage.\textsuperscript{30} In tropical infectious disease models, dopamine and furosemide given at the prerenal failure stage induced diuresis and prevented renal failure. There are no clinical data in the snakebite model. Of interest, hyperparathyroidectomy in a canine prior to Russell’s viper venom administration attenuated the decrease in glomerular filtration and renal blood flow.\textsuperscript{31}

**RENAL PATHOLOGY**

All renal structures can be involved in snake envenomation.

**Glomerular changes**

Glomerular involvement in snakebite is often overlooked. In animal experiments focal mesangial proliferative glomerulonephritis can be induced by injection of Habu snake (**Trimeresurus flavoviridis**) venom.\textsuperscript{32} Mesangiolysis is the early appearance of the glomerular lesion.\textsuperscript{33,34} There is dissolution of the mesangial matrix. Hypercellularity of mesangial cells appears later as a healing reaction. The mesangial proliferative lesion is inhibited in the platelet-depleted animal. Habu snake venom induced proliferation of mesangial cells with a significant elevation of monocyte chemoattractant protein (MCP-1) mRNA. Positive staining of MCP-1 is shown in the marginal area of glomeruli with mesangiolysis. Extracellular matrix glycoprotein tenascin C expression, especially domain D and platelet-derived growth factor, are significant in the healing process of glomerulonephritis.\textsuperscript{35,36} Vascular endothelial growth factor regulates angiogenesis and plays an important role in capillary repair and resolution of glomerulonephritis. Over the course of the disease mesangial cells migrate into the lesion, proliferate and form a confluent cellular mass. Fibronectin derived from platelets and macrophages provides a matrix with mesangial cell migration into glomerular lesions. The venom of Bothrops moojeni can also cause mesangiolysis, glomerular microaneurysm and proteinuria in rats.\textsuperscript{37}

In humans, mild mesangial proliferative glomerulonephritis has been observed following the bite of Russell’s viper, cobra, green pit viper and Habu snake.\textsuperscript{38} There is widening of mesangial areas due to proliferation of mesangial cells or an increase in the amount of mesangial matrix or both. Irregular thickening of the wall of peripheral capillary loops may be observed. In addition, mononuclear leucocytes and a small number of polymorphonuclear cells are occasionally present within glomerular capillary lumens. This is a non-specific finding and may be seen in other snake bites also. Deposition of IgM and C3 in the glomeruli may be intense, negligible or absent depending upon the time renal biopsy is performed. IgM and C3 deposition may be absent when renal biopsy is performed early after the bite. In most cases in which renal biopsy was performed late in the course of the disease, the deposition appears fine and granular and is commonly located in the mesangial areas and sometimes along the capillary loops.\textsuperscript{39} This is different from IgM mesangial proliferative glomerulonephritis in which deposition of IgM and C3 is more intense along the capillary loops. IgM deposits are more prominent in Russell’s viper bite than in cobra bite and green pit viper bite. Deposition of C3 is more intense in cobra cases. Fibrin deposition in the peripheral capillary loops and the Bowman’s space is observed in some green pit viper and Russell’s viper cases. Mesangiolysis is demonstrable in the patient bitten by Russell’s viper or green pit viper.\textsuperscript{40}

In some instances the severe form of glomerulonephritis may occur. Extracapillary proliferative glomerulonephritis with fibrin deposition and crescent formation without immunoglobulins and C3 has been shown in puff adder bite\textsuperscript{40} and Russell’s viper bite.\textsuperscript{41} Diffuse proliferative glomerulonephritis can be observed in occasional green pit viper bite victims.\textsuperscript{42}

**Tubulointerstitial changes**

Degeneration, necrosis, and regeneration of tubular epithelial cells have been observed in renal failure following the bite by either haematoxic or myotoxic snakes.\textsuperscript{27-31} These changes occur in any part of the renal tubule. Interstitial oedema and cellular infiltration are observed. The infiltrates consist of lymphocytes, plasma cells, and mononuclear phagocytic cells. Interstitial lesions are more prominent in the area where there is tubulorhexis. In haemotoxic snakebite fine granules of haemoglobin can be observed in the proximal tubular cells, and haemoglobin casts are seen in the lumen of necrotic tubules. In myotoxic snakebite the tubules contain myoglobin casts. Mild tubular degeneration is present in green pit viper bite and has been observed occasionally in cobra bite. Tubular necrosis is the common cause of acute renal failure in snakebite. Acute diffuse interstitial nephritis has been observed in Russell’s viper bite.\textsuperscript{41-43} There is diffuse and intense interstitial infiltration with mononuclear cells out of proportion to tubular degeneration. Immunofluorescence study shows no deposition of immunoglobulins and complement.
Vascular changes

Segmental necrotizing arteritis of the interlobular arteries has been described in Russell's viper bite. The lesion could have been missed if the renal biopsy was superficial. Segmental thrombophlebitis of the arcuate vein and its tributaries has been reported in both Russell's viper bite and green pit viper bite. Deposition of C3 without immunoglobulins in the wall of necrotizing arteries is demonstrable. Deposition of C3 in the wall of afferent and efferent arterioles without any vascular change has been shown in both viper bite and cobra bite patients.

Renal infarction and cortical necrosis

In animal experiments, renal infarction has been demonstrated following crotalid snake venom administration. In human, haemorrhagic infarct has been observed in the patients bitten by rattlesnake and Russell's viper. Fibrin platelet thrombi appear in interlobular arteries within or near the infarcted areas. Necrotic tubules containing haemoglobin casts are demonstrable.

Cortical necrosis has been observed following the bite of Russell's viper, *Echis carinatus*, *Bothrops jararaca*, and *Agraystrodon hypnale*. The lesion is associated with disseminated intravascular coagulation. There is necrosis of all elements of the kidney with thrombi in the renal vascular bed. In cases with recovery there is residual impairment of renal function, and calcification of the renal cortex may be seen by radiography.

The relationship between renal pathological changes and clinical renal manifestation is shown in Table 2.

PATHOGENESIS

The pathogenesis of renal lesions 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 mediators. Phospholipase A₂, an important toxic component of the venom, stimulates hypothalamus-pituitary and immune axes to increase adrenocorticotrophic hormone, corticosteroid, arginine vasopressin and acute phase response. Histamine, kinins, eicosanoids, platelet activating factor, catecholamines and endothelin are among the involved mediators. Zinc metalloprotease can cleave glutathione-S-transferase-tumour necrosis factor-alpha fusion protein (GST-TNF-α) substrate to generate biologically active TNF-α. In a recent study of vasoactive mediator release following Russell's viper envenomation the plasma concentrations of norepinephrine, epinephrine, dopamine, thromboxane B₂, endothelins and 6 keto PGFα were elevated. Besides these vasoactive mediators cytokines are important in cellular interaction in a variety of immunological and inflammatory processes. Envenomation of mice by the venom of *Bothrops asper* and *Bothrops jararaca* induced striking elevations of serum TNF-α, IL-1, IL-6, IL-10 IFN-γ and NO. The effects of cytokines and vasoactive mediators are reflected by haemodynamic changes and 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. Haemodynamic changes therefore play an important role in snake envenomation. Figure 1 summarizes the pathogenesis of nephropathy in snakebite.

Table 2 Clinico-pathological correlation

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Clinical renal manifestation</th>
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<tbody>
<tr>
<td>Mesangiolysis</td>
<td>Normal renal function, normal urine finding, or haematuria</td>
</tr>
<tr>
<td>Mesangial proliferative glomerulonephritis</td>
<td>Normal renal function, normal urine finding or haematuria, or mild proteinuria, occasional heavy proteinuria with complete resolution</td>
</tr>
<tr>
<td>Diffuse proliferative glomerulonephritis</td>
<td>Mild renal failure, haematuria</td>
</tr>
<tr>
<td>Extracapillary proliferative glomerulonephritis</td>
<td>Severe renal failure and haematuria with prolonged clinical course</td>
</tr>
<tr>
<td>(usually associated with tubular necrosis)</td>
<td>Severe renal failure with prolonged clinical course</td>
</tr>
<tr>
<td>Vasculitis (usually associated with tubular necrosis)</td>
<td>Acute renal failure</td>
</tr>
<tr>
<td>Tubular necrosis</td>
<td>Severe renal failure with prolonged clinical course</td>
</tr>
<tr>
<td>Acute diffuse interstitial nephritis(usually associated with tubular necrosis)</td>
<td>Severe acute renal failure with residual damage or without recovery</td>
</tr>
<tr>
<td>Cortical necrosis</td>
<td></td>
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</table>

Fig. 1 Pathogenesis of nephropathy in snakebite. RBF, renal blood flow.
Haemodynamic alterations

Haemodynamic changes in snakebite vary among snakes involved. In a study of Russell’s viper envenomation in canines, initially the cardiac output was decreased, systemic vascular resistance (SVR) and renal vascular resistance (RVR) was increased; the renal blood flow (RBF) and the glomerular filtration rate (GFR) were decreased.\(^{11}\) Haemodynamic changes are consistent with the effects of vasoconstrictive mediators. The decreased cardiac output is believed to be attributed to the effect of thromboxane A\(_2\) resulting in pulmonary artery constriction and decreased blood return to the heart. Later at 6.00 h after envenomation the cardiac output was increased; SVR was decreased; RVR was markedly increased; RBF and GFR further decreased (Fig. 2). Haemodynamic alteration at this stage is similar to that of sepsis with prominent effects of NO and PGI\(_2\) on systemic circulation and the effect of vasoconstrictive mediators on the kidney. In a sea snake study envenomation by *Lapemis hardwicke* in canines revealed there was no change in cardiac output (CO) and SVR, but RVR was increased.\(^{29}\) RBF and GFR were decreased, and renal failure developed. There was no change in renal haemodynamics when sodium bicarbonate was given before envenomation.\(^{29}\) Renal failure was prevented. It was suggested that decreased RBF and GFR were due to renal tubular obstruction by myoglobin. Sodium bicarbonate prevents tubular obstruction thus maintaining RBF and GFR. Intravenous injection of cobra venom (*Naja kaouthia*) in canines decreased GFR and RBF of short duration without causing renal failure.

In isolated renal perfusion there are great variations in venom effects not only among different snakes but also among snakes of the same genus but different subspecies. However, in most snake envenomations, the glomerular filtration rate and renal blood flow are decreased where renal vascular resistance is increased.\(^{33,34}\) In a study of *Bothrops moojeni* venom, decreased RVR and increased RBF were observed following envenomation.\(^{55}\) GFR remained unchanged, presumably due to the effect of natriuretic peptides in the venom.\(^{55,56}\)

Although haemodynamic changes are basic in the development of ARF, the duration of decreased GFR and RBF and the associated complications are important. There is good correlation between the renal function and the haematologic profile in viper bite.\(^{37}\) Additional insults including haemoglobinuria, myoglobinuria, haemorrhage, complement activation and reactive oxygen species play contributing roles in prolonging the duration of decreased GFR and RBF. Experimentally, cobra envenomation causes the decrease in GFR and RBF of short duration without developing ARF, while Russell’s viper and sea snake envenomation, which cause longer duration of renal ischaemia associated with DIC, haemoglobinuria and myoglobinuria result in the development of acute renal failure.\(^{29,52}\)

**Direct nephrotoxicity**

Renal failure has been observed in patients a few hours after snakebite without hypotension, haemorrhage, intravascular haemolysis and rhabdomyolysis. The clinical evidence suggests direct nephrotoxicity of the venom. Mesangiolysis,\(^{33,34}\) glomerulonephritis,\(^{19,20,39}\) and vasculitis\(^{33,38}\) without immunologic clues indicate direct glomerular and vascular injury by the venom. Venomous snakes have enzymes that can directly cause cellular injury. Metalloprotease can cause proteolysis of the extracellular matrix and disrupts cell-matrix and cellular adhesion. The enzymes are present in the venoms of snakes in the Viperinae and Crotalinae subfamilies,\(^{58}\) and bind with various degrees of specificity to integrin alpha 4 beta 1, alpha 4 beta 7, alpha 5 beta 1, alpha 6 beta 1, alpha 9 beta 1, alpha V beta 3 and alpha V beta 5, expressed on cells.\(^{59-61}\) Integrity of cellular junctions is disrupted as a result of disruption of the actin cytoskeleton resulting in a loss of cell polarity. Integrins which are critical for cellular adhesion, redistribute away from the basal cell surface, contributing to the loss of adhesion to the basement membrane. Metalloproteases can induce apoptosis of vascular endothelial cells.\(^{62}\) Phospholipase A\(_2\) enzymes are present in several poisonous snakes including crotalids, vipersids, elapids and hydrophids. The enzymes are toxic and induce a wide spectrum of pharmacological effects. Phospholipase A\(_2\) interacts with the biological membranes via a distinct molecular region. This active region is likely to be formed by a combination of basic hydrophobic amino acid residues near the C-terminal of the protein. The high affinity interaction of PLA\(_2\) with its target protein is probably due to the interaction of charges, hydrophobicity and van der Waals contact surfaces between the toxin and the binding site as the surface of the cell membrane.\(^{55,64}\) These events lead to membrane destabilization and loss of permeability and cellular necrosis. In animal experiments, *Bothrops moojeni* crude venom decreases transepithelial electrical resistance across the cultured Madin-Darby canine kidney (MDCK) cell monolayers, causes disarray of the cytoskeleton and impairs cell to matrix adhesion.\(^{29}\) *Bothrops* venom decreased neutral

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**Fig. 2** Haemodynamic changes in Russell’s viper envenomation. ■ cardiac output; □ glomerular filtration rate; ■ renal blood flow; □ renal vascular resistance; □ systemic vascular resistance.

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red uptake of vero cells. Notexin from tiger snake venom can cause renal tubular and glomerular damage within 24 h after subcutaneous injection in mice. By isolated renal perfusion, Russell's viper venom decreased potential difference across the proximal tubular membrane and decreased tubular re-absorption of sodium in dose-dependent fashion. The venom causes lysis of vascular smooth muscle, hydropic and vascular degeneration of proximal and distal tubular cells and cortical collecting duct with detachment from the basement membrane. In a cell culture study using LLC-PK₁ cells and MDCK cells representing proximal and distal/collecting tubular cells, Russell's viper venom caused nuclear pyknosis and cellular detachment from the substrate.

**Immunological mechanism**

Immunological mechanism plays a minor role in the pathogenesis of glomerulonephritis. In contrast to glomerulonephritis seen without immunologic evidence in acute animal experiments and in a number of patients bitten by snakes, immune complex glomerulonephritis has been observed later in the course of snakebite regardless of antivenom administration. There is deposition of C₃ and IgM in the glomerular mesangium. The evidence suggests an immune complex glomerulonephritis with implanted antigen, followed by IgM deposit acquired naturally. Alternatively, this could be due to a concealed infection in the snakebite that results in immune complex glomerulonephritis with predominant deposition of IgM and C₃ in the mesangial areas. At present, the evidence is in favour of glomerulonephritis directly induced by the snake venom. The immunological role is rather weak but cannot be entirely denied, especially the role of immune complex in situ. Further studies in animals are required.

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