

Short-term Changes in Urine Beta 2 Microglobulin Following Recovery of Acute Kidney Injury Resulting From Snake Envenomation



Challa Jaswanth¹, P.S. Priyamvada¹, Bobby Zachariah², Sathish Haridasan¹, Sreejith Parameswaran¹ and R.P. Swaminathan³

¹Department of Nephrology, Jawaharlal Institute of Post Graduate Medical Education and Research, Puducherry, India;

²Department of Biochemistry, Jawaharlal Institute of Post Graduate Medical Education and Research, Puducherry, India; and

³Department of Medicine, Jawaharlal Institute of Post Graduate Medical Education and Research, Puducherry, India

Introduction: Urine β_2 microglobulin (β_2m) is a validated marker to diagnose sepsis and toxin-related acute kidney injury (AKI). In the current study, we used urine β_2m as a potential marker to identify persistent tubular dysfunction following a clinical recovery from snake venom-related AKI.

Methods: A total of 42 patients who developed AKI following hemotoxic envenomation were followed up for a period of 6 months. Urine albumin excretion, estimated glomerular filtration rate (eGFR), and urine β_2m levels were measured at 2 weeks, 3 months, and 6 months following discharge.

Results: At the end of 6 months of follow-up, 6 patients (14.3 %) progressed to chronic kidney disease (CKD) (eGFR < 60 ml and/or urine albumin excretion > 30 mg/d). The urine β_2m levels were 1590 $\mu\text{g/l}$ (interquartile range [IQR] 425–5260), 610 $\mu\text{g/l}$ (IQR 210–1850), 850 $\mu\text{g/l}$ (IQR 270–2780) at 2 weeks, 3 months, and 6 months, respectively ($P = 0.020$). The levels of urine β_2m in the study population at the end of 6 months remained significantly higher compared with the levels in healthy control population (850 $\mu\text{g/l}$ [IQR 270–2780] vs. 210 $\mu\text{g/l}$ [IQR 150–480]; $P = 0.001$). The proportion of patients with urine β_2m levels exceeding the 95th percentile of control population (>644 $\mu\text{g/l}$) during the 3 follow-up visits were 70.7% ($n = 29$), 48.8 % ($n = 20$), and 51.2% ($n = 21$). Similar trends were noticed in a sensitivity analysis, after excluding patients with CKD.

Conclusions: Urine β_2m levels remain persistently elevated in approximately half of the individuals who recover from AKI due to snake envenomation.

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KEYWORDS: acute kidney injury; beta 2 microglobulin

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The epidemiology of AKI in the tropical low- and middle-income countries from Southeast Asia is dominated by infectious diseases and envenomation, occurring in relatively healthy individuals without significant comorbidities.¹ Snake envenomation is believed to account for 0.5% of all deaths in India.² It is reported that 12% to 30% of patients develop AKI following hemotoxic envenomation, but the actual rates might be higher due to nonuniform reporting policies. Following an episode of AKI, a complete regeneration of the tubules leads to full recovery of kidney function. A maladaptive

repair leads to acute kidney disease, which may subsequently recover or progress to CKD.^{3,4} Even though envenomation is a major reason for AKI in the tropics, there is only minimal data on the long-term consequences on kidney function. A single-center study reported that approximately one-fourth of patients who develop AKI following a snake bite develop CKD long term.⁵

Most victims of AKI from snake envenomation are young individuals who lack the susceptibility factors putting them at the risk of AKI or the well-known risk factors for CKD or its progression.^{6,7} The current screening recommendations to identify the progression of AKI to CKD include periodic assessment of eGFR, urine albumin excretion, and blood pressure. These investigations are not sensitive enough to detect sub-clinical persistent tubular damage, which might act as a forerunner of CKD. An accurate and sensitive marker to assess the extent of tubular damage would aid in the risk stratification, especially in individuals who lack

Correspondence: P.S. Priyamvada, Department of Nephrology, Jawaharlal Institute of Post Graduate Medical Education and Research, Puducherry 605006, India. E-mail: priyamvadaps@gmail.com

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the conventional risk factors. Data on utility of urine biomarkers to identify persistent tubular damage following AKI are limited. $\beta_2\text{m}$ is a protein secreted by all nucleated cells at a constant rate, filtered by glomerulus, reabsorbed and catabolized by renal tubules, resulting in very low urine concentrations in healthy individuals.⁸ Urine $\beta_2\text{m}$ levels are reported to reflect renal tubular injury following exposure to toxins and drugs.⁹ The utility of urine $\beta_2\text{m}$ as a potential marker for recovery of tubular function following apparent recovery of AKI is still unexplored. Data from animal experiments have shown that urine $\beta_2\text{m}$ rapidly rises following exposure to nephrotoxins and returns to baseline following recovery.¹⁰ We hypothesized that in patients with AKI, if tubular recovery is complete, urine $\beta_2\text{m}$ levels should come down to levels comparable with healthy individuals. In the current study, we assessed the changes in urine $\beta_2\text{m}$ levels in the initial 6 months following snake bite-related AKI.

MATERIALS AND METHODS

Screening of the Study Population

The study period was from January 2016 to December 2017. All patients admitted to medical wards with AKI due to hemotoxic envenomation were screened. Hemotoxic envenomation was defined as the presence of whole blood clotting time >20 minutes in patients who sustained a snake bite. AKI was defined as per Kidney Disease: Improving Global Outcomes 2012 work group criteria. Patients with previous history of CKD were excluded. An ultrasound examination of the abdomen to assess kidney size was performed before recruitment. Patients with bipolar length of kidneys less than 9 cm, cysts, stones, or any other structural abnormalities of kidneys were also excluded. All patients who agreed to recruitment were enrolled at the time of discharge from the hospital. Demographic, clinical, and laboratory details were collected at the time of enrollment. The study protocol was approved by the institute ethics committee, JIPMER.

Follow-up Visits

The follow-up period was 6 months. A total of 3 follow-up visits were scheduled at 2 weeks, 3 months, and 6 months following discharge from the hospital. Serum creatinine, urine albumin excretion, and urine $\beta_2\text{m}$ were checked during the follow-up visits. Serum creatinine was estimated by modified Jaffe's Method. Glomerular filtration rate was estimated using the Chronic Kidney Disease Epidemiology Collaboration creatinine equation (2009). Urine albumin excretion was checked using nephelometry with a Behring BN Prospect analyzer (Dade Behring, Deerfield, IL). Fresh urine samples of 10 ml were collected

for $\beta_2\text{m}$ during each follow-up visit. The sample was frozen immediately at -80°C . The estimation was done at a later date with enzyme-linked immunosorbent assay (Cal Biotech, Inc., El Cajon, CA). The lower limit of detection of the $\beta_2\text{m}$ assay was $20\ \mu\text{g/l}$. All enzyme-linked immunosorbent assay measurements were done in duplicate, the intra-assay coefficient of variation being 7.5%. Sample collection was deferred for 2 weeks in patients with history of any intercurrent urinary or systemic infections during the follow-up visits. Patients were instructed to avoid nephrotoxic medications during the study period. All laboratory measurements were subjected to rigorous external and internal standardization.

Because there were no available data on $\beta_2\text{m}$ in the healthy population from the geographic area, we checked the urine $\beta_2\text{m}$ levels in 25 healthy controls. The controls were aged between 20 and 60 years without history of diabetes mellitus or hypertension with an eGFR $>60\ \text{ml/min per } 1.73\ \text{m}^2$ and urine albumin excretion $<30\ \text{mg/d}$.

Statistical Methods

All categorical variables were expressed as frequencies and percentages and continuous variables were expressed as means with confidence intervals or median with IQR wherever appropriate. The changes in eGFR, urine $\beta_2\text{m}$, creatinine and urine albumin in the AKI cohort over 6 months of follow-up were compared using repeated measures analysis of variance with a Bonferroni correction. The urine $\beta_2\text{m}$ levels of the AKI cohort at 6 months were compared with controls using a Student's *t*-test. Urine $\beta_2\text{m}$ levels, which did not follow a normal distribution, were log transformed and compared. The related categorical variables were compared with Cochran's Q test. The analysis was done with IBM SPSS Statistics, V 19.0 (IBM Corp., Armonk, NY).

RESULTS

A total number of 70 patients were recruited; 42 patients attended all 3 scheduled follow-up visits. The median duration of first follow-up was 29 days (IQR 23–35 days) from diagnosis of AKI. The clinical characteristics of the cohort at the time of hospitalization is given in Table 1. There were no significant comorbidities except for diabetes in 1 patient.

The proportion of patients who had GFR $<60\ \text{ml/min per } 1.73\ \text{m}^2$ and urine albumin excretion $>30\ \text{mg/d}$ on each follow-up visit is given in Table 2. Among 42 patients, 1 patient did not recover from AKI and remained dialysis dependent. A renal biopsy from the patient showed thrombotic microangiopathy. Kidney biopsy was done in another 2 patients with low GFR. The biopsies revealed chronic thrombotic microangiopathy and

Table 1. Demographic and clinical characteristics of study population ($n = 42$)

Parameter	Value
Age (yr), mean (CI)	41.83 (38.31–45.36)
Male gender, n (%)	27 (64.3)
Renal replacement therapy, n (%)	32 (76.2)
Mechanical ventilation, n (%)	01 (02.4)
Inotropic support, n (%)	05 (11.9)
Capillary leak syndrome, n (%)	03 (7.1)
Haemoglobin, g/dl	10.99 (10.14–11.89)
Platelet count (ml), median (IQR)	40.5×10^3 (21.0–56.2)
Quantity of ASV received in ml, median (IQR) ^a	150 (80–240)
Serum creatinine at admission (mg/dl), median (IQR)	3.00 (1.29–4.71)
Peak serum creatinine (mg/dl), median (IQR)	8.30 (4.8–11.4)
Serum creatinine at discharge (mg/dl), median (IQR)	3.8 (2.1–5)
Hospital stay in days, median (IQR)	15 (09–21)
Proteinuria $\geq 1+$, n (%)	28 (66.6)
AKI stage at admission, n (%)	
AKI 1	05 (11.9)
AKI2	02 (04.7)
AKI 3	35 (83.3)

AKI, acute kidney injury; ASV, anti-snake venom; CI, confidence interval; IQR, inter-quartile range.

^aASV available as lyophilized vials, which is reconstituted to 10 ml. Each vial contains antivenom against *N Naja* 0.60 mg, *Bungarus caeruleus* 0.45 mg, *Daboia russelli* 0.60 mg, and *Echis carinatus* 0.45 mg.

persistent acute tubular necrosis. Three other patients who progressed to CKD by 6 months did not give consent for kidney biopsy.

The patient who remained dialysis dependent was excluded from further analysis. The individual trends in serum creatinine of the remaining 41 patients are given in Figure 1. The serum creatinine, GFR and urine β 2m levels during follow-up are given in Table 3. The creatinine, eGFR improved, and urine β 2m levels declined significantly between 2 weeks and 3 months, whereas the changes between 3 months and 6 months were not significant (Figure 2). The proportion of patients with urine β 2m levels exceeding the 95th percentile for controls ($> 644 \mu\text{g/l}$) during the 3 follow-up visits are given in Table 3. The results were similar when the analysis was repeated after excluding the patients who developed CKD (Table 3). The renal function and urine β 2m of all recruited patients are given as Supplementary Table S1.

The levels of urine β 2m in the study population at the end of 6 months remained significantly higher compared with the levels in controls (Table 4).

Table 2. Proportion of patients with impaired renal function on follow-up visits ($n = 42$)

Parameter	2 wk, n (%)	3 mo, n (%)	6 mo, n (%)	P
GFR < 60 ml/min per 1.73 m^2 and/or urine albumin > 30 mg	15 (35.7)	07 (16.7)	06 (14.28)	0.010
GFR < 60 ml/min per 1.73 m^2	15 (35.7)	05 (11.9)	05 (11.9)	0.001
Urine albumin > 30 mg	03 (7.1)	04 (9.5)	03 (7.1)	0.819

GFR, glomerular filtration rate.

The patients who had elevated urine β 2m levels ($> 644 \mu\text{g/l}$) at 6 months had marginally lower GFR throughout the follow-up period ($P = 0.60$; Figure 3). The creatinine levels on admission and discharge, duration of hospital stay, and dose of anti-snake venom was comparable between the patients with normal and elevated urine β 2m levels ($> 644 \mu\text{g/l}$). The urine β 2m levels at first visit strongly correlated with serum creatinine at 6 months ($r = 0.965$; $P < 0.001$). There was a moderate correlation with urine β 2m at 3 months and serum creatinine at 6 months ($r = 0.579$, $P < 0.001$).

DISCUSSION

Snake envenomation is a major reason contributing to renal failure in Southeast Asia, especially in India. In India, most envenomations result from hemotoxic snakes, such as *Daboia russelli* and *Echis carinatus*. Recently, bites from other species, including various pit vipers, are also reported to lead to hemotoxic envenomation. Hemotoxic envenomations produce local reaction with bleeding tendencies, hemolysis, disseminated intravascular coagulation, and renal failure. The classic renal lesions described in hemotoxic snakebite are acute tubular necrosis and cortical necrosis. The reported prevalence rates of AKI following hemotoxic envenomation in India varies from 14% to 44%.¹¹ Retrospective study designs, and lack of uniform definitions of AKI and accessibility to health care facilities might account for these wide variations. Even though snake bite is an important cause of AKI in the tropics, there is only limited information on the renal recovery patterns following envenomation. Most of the published literature focuses on the in-hospital mortality of snake bite-related AKI. There are only 2 longitudinal studies that looked into the long-term recovery patterns following snake bite-related AKI. Waikhom *et al.*⁵ reported that 40% and 5% of patients with snake bite-related AKI progressed to CKD and end-stage renal disease, respectively, over a period of 4 years. These figures are of concern, as the patients were previously healthy individuals in their early 40s without any comorbidities. Another study from Sri Lanka reported that 37% of patients who sustain AKI following envenomation develop CKD by the end of 1 year, but most patients had significant comorbidities like hypertension and diabetes.¹²

Most of the data on AKI as a risk factor for CKD come from high-income countries where the patients are in the sixth or seventh decade with multiple comorbidities like metabolic syndrome, diabetes, hypertension, and cardiac diseases. These risk factors might predispose to AKI and would persist even after an apparent recovery of AKI

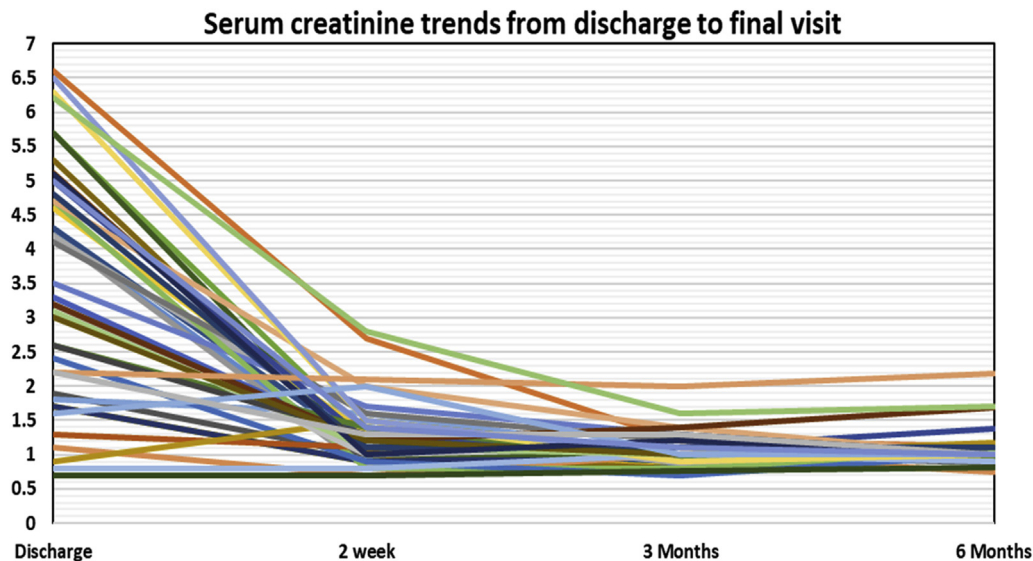


Figure 1. Serum creatinine trends of the study population.

and may independently contribute to progression of CKD.¹³ The severity of acute kidney disease and concomitant comorbidities act as the key determinants of risk of progression to CKD¹⁴; however, data from relatively younger patients with AKI, without comorbid conditions, reported lower rates of progression to CKD.¹⁵ The existing risk stratification strategies with weightage on underlying comorbidities might be of limited utility in relatively healthy younger individuals who sustain AKI. Serum creatinine-based measurements might not be sensitive enough to detect mild degrees of tubular dysfunction. There is an unmet need of serum or urine markers that could detect early subclinical tubular dysfunction following an episode of AKI.

Multiple urine markers are reported to remain elevated within a few hours following an acute insult to kidney, facilitating an early diagnosis of AKI. In addition to early diagnosis, urine biomarkers like liver fatty acid binding protein, neutrophil gelatinase-associated lipocalin, interleukin-18, and kidney injury

molecule 1 are found to be useful for predicting the in-hospital mortality as well as the progression of AKI to higher grades.¹⁶ Whether elevated biomarkers at the time of diagnosis of AKI are predictive of adverse outcomes in the long term remains controversial. In critically ill adults, higher levels of various urine biomarkers like neutrophil gelatinase-associated lipocalin, tissue inhibitor of metalloproteinase-2, and insulinlike growth factor binding protein-7 at time of diagnosis of AKI were found to be associated with adverse outcomes like progression to CKD.^{17,18} However, long-term follow-up studies in pediatric cardiac surgery cohorts have failed to show an association between the level of postoperative urine biomarkers and future development of CKD.¹⁹

There are only minimal data on the temporal patterns and trends of urine biomarkers following an apparent recovery from AKI. The biomarkers can remain elevated if there is persistent tubular injury. Identification of a sensitive marker for tubular

Table 3. Renal function and urine β 2 microglobulin levels on follow-up

<i>n</i> = 41				
Parameter	2 wk (<i>n</i> = 41)	3 mo (<i>n</i> = 41)	6 mo (<i>n</i> = 41)	<i>P</i>
eGFR (ml/min per 1.73 m ²), mean (CI)	69.37 (62.09–76.64)	82.76 (77.04–88.47)	83.26 (77.09–89.44)	0.001
Creatinine (mg/dl), mean (CI)	1.29 (1.14–1.44)	1.05 (0.98–1.14)	1.06 (0.98–1.13)	0.001
Urine β 2 microglobulin (μ g/l), median (IQR) ^a	1590 (425–5260)	610 (210–1850)	850 (270–2780)	0.020
Urine β 2 microglobulin (μ g/l) > 644 μ g/l, <i>n</i> (%)	29 (70.7%)	20 (48.8%)	21 (51.2%)	0.042
<i>n</i> = 36 (Excluding patients with CKD at 6 months)				
eGFR (ml/min per 1.73 m ²), mean (CI)	70.56 (63.18–77.93)	85.87 (80.67–91.27)	87.27 (80.02–92.53)	<0.001
Creatinine (mg/dl), mean (CI)	1.25 (1.11–1.39)	1 (0.94–1.06)	0.99 (0.96–1.03)	<0.001
Urine β 2 microglobulin (μ g/l), median (IQR) ^a	1235 (417–5355)	575 (172–1677)	565 (250–1607)	0.012
Urine β 2 microglobulin >644 μ g/l	25 (69.4%)	16 (44.4%)	17 (47.2%)	0.036

eGFR, creatinine, and urine β 2 microglobulin levels were significantly different between 2 weeks and 3 months (repeated measures analysis of variance with Bonferroni correction). CI, confidence interval; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; IQR, interquartile range.

^aValues of urine β 2 microglobulin were log transformed and means were compared using a repeated measures analysis of variance.

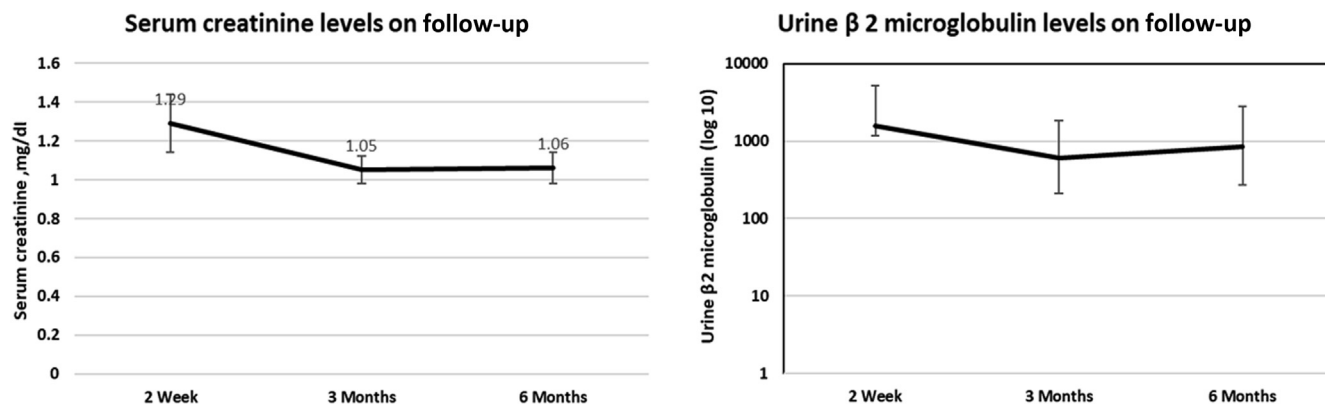


Figure 2. Serum creatinine and urine β2 microglobulin on follow-up visits.

dysfunction in patients with AKI would facilitate identifying individuals who are at risk of future CKD. Transcriptional analysis in animals have suggested urine kidney injury molecule 1 and neutrophil gelatinase-associated lipocalin as potential markers of transition from AKI to CKD.²⁰ The only available long-term data on urinary biomarkers following AKI are from pediatric patients who developed AKI following cardiopulmonary bypass.²¹ Follow-up data after 7 years revealed that in patients who developed AKI following cardiopulmonary bypass, the eGFR and urine albumin excretion were comparable with healthy controls. However, despite a normal eGFR and urine albumin excretion, the urine levels of interleukin-18 and liver fatty acid binding protein remained considerably elevated throughout the follow-up period in patients who developed AKI following cardiopulmonary bypass. There are no other published human data on the long-term trends in any of the available biomarkers following an episode of apparent recovery from AKI. A multicentric trial that assesses the utility of a battery of biomarkers in the AKI-to-CKD transition is under way.²²

β2m is a protein that was discovered in 1964 in the urine of patients with Wilson disease and cadmium poisoning.⁹ It is a 100-amino acid protein secreted by all nucleated cells at a constant rate. It is filtered by the glomerulus and reabsorbed and catabolized by renal tubules, resulting in urine concentrations less than 360

μg/l. A few population-based studies had shown that urine β2m levels can be used to diagnose tubular toxicity by heavy metals, lithium, tenofovir, aminoglycosides, and sepsis^{23–28}; however, it was not found to be useful in predicting progression or severity of AKI.²⁹ Zeng *et al.*³⁰ demonstrated that urine β2m levels correlated well with kidney injury molecule 1 immunostaining on kidney biopsy. A meta-analysis has shown urine β2m can accurately predict AKI in patients with sepsis.³¹ β2m also has been found to be useful for diagnosis of tubulointerstitial nephritis in children.³² However, there are potential concerns on the utility of β2m as a marker for tubular injury. Because β2m is filtered by the glomerulus, structural alterations resulting from various glomerular diseases can lead to enhanced glomerular leakage with resultant overflow proteinuria.³³

In rat models, urine β2m is reported to increase early in the course of nephrotoxic AKI during the recovery phase and return to control range during the recovery phase.⁸ There are no data on the utility of urine β2m as a marker of tubular function recovery following AKI in humans. In the current study, we used urine β2m as a potential marker to identify persistent tubular dysfunction following AKI resulting from snake envenomation. We observed that 15% of patients had low GFR or albuminuria at the end of 6 months of follow-up. However, approximately 50% of the subjects had urine β2m excretion exceeding the 95th percentile of the control population. There was an improvement in GFR and fall in urine β2m between 2 weeks and 3 months. However, the mean levels of urine β2m in the study population remained higher compared with healthy controls even at the end of 6 months. Snake bite-related AKI represents the prototype of toxin-related AKI, with a predictable time frame for development of AKI following exposure. The patients included in this study were relatively young without significant comorbidities except for a single patient with diabetes mellitus. A persistently elevated urine

Table 4. Urine β2 microglobulin levels in the study population (at 6 months) and controls

Parameter	Study population	Controls (n = 25)	P
Age, mean (CI)	41.83 (38.31–45.36)	38.04 (32.95–43.13)	0.23
Urine β2 microglobulin (μg/l), median (IQR) ^a (n = 41)	850 (270–2780)	210 (150–480)	0.001
Urine β2 microglobulin (μg/l), median (IQR) ^a (n = 36)	565 (250–1607)	210 (150–480)	0.001

^aValues of urine β2 microglobulin were log transformed and means were compared using a Student's *t*-test.

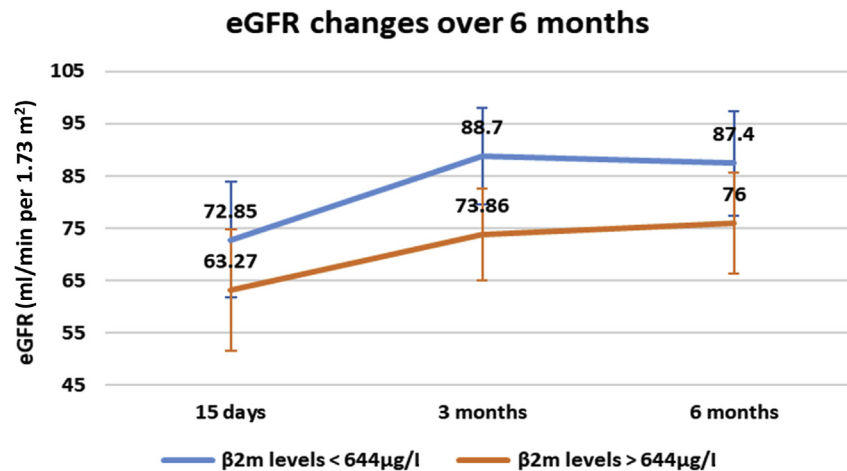


Figure 3. Estimated glomerular filtration rate (eGFR) trends in patients with urine $\beta 2$ microglobulin > 644 $\mu g/l$ versus urine $\beta 2$ microglobulin < 644 $\mu g/l$ at 6 months.

$\beta 2m$ excretion is likely to reflect incomplete renal tubular repair following AKI. Even though not statistically significant, we observed that patients with urine $\beta 2m$ exceeding the 95th percentile of controls had lower GFRs throughout the follow-up. The results were similar even after excluding the patients with albuminuria and low GFR. None of the subjects had any systemic conditions or medications that are known to be associated with increased production of $\beta 2m$ and excretion in urine. There was no evidence of significant proteinuria or other features to suggest any significant glomerular damage.

Urine $\beta 2m$ is a validated marker for diagnosing proximal tubular injury. There are limited data on its prognostic utility. A recent study by Barton *et al.*³⁴ showed that serum and urine $\beta 2m$ correlates with severity of AKI. To the best of our knowledge, the current study is the first one that attempted to look into the recovery patterns of AKI using urine $\beta 2m$ as a candidate marker. The study has a few limitations. As there is no published literature to guide the sample size calculation, we resorted to a convenience sampling. The attrition rates were high; only 60% of patients attended all 3 follow-up visits. Three-fourths of the patients had severe AKI requiring renal replacement therapy, which might explain the persistent tubular injury. We did not find any differences in the severity of AKI, dose of anti-snake venom, or duration of hospitalization between the high and normal urine $\beta 2m$ groups. The study might be underpowered for a subgroup analysis, because of a relatively smaller sample size. The results might not be generalizable to less severe forms of AKI. Measured GFR rates would have provided better insights into the recovery patterns. Longer follow-up will be required to ascertain the association between elevated urine $\beta 2m$ and development of CKD in the long term. Even though the patients had no comorbidities or risk factors of CKD,

early CKD could not be confidently excluded in the patients because of lack of previous documentation of kidney function.

CONCLUSIONS

Urine $\beta 2m$ levels are persistently elevated even at 6 months after apparent recovery from AKI following snake envenomation. Urine $\beta 2m$ level may be a potential early marker for persistent tubular dysfunction in patients who recover from AKI following snake envenomation. Long-term follow-up studies are required to correlate the short-term changes in urine $\beta 2m$ with later development of CKD.

DISCLOSURE

All the authors declared no competing interests.

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All procedures performed in studies involving human participants were in accordance with the ethical standards of the institution and Indian Council of Medical Research at which the studies were conducted (institutional review board approval no: PGRMC/DM/NEPHRO/01/2015) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent was obtained from all individual participants included in the study.

SUPPLEMENTARY MATERIAL

Table S1. Renal function and urine $\beta 2$ microglobulin levels on follow-up.

Supplementary material is linked to the online version of the paper at www.kireports.org.

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