URINE BETA 2 MICROGLOBULIN AS A PREDICTIVE BIOMARKER FOR ACUTE KIDNEY INJURY TO CHRONIC KIDNEY DISEASE PROGRESSION IN PATIENTS WITH ACUTE RENAL FAILURE FOLLOWING HEMOTOXIC SNAKE BITE"

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CERTIFICATE FROM DEAN

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DECLARATION

I, **Dr. A. VIGNESH** solemnly declare that, this dissertation **"URINE BETA 2 MICROGLOBULIN AS A PREDICTIVE BIOMARKER FOR ACUTE KIDNEY INJURY TO CHRONIC KIDNEY DISEASE PROGRESSION IN PATIENTS WITH ACUTE RENAL FAILURE FOLLOWING HEMOTOXIC SNAKE BITE"** is a bonafide record of work done by me at the Department of General Medicine, Govt. Rajaji Hospital, Madurai, under the guidance of **Dr. DAVID PRADEEP KUMAR, M.D.,** Professor, Department of General Medicine, Madurai Medical College, Madurai.

This dissertation is submitted to The Tamil Nadu Dr. M.G.R Medical University, Chennai in partial fulfillment of the rules and regulations for the award of **Doctor of Medicine (M.D.), General Medicine Branch-I** examination to be held in MAY 2023**.**

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Dr. A. VIGNESH

ABSTRACT

INTRODUCTION:

Acute kidney injury (AKI) secondary to hemotoxic snake bite is a significant public health concern, showing a steadily increasing prevalence in the last few decades . snake bite acute kidney injury outcomes depend on multiple factors. Patients who recovered from an snake bite AKI episode have a long-term risk of developing sequelae like chronic kidney disease (CKD) and end-stage renal disease (ESRD). In this study we are going to predict the kidney disease progression through urine beta 2 microglobulin monitoring.

AIMS AND OBJECTIVES:

- To estimate the percentage of individuals with hemotoxic snake envenomation with Acute Kidney Injury progressing to Chronic Kidney Disease.
- To identify an accurate, reliable and sensitive marker to assess the extent of tubular damage would aid in the risk stratification, especially in individuals who lack the conventional risk factors.

MATERIALS AND METHODS:

- All patients admitted to medical wards with AKI(defined as per KDIGO 2012) due to hemotoxic snake envenomation were screened.

Hemotoxic envenomation was defined as the presence of whole blood clotting time >20 minutes in patients who sustained a snake bite.

- Demographic, clinical, laboratory details and periodic assessment of eGFR, urine albumin excretion,sr.creatinine and urine beta 2 microglobulin are collected at the time of enrollment and periodically at end of 2 weeks , 12 weeks and 24 weeks respectively.
- An ultrasound examination of the abdomen to assess kidney size was performed before recruitment.
- Urine b2m will be estimated by CLIA method , serum creatinine by modified jaffe's method, eGFR by CKD-EPI formula,urine albumin by nephelometry.

RESULTS:

In this study majority of patient's age were between 31-40 years (36%), 22% were between 51-60 years, 20% were between 21-30 years, 14% between 41-50years and 8% were between the age of 10-20years.

Mean age in this study group was37.72±13.07years. In Jaswanth et al., study mean age was 41.83 years. In our study majority of the patients were male (54%) and 46% were females.

Urine Albumin 1+ in 2 week (n=15), 12 weeks (n=6) and in 24 weeks (n=6). 2+ in 2 week (n=3), 12 weeks (n=2) and in 24 weeks (n=2). 3+ in 2

week (n=2), 12 weeks (n=0) and in 24 weeks (n=0). In Jaswanth et al., study number of patients having urine albumin >1+ was 28, in our study urine albumin >1+ was 20 patients.

Mean serum Urea level in 2 weeks 46.34±9.55 mg/dl, 12 weeks 38.42±8.52 mg/dl, in 24weeks 34.84±9.22mg/dl. There was decrease in serum urea level noted in followup weeks.

Mean serum creatinine level in 2 weeks 1.418±0.32mg/dl, in 12 weeks 1.278±0.31mg/dl, in 24 weeks 1.168±0.43mg/dl. Creatinine level was decreasing constantly in following weeks.

Mean eGFR in 2weeks was 57.38±7.004, in 12 weeks was 61.38±8.85, in 24weeks was 65.7±9.78. Mean urine beta 2 microglobulin in 2 weeks was 1391.36, in 12 weeks 710.52, in 24 weeks 485.78.

The serum creatinine, urea, eGFR improved and beta 2 microglobulin level declined significantly between 12 weeks and 24 weeks.

Urine beta 2 microglobulin level at 2weeks were correlated with serum creatinine level in 24 weeks, there was a strong correlation between microglobulin level and development of kidney disease.

In our study urine bête 2 microglobulin have 80.00%sensitivity and 77.1% specificity in predicting kidney injury.

CONCLUSION

Urine beta 2 microglobulin is one of the validated marker for acute kidney injury detection. From this study, we conclude that urine beta 2 microglobulin level may be a potential, reliable early marker for detecting acute kidney injury to chronic kidney disease progression in hemotoxic snake bite patients suffered from acute kidney injury. Long term follow up is further needed to confirm the exact correlation between urine beta 2 microglobulin and chronic kidney disease progression.

TABLE ON CONTENTS

INTRODUCTION

Acute kidney injury (AKI) is a significant public health concern, showing a steadily increasing prevalence in the last few decades [1, 2]. AKI outcomes depend on multiple factors, including the aetiology, comorbidities, critical illness, stage of AKI, and patient age. Patients who recover from an AKI episode have a long-term risk of developing sequelae like chronic kidney disease (CKD) and end-stage renal disease (ESRD) [3, 4]. Snake envenomation is believed to account for 0.5% of all deaths in India [5]. 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 [6, 7]. 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 [8]. 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 [9, 10]. 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 subclinical 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 the conventional risk factors. Data on utility of urine biomarkers to identify persistent tubular damage following AKI are limited.

β2m 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 [11]. Urine β 2m levels are reported to reflect renal tubular injury following exposure to toxins and drugs [12]. The utility of urine β2m 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 β2m rapidly rises following exposure to nephrotoxins and returns to baseline following recovery [13]. We hypothesized that in patients with AKI, if tubular recovery is complete, urine β2m levels should come down to levels comparable with healthy individuals [14].

In this current study, determined an accurate, reliable and sensitive marker to assess the extent of tubular damage would aid in the risk stratification, especially in individuals who lack the conventional risk factors.

AIMS AND OBJECTIVES

To estimate the percentage of individuals with hemotoxic snake envenomation with Acute Kidney Injury progressing to Chronic Kidney Disease.

To identify an accurate, reliable and sensitive marker to assess the extent of tubular damage would aid in the risk stratification, especially in individuals who lack the conventional risk factors.

REVIEW OF LITERATURE

Urine β2 microglobulin (β2m) is a validated marker to diagnose sepsis and toxin-related acute kidney injury (AKI). In this present study, we used urine β2m as a potential marker to identify persistent tubular dysfunction following a clinical recovery from snake venom–related AKI.

Challa Jaswanth et al. [15] studied the urine β2m as a potential marker to identify persistent tubular dysfunction following a clinical recovery from snake venom–related AKI. 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 β2m levels were measured at 2 weeks, 3 months, and 6 months following discharge. 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 β2m levels were 1590 μg/l (interquartile range [IQR] 425–5260), 610 μg/l (IQR 210–1850), 850 μg/l (IQR 270–2780) at 2 weeks, 3 months, and 6 months, respectively (P = 0.020). The levels of urine β2m in the study population at the end of 6 months remained significantly higher compared with the levels in healthy control population (850 μg/l [IQR 270–2780] vs. 210 μg/l [IQR 150–480]; P = 0.001). The proportion of patients with urine β2m

levels exceeding the 95th percentile of control population (>644 µg/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. Urine β2m levels remain persistently elevated in approximately half of the individuals who recover from AKI due to snake envenomation.

Puthiyottil et al. [16] assessed whether urinary β2m/Creatinine and kidney injury molecule-1/creatinine (KIM-1/creat) ratios, measured in the early recovery phase of AKI, are predictive of kidney function at one year. Adult patients who survived an episode of AKI were followed up for one year (n=125). β2m/creat and KIM-1/creat ratio were measured at two weeks and three months following AKI. In the AKI survivors, the β2M/creat ratio at 2 weeks [18.3mg/g (IQR 2.3, 52.9)] and KIM-1/creat ratio [1.1 µg/g (IQR 0.5, 4.0) at two weeks were higher compared to healthy controls [β2M/creat ratio 0.35 mg/g (0.17, 0.58) and KIM-1/creat ratio 0.40 µg/g (0.23, 1.00); P=<0.001]. After adjusting for covariates, the eGFR and urinary B2M/creat ratio at two weeks following AKI were predictive of eGFR at one year (P<0.001). KIM-1/ creat ratios were not predictive of eGFR at one year. A urinary β2M/creat ratio of 10.85 at two weeks following AKI had 85.5% sensitivity (95% CI 74, 93) and 64.3% (95% CI 53, 75) specificity to predict CKD at one year. An eGFR cutoff of

60 mL/min/1.73 m^2 at two weeks had a sensitivity of 81.8% (95% CI 69, 90) and specificity of 71.4% (95% CI 60, 81) for predicting CKD. The presence of either one criteria (urinary B2M/creat ratio >10.85 (mg/g) or eGFR <60 mL at two weeks) had a sensitivity of 100% (95% CI 94%, 100%) in predicting CKD at one year. An eGFR <60 mL/min/1.73m^2 and elevated urinary β2M/creat ratio at two weeks following AKI is predictive of low eGFR at one year. Urinary KIM-1/creat ratios do not predict CKD progression.

Westhoff et al. [17] compared urinary calprotectin, kidney injury molecule-1, and neutrophil gelatinase-associated lipocalin for their performance in predicting mortality and need for renal replacement therapy in pediatric AKI patients. Urinary biomarker concentrations were assessed prospectively in 141 subjects aged 0-18 years including 55 patients with established AKI according to pediatric Risk, Injury, Failure, Loss, and End-stage kidney disease criteria, 27 patients without AKI, and 59 healthy children. Within the AKI group, receiver operating characteristic curve analysis revealed moderate to poor performance of calprotectin and KIM-1 in the prediction of 30-day mortality 0.55; KIM-1 AUC 0.55) and 3-month mortality and fair performance in the prediction of RRT requirement. Urinary NGAL showed good performance in predicting 30-day and 3-month mortality and moderate performance in predicting RRT.

Urinary NGAL is a good predictor of mortality performing better than pRIFLE stage, eGFR, or Creatinine, but it shows moderate performance in the prediction of dialysis.

Peralta et al. [18] investigated the levels of urinary biomarkers of tubular injury. 86 participants in the multi-ethnic study of atherosclerosis. NGAL and KIM-1 were measured at baseline, expressed as log-transformed continuous variables, and categorized into deciles. Out of 343 cases, 145 had incident CKD stage 3, 141 had rapid kidney function decrease and 57 had both. Mean eGFR for controls was 81 ± 10 mL/min/1.73 m(2) at baseline and 80 ± 10 mL/min/1.73 m(2) at follow-up compared with 82 ± 13 and 58 ± 10 mL/min/1.73 m(2) for cases. Each doubling of KIM-1 level was associated with an OR of 1.15 (95% CI, 1.02-1.29) for incident CKD stage 3 and/or rapid kidney function decrease. Compared with the lowest 90%, the highest decile of KIM-1 level was associated with an OR of 2.02 (95% CI, 1.15-3.56) for the outcome; these associations were independent of albuminuria. NGAL levels (in nanograms per milliliter) were not associated with incident CKD stage 3 and/or rapid kidney function decrease (OR, 1.04; 95% CI, 0.99-1.10). Results were similar when KIM-1 and NGAL levels were standardized for urine Creatinine. Urinary KIM-1 level is associated with future risk of

kidney disease independent of albuminuria. Urinary biomarkers of tubular injury are a promising tool for identifying persons at risk of CKD.

Seibert et al. [19] conducted a prospective study in 490 patients undergoing coronary angiography. An increase of serum Creatinine concentration ≥ 0.3 mg/dl from baseline to day 2-3 was defined as primary endpoint (CI-AKI). Urinary neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), and calprotectin were assessed < 24h before coronary angiography. Prognostic accuracy was assessed by receiver operating characteristics (ROC) calculations. 30 (6.1%) patients suffered from CI-AKI (27 AKIN stage I, 3 AKIN stage II, 0 AKIN stage III). Those subjects who developed CI-AKI had 3.1 fold higher baseline urinary NGAL/Creatinine ratios than those without CI-AKI (60.8 [IQR 18.7-93.1] μg/mg vs. 19.9 [IQR 12.3-38.9] μg/mg, p = 0.001). In those subjects without clinically overt CKD (eGFR > 60 ml/min, urinary albumin Creatinine ratio <30 mg/g), the NGAL/Creatinine ratio was 2.6 higher in CI-AKI vs. no CI-AKI (47.8 [IQR 11.8-75.3] vs. 18.6 [IQR 11.7-36.3] μg/mg). No significant differences were obtained for KIM-1 and calprotectin (p>0.05 each). ROC analyses revealed an area under the curve (AUC) of 0.68 (95% CI 0.60-0.81) for NGAL/Creatinine. An NGAL/Creatinine ratio < 56.4 μg/mg has a negative predictive value of 96.5%. In this study is the largest investigation on the use of urinary

biomarkers for CI-AKI risk stratification so far. It shows that NGAL provides prognostic information beyond the glomerular biomarkers eGFR and proteinuria.

Xue et al. [20] investigated the diagnostic performance of urinary kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) for acute kidney injury (AKI) in 90 obstructive nephropathy patients. Urine samples were obtained preoperatively and 4, 8, 12, 24, 48 and 72 h postoperatively, and urinary KIM-1 and NGAL contents were measured by enzyme linked immunosorbent assay and corrected against urine creatinine content. The receiver operating characteristic (ROC) curves were used to determine the area under the curve (AUCs) of urinary KIM-1 and NGAL for AKI. The baseline urinary KIM-1 contents were higher in AKI patients than non-AKI patients ($P < 0.01$). Urinary NGAL contents were also higher in AKI patients than non-AKI patients ($P < 0.001$). The area under the curve (AUC) of urinary KIM-1 was 0.900 ($P = 0.004$) and at a cutoff of 338.26 pg/mg Cr, the sensitivity was 90% and the specificity was 75%. The AUC of urinary NGAL was 0.900 ($P = 0.004$) and at a cutoff of 261.76 ng/mg Cr, the sensitivity was 90% and the specificity was 87.5%. The combined AUC of urinary KIM-1 and NGAL was 0.938 ($P = 0.002$) with a sensitivity of 90% and a specificity of 100%. Cox regression analysis revealed that urinary KIM-

1content 72 h after operation correlated with the prognosis of AKI patients (P = 0.009). When kidney viability was stratified by urinary KIM-1 content 72 h postoperatively, Kaplan-Meier analysis showed that patients with a urinary content of KIM-1 < 138.20 pg/mg had a higher kidney viability rate than those with a urinary content of KIM-1 > 138.20 pg/mg. Finally concluded, the urinary KIM-1 and NGAL had a good accuracy for detecting AKI. KIM-1 72 h postoperatively can predict the renal outcome of obstructive nephropathy.

Kevin et al. [21] elevated levels of B2M would associate with either the diagnosis of AKI [under current Kidney Disease: Improving Global Outcomes (KDIGO) criteria] or recovery from AKI. We performed a retrospective study, including children who had urine B2M (uB2M) and/or serum B2M (sB2M) measured by immunoturbidimetry in our clinical laboratory between January 2011 and December 2015. We defined AKI based on KDIGO criteria [increase of serum creatinine (sCr) 0.3 mg/dL over 48 h or >50% baseline over 7 days] or urine output <0.5 mL/kg/h for 24 h. Recovery from AKI was defined as a return to baseline sCr within 6 months. We calculated receiver operating characteristics (ROC) area under the curve (AUC). Of 529 patients, 245 developed AKI. Serum and uB2M associated with AKI development (AUCs 0.84 and 0.73, respectively). Patients had a graded higher median sB2M and uB2M with each higher

AKI stage. sB2M differentiated Stage I from Stage III AKI ($P < 0.001$) and Stage II from Stage III AKI ($P = 0.004$). However, neither uB2M nor sB2M levels associated with recovery from AKI. Only older age {hazard ratio [HR] 0.97, [95% confidence interval (CI) 0.94-0.99]} and need for dialysis [HR 0.39 (95% CI 0.23-0.61)] predicted incomplete recovery after AKI. Using KDIGO criteria, sB2M and uB2M associate with the severity of AKI. Given its relative ease and lower cost, we suggest more widespread use of B2M for AKI detection.

Li et al. [22] investigated whether human urinary kidney injury molecule-1 (KIM-1) is an early marker to predict CI-AKI in patients with diabetes mellitus undergoing percutaneous coronary intervention (PCI). The present study includes the general clinical data of 145 patients with diabetes mellitus who underwent PCI between March 1, 2013 and December 31, 2013. A non-ionic, low osmolarity contrast agent was used during the present study. The Scr levels and estimated glomerular filtration rate were measured prior to and within 24 and 48 h after the injection of contrast agents. Urinary samples were collected prior to and within 2, 6, 12, 24 and 48 h after the coronary interventional procedure. Simultaneously, the urinary KIM-1 values were measured using an ELISA kit. CI-AKI was diagnosed as an increase of ≥0.5 mg/dl or ≥25% in Scr concentration over baseline, 24-48 h after the procedure. In total, 19 of 145

(13.1%) patients exhibited CI-AKI. There was a significant difference ($P<0.05$) between the urinary KIM-1 levels measured 2, 6, 12, 24 and 48 h after the procedure and those prior to the procedure in the CI-AKI group. There was no significant difference between the Scr values measured 24 h after the procedure and those prior to the procedure. Evidently, using KIM-1 values to predict CI-AKI was <24 h earlier compared to using Scr values. The area under the receiver operating characteristic curve of KIM-1 24 h after the procedure was 0.856 and the 95% confidence interval of the corresponding area was 0.782-0.929. When the pivotal point of CI-AKI diagnosis was 6,327.755 pg/ml, the specificity was 85.7% and the sensitivity was 73.7%. Univariate analysis showed that the Scr concentration was positively correlated with the urinary KIM-1 level during the time prior to the procedure and 24 and 48 h after the procedure. In conclusion, the urinary KIM-1 may be a potential indicator for the early diagnosis of CI-AKI.

Kohl et al. [23] evaluated novel urinary kidney injury biomarkers focusing on early detection and better prediction of AKI with higher sensitivity and specificity. Levels of excreted urinary biomarkers correlated with severity of AKI, exhibited a dose-dependent response to sucrose treatment, and demonstrated evidence of recovery from kidney injury with transient and reversible changes. The exceptions were KIM-1

and NGAL, which showed later responses following CM and iron-induced renal injury. All biomarkers outperformed plasma Creatinine (PCr), BUN, and histopathology, with regard to practicability and/or detection of proximal tubular injury. The use of a panel of urinary kidney injury biomarkers emerged as an early, sensitive, and predictive tool to detect AKI showing enhanced sensitivity compared to current state-of-the-art markers.

Malhotra et al. [24] studied the random sample of SPRINT participants with prevalent chronic kidney disease (CKD) defined as eGFR<60mL/min/1.73m^2 by the CKD-EPI (CKD Epidemiology Collaboration) Creatinine - cystatin C equation at baseline. Urine biomarkers of tubule function and uromodulin), injury, inflammation, and repair at baseline, year 1, and year 4. Biomarkers were indexed to urine Creatinine concentration and changes between arms were evaluated using mixed-effects linear models and an intention-to-treat approach. 78 SPRINT participants (519 in the intensive and 459 in the standard arm) with prevalent CKD were included. Mean age was 72±9 years and eGFR was 46.1±9.4mL/min/1.73m^2 at baseline. Clinical characteristics, eGFR, urinary albumin-Creatinine ratio, and all 8 biomarker values were similar across arms at baseline. Compared to the standard arm, eGFR was lower by 2.9 and 3.3mL/min/1.73m^2 in the intensive arm at year 1 and year 4.

None of the 8 tubule marker levels was higher in the intensive arm compared to the standard arm at year 1 or year 4. Two tubule function markers lower at year 1 in the intensive versus standard arm, respectively.

Ghadrdan et al. [25] compared the sensitivity and specificity of urinary NGAL and KIM-1 with serum Creatinine in cisplatin related AKI. Patient's ≥18 years with solid tumors who received cisplatin-based chemotherapy were included. Urine samples were collected 0, 6 and 24 h after cisplatin infusion and the urinary NGAL, KIM-1, and Creatinine concentrations were evaluated. NGAL and KIM-1 concentrations were adjusted based on urine Creatinine to eliminate hydration effects. Serum Creatinine levels were assessed at the base and 72 h after cisplatin administration. Seven out of the 35 recruited patients (20%) suffered from AKI defined by Acute Kidney Injury Network criteria. In AKI patients, the ratio of urinary KIM-1-creatinine at 24 h compared to baseline (24 h/baseline) and NGAL-Creatinine 24 h/baseline were significantly higher than those of non-AKI group (p = 0.037 and 0.047 respectively). The area under the receiver-operating characteristic curve for KIM-1-creatinine 24 h/baseline and NGAL-Creatinine 24 h/baseline were 0.78 (0.59-0.96, p = 0.032) and 0.77 (0.57-0.97, p = 0.036) respectively. The changes in urinary NGAL-Creatinine and KIM-1-creatinine ratios, 24 h after cisplatin administration can be utilized to predict AKI in cisplatin recipients.

Latoch et al. [26] studied was to test the hypothesis whether urinary kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) are increased in acute lymphoblastic leukemia (ALL) survivors. The median time after cessation of treatment was 6.55 (IQR: 1.96-9.93) years and median age at the time of study: 12 (IQR: 6.76-16.00). The control group included 53 healthy peers. Immunoenzymatic ELISA commercial kits were used to measure urine KIM-1 and NGAL levels. The median levels of urine uNGAL ($p < 0.05$), uNGAL/Creatinine (cr.) ratio ($p < 0.0001$) and uKIM-1/Creatinine ratio ($p < 0.0001$) were significantly higher in ALL survivors in comparison with healthy controls. Female patients had significantly higher levels of NGAL and NGAL/cr. than males (mean 8.42 ± 7.1 vs. 4.59 ± 4.5 ng/mL and 86.57 ± 77 vs. 37.7 ± 37 ng/mg, respectively; $p < 0.01$). Of all the study participants, 11 (13%) presented eGFR below 90 mL/min/1.73 m^2. The NGAL/cr. ratio seemed to be the best predictor of decreased eGFR (AUC = 0.65). The cumulative dose of methotrexate and cyclophosphamide did not predict the values of the urine NGAL, NGAL/cr., KIM-1/cr. and eGFR. Five years after the end of treatment, the patients had higher levels of uKIM-1 (1.02 ± 0.8 vs. 0.62 ± 0.6 ng/mL, $p < 0.01$), uNGAL (7.9 ± 6.7 vs. 4.6 ± 5 ng/mL, $p < 0.01$) and lower eGFR (114 ± 29 vs. 134 ± 35 mL/min/1.73 m^2, $p < 0.01$) in comparison with ALL survivors with the observation period of less than 5 years. We demonstrated that ALL survivors have higher levels of urine

NGAL, NGAL/cr. and uKIM-1/cr. ratio as compared to the control group. Further long-term follow-up studies are necessary to assess the significance of the NGAL and KIM-1 and their relationship to kidney damage after anticancer treatment in childhood.

Wajda et al. [27] studied the Acute pancreatitis (AP) may be associated with severe inflammation and hypovolemia leading to organ complications including acute kidney injury (AKI). 69 patients with mild to severe AP admitted to a secondary care hospital during the first 24 h from initial symptoms of AP. KIM-1 was measured in urine samples collected on the day of admission and two subsequent days of hospital stay. AKI was diagnosed based on Creatinine increase according to Kidney Disease: Improving Global Outcomes 2012 guidelines. Urinary KIM-1 on study days 1 to 3 was not significantly higher in 10 patients who developed AKI as compared to those without AKI and did not correlate with serum Creatinine or urea. On days 2 and 3, urinary KIM-1 correlated positively with urinary liver-type fatty acid-binding protein, another marker of tubular injury. On days 2 and 3, urinary KIM-1 was higher among patients with systemic inflammatory response syndrome and several correlations between KIM-1 and inflammatory markers (procalcitonin, urokinase-type plasminogen activator receptor, C-reactive protein) were observed on days 1 to 3. With a limited number of patients, our study cannot exclude the

diagnostic utility of KIM-1 in AP, however, our results do not support it. We hypothesize that the increase of KIM-1 in AKI complicating AP lasts a short time, and it may only be observed with more frequent monitoring of the marker. Moreover, urinary KIM-1 concentrations in AP are associated with inflammation severity.

SNAKEBITE AND ACUTE KIDNEY INJURY

The World Health Organization (WHO) estimates that about 5 million snakebites occur each year, resulting in up to 2.7 million envenomings. Published reports suggest that between 81,000 and 138,000 deaths occur each year. Snakebite envenoming causes as many as 400,000 amputations and other permanent disabilities. Many snakebites go unreported, often because victims seek treatment from non-medical sources or do not have access to health care. As a result it is believed that many cases of snakebite go unreported.

Snake antivenoms are effective treatments to prevent or reverse most of the harmful effects of snakebite envenoming. They are included in the WHO Essential Medicines List and should be part of any primary health-care package where snake bites occur.

Unfortunately many people either lack access to antivenom, or cannot afford to pay for them. Many families sell possessions or go into debt in order to obtain antivenom after someone is bitten. Difficulties in

ensuring proper regulation and testing of antivenoms also affect the availability of good quality, effective products.

WHO added snakebite envenoming to its priority list of neglected tropical diseases (NTDs) in June 2017. A nationally representative study(Million Death study) noted--45,900 annual snakebite deaths nationally. In India, around 90% of snakebites are caused by the 'big four' among the crawlers - common krait, Indian cobra, Russell's viper and saw scaled viper. Effective interventions involving education and antivenom provision would reduce snakebite deaths in India.

Epidemiology of snakebite:-

Snakebite is observed all over the country with a rural / urban ratio of 9:1. They are more common during monsoon and post monsoon seasons. Snakebites are seen often among agricultural workers and among those going to the forest. Many of the susceptible populations are poor living below poverty line, living in rural areas with less access to health care.

The male / female ratio among the victims is approximately 3:2. Majority are young and their age is between 25 to 44 years. Most of the bites (90 to 95%) are noticed on the extremities (limbs). The hospital stay varies from 2 to 30 days, with the median being 4 days. The in-hospital mortality varies from 5 to 10%, and the causes are acute renal failure, respiratory failure, sepsis, bleeding and others.

Classification of Snakes:-

There are more than 3000 spices of snakes in the world. For the purpose of clinical practice, snakes are classified into poisonous (venomous) and non-poisonous (nonvenomous) snakes. Poisonous snake are classified into three families and they are

- Cobra group [Elapidae]
- Viper group [Viperidae]
- Sea snake group [Hydrophidae]

For many decades, the concept of the “Big 4” snakes of medical importance has reflected the view that 4 spices and responsible for Indian snakebite mortality. They are the Indian Cobra (Naja naja), the common Krait (Bungarus caeruleus), the Russell’s viper (Daboia russelii) and the Saw scaled viper (Echis carinatus). However, recently another species, the Hump-nosed pit viper (Hypnale hypnale), has been found to be capable of causing lethal envenomation, and that this problem had been concealed by systematic misidentification of this species as the saw-scaled viper.

The concept of the “Big 4” snakes has failed to include all currently known snakes of medical significance in India. This has a negative effects on clinical management of snakebite and the development of effective snake anti venoms.

Categorisation of snakes (W.H.O. 1981)

Class	Details	Name of the snakes
I	Commonly cause death or serious disability	Russell’s viper / Cobra / Saw scaled viper
II	Uncommonly cause bites but are recorded to cause serious effects (death or local necrosis)	Krait / Hump-nosed pit viper / King cobra / Mountain pit viper
III	Commonly cause bites but serious effects are very uncommon.	Water snakes, Green snakes

Russell’s viper (Daboia russelii):-

The Russell’s viper is a stout bodied snake, the largest of which grows to approximately 1.8 meters in length. Like all vipers it is a nocturnal snake, but unfortunately for humans, during the daytime it rests up under

bushes, at the base of trees and in leaf litter. It is therefore frequently encountered by rural workers, as they are carrying out general agricultural activities.

There are two key identification features that are worth nothing. The first is a series of chain –like or black edged almond shaped marks along the snakes back and flanks. The second distinguishing mark is a white triangular mark on the head with the apex of the triangle pointing towards the nostrils.

Saw scaled Viper:-

The southern Indian Saw Scaled Viper is a small snake, usually between 30 and 40 centimeters long. The Northern Indian species (Echis sochureki) is much larger, with an average size of 60 centimeters. It in habits mainly dry arid climates but can also be found in scrub land.

One of the key identification features of this species is the posture it adopts when it is agitated. It moves its body into a figure of eight like arrangements with its head at the center. It rapidly moves its coils against each other and produces a hissing like sound which gives its name of ‘Saw scaled’ in addition, there are often wavy hoop like markings down both sides of the saw scales body. On the head, there is usually a white or cream arrow shaped mark, pointing towards the front of the head, often compared to the shape of the birds foot.

The Hump-Nosed Pit Viper (Hypnale hypnale):-

The hump nosed pit viper is one of India's tiniest venomous snakes, its total length ranging from 28.5 to 55cm. Its distinctive features include the presence of five large symmetrical plate scales on the top of the head in addition to the smaller scales typical of all vipers. There are heat sensitive pits between the nostril and the eye.

Spectacled Cobra (Naja naja):-

The spectacle Cobra, is probably India's most well recognized snake. The hood markings of the spectacle like mark, distinguishes this snake from other species, and its habit of rearing up when alarmed makes it distinctive but not definitive as other species do this, notably the Trinket snake. The cobra's coloration may vary from pale yellow to black.

Common Krait (Bungarus caeruleus):-

The Common krait is a nocturnal snake which usually grows to approximately 1.0 to 1.2 meters in length. Its primary diet is other snakes. It can be found all over peninsular India and often seeks habitation near human dwellings. During the day it rest up in piles of bricks, rat burrows or other buildings. The common krait is the most poisonous snake in India and its venom is pre-synaptic neurotoxic in nature.

There are number of key identifiers which are worth remembering. The krait is black, sometimes with the bluish tinge, with a white belly. Its

markings consist of paired white bands which may be less distinct anteriorly. These paired white bands distinguish the snake from another black nocturnal snake, The Common wolf snake. The wolf snake's white bands usually are thicker and are singular bands equidistant from each other. The useful distinguishing feature is a series of hexagonal scales along the top of the snakes back. This feature is really useful if the dead snake has been brought to the hospital and examined.

King Cobra:-

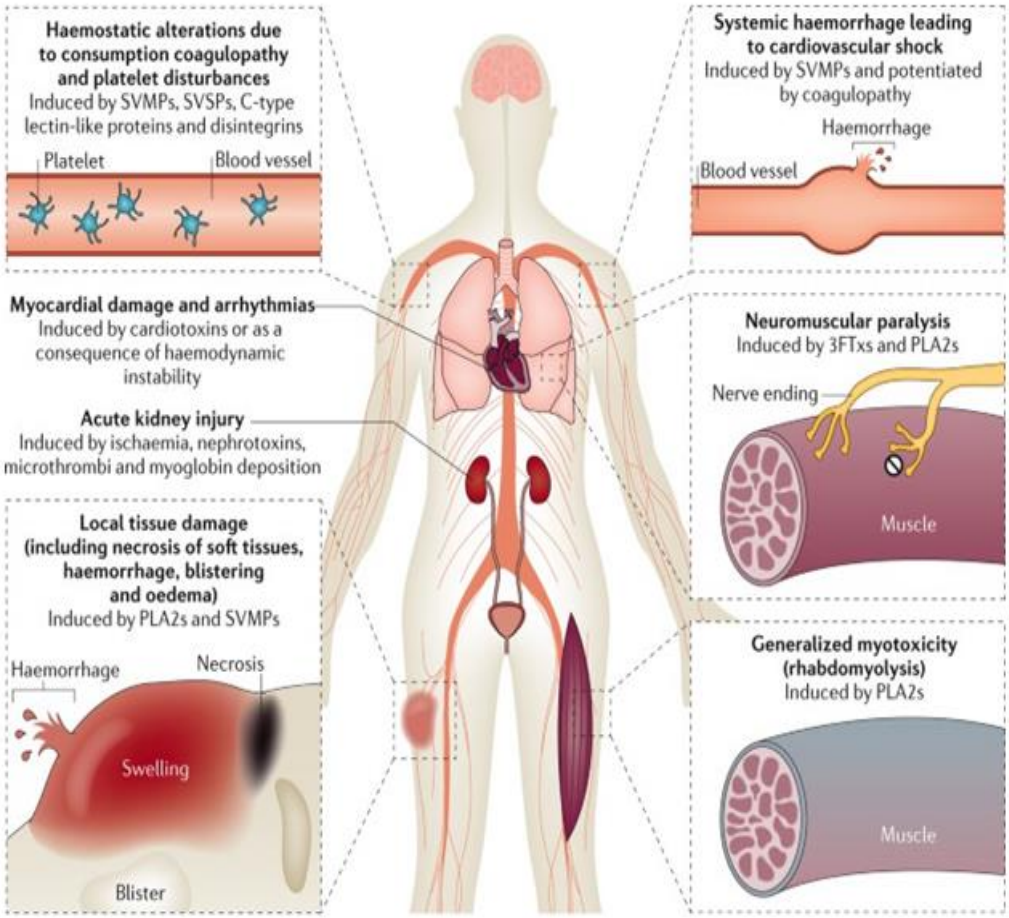
The King Cobra is the least medically significant of the venomous snakes in India in terms of both bites and fatalities. Hence, descriptive features of this are not provided here.

Clinical aspects of Snake Bite:

Snake venom is mostly watery in nature. It consists of numerous enzymes, proteins, amino acids, etc., Some of the enzymes are proteases, collagenase, arginine ester hydrolase, hyaluronidase, phospholipidase, metallo-proteinases, endogenases, autocoids, thrombogenic enzymes, etc., These enzymes also act like toxins on different tissues of the body, and are grouped under neurotoxins, nephrotoxins, hemotoxins, cardiotoxins, cytotoxins etc., resulting in organ dysfunction / destruction. Enormous clinical and experimental works have been published on the pathophysiology of snake bite in relation to different species of snakes.

Hyaluronidase allows rapid spread of venom through subcutaneous tissues by disrupting mucopolysaccharides, and phospholipase A2 has esterolytic effect on the red blood cell membrane and causes hemolysis. It also promotes muscle necrosis.

Thrombogenic enzymes promote formation of weak fibrin clot, which activities plasmin and results in consumptive coagulopathy and hemorrhagic consequences. Venom of some snakes causes neuromuscular blockade at pre or post synaptic level. In addition to above it causes endothelial cell damage which results in increased vascular permeability. In short, snake venom acts on various parts / systems / organs of the body. Venom also causes endothelial cell damage which results in increased permeability



Symptoms and signs:-

An international expert on snakebite, the late Dr. Alistair Reid of the Liverpool School of Tropical Medicine found out that only 10 to 15% of venomous bites end in death. The possibility of survival, even without treatment, is incredibly good in 80-90% of cases. One of the reasons for this is that much snakebite are by non-venomous snakes. Secondly, a large percentage of venomous snakebites are dry bites i.e., the snake does not always inject venom. Sometimes, it might inject only a tiny quantity of venom.

The snake can inject the quantity of venom it wants. This is an entirely voluntary process. Hence, one can never know how much venom was injected except by observing the progression of the symptoms. In other words the recovery in snakebite without even treatment is great. Every traditional healer uses this fact to his / her advantage and propagates his /her own method to treat snakebite viz., herbal details, “snakestone” or mantra, or plain soda water and most villagers would be happy to go to him.

Also, everyone should remember the systemic action of venom and the extent varies from one snake to another. Complications and outcome due to snakebite may also vary from each other and can’t be predicted by any means.

Moreover, the status of poisoning cannot be judged by the bite mark, reaction to envenomation, size or the type of snake. Hence, one has to observe for signs and symptoms which may develop within 24 to 48 hours. The symptoms and signs of Viperine and Elapid envenomation as well late-onset envenomation are listed below.

General symptoms and signs of Viperine envenomation:

Local effects:

Swelling and local pain with or without erythema or discoloration at the site if bite.

Tender enlargement of local lymphnodes as large molecular weight Viper venom molecules enter the system via the lymphatics.

- ✓ Effects due to coagulopathy and hemorrhagic consequences
- ✓ Bleeding from the gingival sulci and other orifices.
- ✓ Epistaxis.
- ✓ The skin and mucous membranes may show evidence of petechiae, purpura and ecchymoses.
- ✓ The passing of reddish or dark-brown urine or declining or no urine output.
- ✓ Lateralising neurological symptoms and asymmetrical pupils may be indicative of intra-cranial bleeding.
- ✓ Vomiting.
- ✓ Acute abdominal tenderness which may suggest gastro-intestinal or retro peritoneal bleeding.
- ✓ Hypotension resulting from hypovolaemia or direct vasodilation.
- ✓ Low back pain, indicative of early renal failure or retroperitoneal bleeding.

Other effects:-

- ✓ Muscle pain indicating rhabdomyolysis.
- ✓ Parotid swelling, conjunctival oedema, sub-conjunctival haemorrhage.
- ✓

General symptoms and signs of Elapid envenomation:-

Local effects:-

- Swelling and local pain with or without erythema or discoloration at the site if bite.(Cobra).
  - Local necrosis and / or blistering / (Cobra).

Neurotoxic effects

- ✓ Descending paralysis, initially of muscles innervated by the cranial nerves, commencing with ptosis, diplopia, or ophthalmoplegia. The patient complains of difficulty in focusing and the eyelids feel heavy. There may be some involvement of the senses of taste and smell.
- ✓ Problems of vision, breathing and speech.
- ✓ Paralysis of jaw and tongue may lead to upper airway obstruction and aspiration of pooled secretions because of the patient's inability to swallow.
- ✓ Numbness around the lips and mouth, progressing to pooling of secretions, bulbar paralysis and respiratory failure.
- ✓ Hypoxia due to inadequate ventilation can cause cyanosis, altered sensorium and coma. This is a life threatening situation and needs urgent intervention.
- ✓ Paradoxical respiration, as a result of the intercostal muscles paralysis is a frequent sign.

- ✓ Krait bites often present in early morning with paralysis that can be mistaken for a stroke. Stomach pain which may suggest submucosal haemorrhages in the stomach

Other effects

- ✓ Stomach pain which may suggest submucosal haemorrhages in the stomach (Krait).
- ✓ Eye pain and damage due to ejection of venom into the eyes by spitting cobra(as observer in Africa)
- ✓ [If failure of renal failure are noted search for others causes / mechanisms]

Late-onset envenomation:-

The patient should be kept under close observation for at least 24 hours. Many species, particularly the Krait and the Hump-nosed pit viper are kown for the length of time it can take for symptoms to manifest. Often this can take between 6 to 12 hours. Late onset envenoming is a well-documented occurrence. This is also particularly pertinent at the start of the rainy season when generally give birth to their young. Juvenile snakes(young ones), 8-10 inches long, tend to bite the victim lower down on the foot in the hard tissue area, and thus any signs of envenomation can take much longer to appear.

Overlapping symptoms and signs:-

Russell's viper envenomation can also manifest with neurotoxic features. This can sometimes cause confusion and further work is necessary to establish how wide this might be. Development of neurotoxic features in Russell's viper bite are believes to be pre synaptic or Krait like in nature. It is for this reason that a doubt is expressed over the response of both species to Neostigmine

Feature	Cobras	Kraits	Russell's Viper	Saw Scaled Viper	Hump Nosed Viper
Local pain/Tissue Damage	YES	NO	YES	YES	YES
Ptosis / Neurological Signs	YES	YES	YES!	NO	NO
Haemostatic abnormalities	NO*	NO!	YES	YES	YES
Renal Complications	NO	NO*	YES	NO*	YES
Response to Neostigmine	YES	NO?	NO?	NOT applicable	NOT applicable
Response to ASV	YES	YES	YES	YES	NO

Sea snakes:-

Sea snake bites are reported rarely among fishermen and / or their family members living in the seashore area as well as among those who walk on the seashore. To begin with there may be local pain which may be insignificant which appears within 60 to 90 minutes. There may not be obvious local swelling. Systemic manifestations noticed among poisonous sea snake bite are neurological involvement, severe muscle pain, rightly, renal failure, hyperkalemia and finally cardiac arrest.

Criteria for diagnosis

- Pain-pain at the site of bite, swelling and regional lymphnode
- Oozing - sero / sanguinous oozing from the site of bite
- Node- development of an enlarged tender lymphnode draining the bitten limb
- Discoloration- discoloration at the site of bite
- Swelling – swelling is seen at the site of the bites on the digits (toes and especially fingers); local swelling develops in more than half of the bitten limb immediately (in the absence of the tourniquet) and swelling extends rapidly beyond the site of bite (eg. beyond the wrist or ankle within a few hours of bites on the hands or feet).

Complications and Outcome:

Complications in snake envenomation are due to the hetrogenous composition of the venom. In addition the quantity and quality of the venom and the response of the individual to the components of venom influence the clinical course, complications and outcome. The complications of venom are observed in various systems viz., the hematological, vascular, renal, respiratory, cardiovascular, endocrine, gastrointestinal muscular and dermatological system.

In addition to the anti-snake venom, the envenomed individual requires supportive treatment for the complications arising out of snakebite as well as the consequences of the complication. One must also remember to look for complications developing after infusion of Inj. anti snake venom and get prepared to treat them also.

The outcome of snakebite depends upon amount of envenomation, bite to needle time, individual's response to envenomation, the complications that develop following snakebite and response to treatment. Till the patient has recovered, one cannot predict the complications and outcome.

Investigations:-

- ✓ 20 Minutes Whole Blood Clotting Test (20WBCT
- ✓ If the 20WBCT is normal in a suspected case of poisonous snakebites, the test should be carried out every 30 minutes from admission for three and then hourly after that. If incoagulable blood is discovered, the 6 hourly cycles will then be adopted to test for the requirement of repeat doses of ASV. This is due to the inability of the liver to replace clotting factors under 6 hrs.
- ✓ Other Useful Tests:
- ✓ Clinical test:
- ✓ PP /BP/ RR / Postural Blood Pressure
- ✓ Laboratory studies:
- ✓ Haemoglobin /PCV / Platelet Count / PT / APTT/FDP/D-Dimer
- ✓ Peripheral Smear/ Blood grouping/ Rh typing
- ✓ Urine Tests for Proteinuria/RBC/Haemoglobinuria/Myoglobinuria
- ✓ Biochemistry for Serum Creatinine/Urea / Electrolytes / Oxygen Saturation.

Imaging studies:

- ✓ X-Ray Chest /CT / Ultrasound(whenever required)

Others:

- ✓ Electrocardiogram
- ✓ Special investigations depending upon clinical status
- ✓ Ocular fundus examination

Treatment:

First aid for snake bite

R.=Reassure the patient

(70% of all snakebites are from non-venomous species. Only 50% of bites by venomous species actually envenomate the patient)

I=Immobilise in the same way as a fractural limb.

(Use bandage or cloth to hold the splints, not to block the blood supply or apply pressure. Do not apply any compression in the form of tight ligatures, they don't work and can be dangerous!)

G.H.=Get to Hospital Immediately.

(Traditional remedies have NO PROVEN benefit in training snakebite).

T=Tell the doctor of any systemic symptoms such at ptosis that manifest on the way to hospital.

This method will get the victim to the hospital quickly, without recourse to traditional medical approaches which can delay effective treatment.

Traditional first aid methods followed for snakebite:

The traditional methods such as application of tourniquet, cutting (incision) and suction, washing the wound, snake stone or other methods have adverse effects and hence, they have to be discarded and these are described below

Washing the Wound:

Victims and bystanders have a tendency to wash the wound to remove any venom on the surface. This should not be done as the action washing increases the flow of venom into system by stimulating the lymphatic system.

Household remedies:

Various forms of household remedies are applied to the site of bite which may enhance absorption of venom

Cutting and Suction:

Cutting the site of bite and suctioning incoagulable blood increases the risk of bleeding to death as well as increases the risk of infection. Venom is not cleared or removed from the snakebite by this method.

Snake stone:

Snake stone is applied to the site of bite saying that it will absorb the venom and falls once the venom is absorbed. This contributes to delay in seeking appropriate health care.

Tourniquets:

Tight tourniquets made of rope, string and cloth, have been followed traditionally to stop venom flow into the body following snakebite.

The problems noticed with tourniquets are:-

- Risk of ischemia and loss of the limb
- Risk of necrosis
- Risk of massive neurotoxic blockade
- Risk of embolism if used in viper bites
- Release of tourniquet may lead to hypotension
- Gives patient a sense of false security, which encourages them to delay their journey to hospital.

Treatment:-

- While dealing with a case of snake bite consider the mnemonic 'RASI'
- Remember principles("12 As")
- Address the problems – clinical and social
- Seek help from others when required and
- Inform the patient and/or case givers on the status of illness, clinical course, management, outcome, welfare measures and follow up clearly with empathy.

1. Admit the victim immediately
2. Ask effectively
3. Assess quickly
4. Act swiftly
5. Administer medication meticulously.
6. Address to the wound properly
7. Anticipate complications keenly
8. Avoid errors carefully
9. Ascertain the status repeatedly
10. Amicable with patients and care givers and show empathy
11. Advise on follow up accordingly

Pharmacological aspects of Anti snake venom:-

The goals of pharmacotherapy with injection Anti snake venom (ASV) are to neutralise the venom, reduce morbidity and mortality, and prevent complications. Currently available Anti Venom (ASV) in India polyvalent i.e., it is effective against all the four common species; Russell's viper (Daboia russilii,) common Cobra (Naja naja), Common Krait (Bungarus caeruleus) and Saw Scaled Viper (Echis carinatus). Indian ASV is a F (ab)2 product derived from horse serum and has a half-life of over 90 hours. Though it is purified, it still can be immunogenic.

At present, no monovalent ASV is available primarily because there are no objective means of identifying the snake species, in the absence of the dead snake. Moreover it is difficult for the physician to determine which type of Monovalent ASV to employ in treating the patient. In addition there are difficulties to prepare, supply and maintain adequate stock of species monovalent ASV.

There are other known species such as the Hump-nosed pit viper (Hypnale hynale) where polyvalent ASV is known to be ineffective.

The recommended dosage level has been based on published research that Russell's viper injects on average 63mg (SD 7) of venom. Logic suggests that our initial that the majority if victims should be covered

by the initial dose and keeps the cost os ASV to acceptable levels. The range of venom injected is 6mg to147mg.

One vial of ASV neutralizes 6mg of Russell's viper venom. So, to neutralize 63mg of venom, 10 vials are needed. Not all victims will require 10 vials as some may be injected with less than 63mg.

They may also pre-sensitise the patient and thereby create greater risk. For Neurotoxic /Anti Haemmostatic envenomation, 8 to 10 vials of ASV are recommended to be administered as in initial dose. Children receive the same ASV adults, as snakes inject the same amount of venom into adults and children. The ASV targeted at neutralizing the venom

ASV may be administered in two ways over a period of one hour at a constant speed and the patient should be closely monitored for 2 hours.

ACUTE KIDNEY INJURY

Epidemiology

AKI complicates 5-7% of acute care hospital admissions and upto 30 % of admission to intensive care unit. In India acute kidney injury constitutes 1.5% all general hospital admissions of which 60 % are due to medical causes, most common causes are acute diarroheal diseases, sepsis, infection, malaria, urinary tract infections, pneumonia, viral hepatitis, snake bite, cardiac failure, renal failure, Diabetes mellitus, nephrotoxic drugs, malignancy , hypertension, major surgery like whipples procedure.

Advanced age, liver diseases, underlying comorbid illness (diabetes mellitus/hypertension/ischemic heart

Disease/chronic obstructive pulmonary disease & cirrhosis) have been implicated as a risk factor for the development of AKI. AKI increases the risk for the development or worsening of chronic kidney disease (AKI). Patients who recover or survive from an episode of AKI are at increased risk for dialysis requiring end stage kidney disease. AKI is major medical complication in the developing world, where the epidemiology differs from the developing countries due to differences in demographics, economics, environmental factors and comorbid disease burden. The pathophysiology of AKI leads to increased hydrostatic pressure in renal tubules and decreased GFR. Inspite of recent advances in renal replacement therapy, mortality due to AKI is high. Poor prognostic factors of AKI are Age >65 years, Acute Respiratory Distress Syndrome, Multi organ dysfunction syndrome.

CAUSES OF AKI

AKI can be community acquired or hospital acquired. Community acquired AKI causes;

- Volume depletion
- Heart failure
- Adverse effects of medications

- Obstruction to urinary tract
- Malignancy
- Hospital acquired AKI causes - sepsis
- Major surgical procedures
- Heart or liver failure
- Nephrotoxic medications

AKI is divided into three categories:

1. Diseases characterized by renal hypoperfusion where integrity of renal parenchyma is preserved (Prerenal)
2. Diseases involving renal parenchyma (Intrinsic)
3. Diseases involving urinary tract obstruction (Postrenal)

AKI is often reversible, but it can lead to significant irreversible damage that leads to chronic kidney disease with associated in hospital morbidity & mortality based on the presence or absence of non renal organ dysfunction. There is a lack of a uniform clinical presentation with variation in nature of injury. AKI can be oliguric or non oliguric which depends upon daily urine excretion which has got a prognostic significance.

- ✓ OLIGURIA-urine output less than 400 ml/day.
- ✓ ANURIA-urine output less than 100 ml/day; abrupt onset suggests bilateral obstruction and catastrophic injury to both kidneys.

Etiology and Pathophysiology

Prerenal

Adaptive response to severe volume depletion & hypotension, with structurally intact nephrons

Intrinsic

Occurs in response to ischemic, cytotoxic or inflammatory insult to the kidney, with structural and functional organ damage.

Postrenal

Arises due to obstruction to outflow of urine.

Increased Extracellular Fluid Loss

Gastrointestinal fluid loss (diarrhoea, vomiting), hemorrhage, renal fluid loss, nephrogenic diabestes insipidus, hypoadrenalism, osmotic diuresis.

Reduced Intake

Altered mental status, hypovolemia,

Extravascular Sequestration

Burns, severe hypoproteinemia, pancreatitis.

Low Cardiac Output State

- ✓ Pericardial diseases (cardiac tamponade)
- ✓ Diseases of the myocardium or valves
- ✓ Impaired venous return (positive pressure ventilation or abdominal compartment syndrome)
- ✓ Pulmonary hypertension
- ✓ Massive pulmonary embolism

Renal Vasoconstriction

- ✓ Hypocalcemis
- ✓ Calcineurin inhibitors
- ✓ Catecholamine
- ✓ Amphotericin B

Systemic Vasodilatation

- ✓ Sepsis
- ✓ Antihypertensive
- ✓ Anaphylaxis

Impaired Renal Auto regulation

- ✓ Cyclooxygenase inhibitors
- ✓ Angiotensin converting enzyme inhibitors
- ✓ Hepatorenal syndrome

- ✓ Hypotension and altered hemodynamics- hemorrhage, vasodilation and increased vascular permeability.

Prerenal Azotemia

Prerenal azotemia (azo meaning nitrogen; emia meaning in the blood) is the most commom form of AKI. By definition prerenal azotemia involves no parenchymal damage to the kidney and it is rapidly reversible once intraglomerular hemodyanamics and parenchymal blood flow is restored. The common clinical conditions associated with prerenal azotemia are hypovolemia, and medications that interfere with renal auto regulatory responses such as non steroidal anti-inflammatory drugs (NSAIDS) and inhibitors of angiotensin. It is caused due to inadequate plasma flow with rise in blood urea and serum Creatinine. Prerenal azotemia may coexist with other forms of intrinsic AKI with processes affecting directly the renal parenchyma.

Prerenal AKI represents the most common of kidney injury and leads to intrinsic AKI if not promptly treated. Volume loss from gastrointestinal tract, renal, burns, hemorrhage can cause AKI. It is caused due to decreased renal perfusion.

Pathophysiology

In prerenal AKI, restoration of normal renal perfusion results in early recovery of renal function but sustained renal hypoperfusion results

in irreversible renal injury. Arterial blood volume depletion or decreased renal perfusion is explained by activation of renin angiotensin system and sympathetic nervous system.

Increased angiotension 2 level causes constriction of pre-glomerular and post-glomerular arterioles (predominance of which maintain intraglomerualr capillary pressure to normal and maintains normal GFR) which is opposed by vasodilator prostaglandins.

Hemodyanamic factors, sympathetic nervous system activation, increased levesl of angiotensin 2, will increase proximal tubular sodium and water reabsorption. In prerenal AKI, the regulatory mechanisms are unable to compensate fully for more severe degrees of hypoperfusion, which leads to decline in glomerular filtration rate. Auto-regulation is the first line of defence by kidney against fluctuations in arterial blood pressure. When renal perfusion decreases, the afferent arteriole senses the degree of stretch and thus relaxes. This is called “myogenic reflex”.

Tubulo-Glomerular Feedback

Also plays an important role in autoregulation. Macula densa presenting in the cortical collecting ducts senses the decrease in solute delivery to the distal tubules and leads to dilatation of afferent arteriole by nitic oxide release.

CAUSES OF INTRINSIC AKI

The hallmark of intrinsic AKI is structural injury in the kidney acute tubular injury (ATN), ischemic or cytotoxic is the most common form. Frank necrosis is not obvious in most cases, it can be patchy. These changes are predominately observed in glomeruli.

RENOVASCULAR CAUSES: (bilateral/unilateral in the setting of one kidney)

• Atherosclerotic plaque

• Large vessel vasculitis

• Dissecting aneurysm

• Thrombosis

• Embolism

• Obstruction of renal vein –thrombosis and compression

DISEASES OF GLOMERULAR VASCULATURE:

• Glomerulonephritis

• Disseminated intravascular coagulation

• Thrombotic microangiopathy

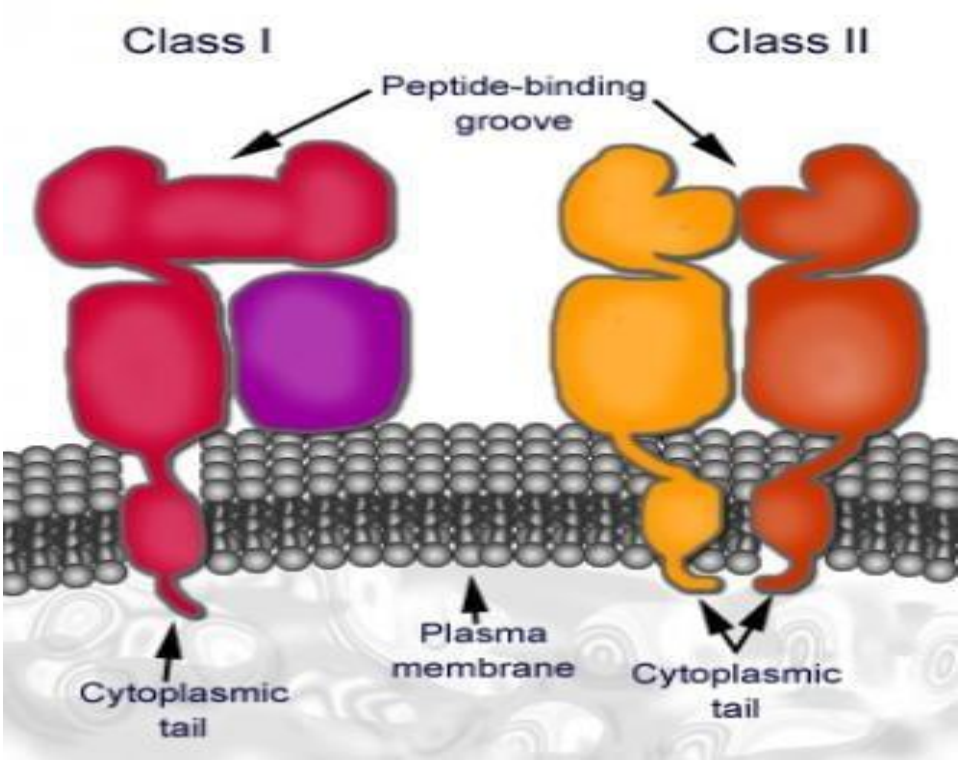
• Preeclampsia

• Collagen vascular diseases (systemic lupus erythematosus, scleroderma)

• Malignant hypertension

BETA 2 MICROGLOBULIN

Beta 2 microglobulin (β2M) is a small protein (11,800 Dalton), presenting in nearly all nucleated cells and most biological fluids, including serum, urine, and synovial fluid. No genetic variant of β2M is known in human. The human β2M shows 70% amino acid sequence similarity to the murine protein and both of them locate on the syntenic chromosomes.The secondary structure of β2M consists of seven β-strands which are organized into two β-sheets linked by a single disulfide bridge, presenting a classical β-sandwich typical of the immunoglobulin (Ig) domain. β2M has no transmembrane region and contains a distinctive molecular structure called a constant-1 Ig superfamily domain, sharing with other adaptive immune molecules including major histocompatibility complex (MHC) class I and class II.



Two evolutionary conserved tryptophan (Trp) residues are important for correct structural fold and function of β2M. Trp60 is exposed to the solvent at the apex of a protein loop and is critical for promoting the association of β2M in MHC I. The mutation of Trp60 increases the stabilization of β2M, inhibits β2 amyloidogenic propensity, and weakens the interaction with the heavy chain of MHC I. Trp95 is buried in the β2M core, and the mutation of Trp95 destabilizes the protein, yielding nonfibrillar β2M aggregates. Both Trp residues play differential and complementary roles in the structure of β2M, distinctly affecting β2M toward self-aggregation into amyloid fibrils. Once the aspartate residue is replaced by asparagine residue at position 76, β2M becomes

thermodynamically unstable and remarkably fibrillogenic *in vitro* under physiological conditions.

Normally, β2M is noncovalently linked with the other polypeptide chain (α chain) to form MHC I or like structures, including MHC I, neonatal Fc receptor (FcRn), a cluster of differentiation 1 (CD1), human hemochromatosis protein (HFE), Qa, and so on. β2M makes extensive contacts with all three domains of the α chain. Thus, the conformation of α chain is highly dependent on the presence of β2M. Although α1 and α2 domains differ among molecules, α3 domain and β2M are relatively conserved, where the intermolecular interaction occurs. A number of residues at the points of contact with β2M are shared among MHC I or like molecules. Furthermore, interactions with α1 and α2 domains are important for the paired association of α3 domain and β2M in the presence of native antigens.β2M could dissociate from such molecules and shed into the serum, where it is transported to the kidneys to be degraded and excreted. An 88-kD protein (calnexin) associates rapidly and quantitatively with newly synthesized murine MHC I molecules within the endoplasmic reticulum. Both β2M and peptide are required for efficient calnexin dissociation and subsequent MHC I transport.

Not only β2M is to interact with and stabilize the tertiary structure of the MHC I or like molecules, but also it is extensively involved in the

functional regulation of survival, proliferation, apoptosis, and even metastasis in cancer cells.As well as a cancer prognostic marker, β2M is also a promising cancer therapeutic target. Although β2M acts as both a positive and negative growth factor in different cancer cells, the application of anti-β2M antibodies induces cancer cell apoptosis and do not block the down-regulation effect of β2M in myeloma cells. Moreover, systemic β2M accumulation in aging blood promoted age-related cognitive dysfunction and impairs neurogenesis, suggesting that β2M may be targeted therapeutically in old age. Thus, targeting β2M will shed light on the modulatory activity in the immune system and provide new pathways on cancer or aging-related therapeutics.

Indications from the Level of Free Beta 2 Microglobulin

The abnormal level of β2M in blood or urea is associated with multiple diseases, such as some acute and chronic inflammations, liver or renal dysfunctions, some viral infections, and several malignancies. Furthermore, amyloidosis associated to hemodialysis is related with persistently high β2M serum levels. In rare cases, a cerebrospinal fluid β2M level is used to assess a disease involved with the central nervous system.

Serum and plasma β2M values reflect the activation of the cellular immune system, as well as a tumor marker in certain hematologic

malignancies. For the inflammatory bowel disease, β2M was suggested to be used as an activity parameter. β2M levels also rise during infection with some viruses, including cytomegalovirus and human immunodeficiency virus (HIV). Strong evidence showed cytomegalovirus could directly bind β2M via two envelope proteins. Recently, soluble β2M was proposed as a possible serologic marker of neurologic disease during the infection of human T-cell leukemia virus. On the other hand, abnormality of urine β2M values indicates renal filtration or reabsorption disorders. The small size of β2M allows it pass through the glomerular membrane, however, it can be reabsorbed in the proximal tubules by specific receptors. The disorder of kidney's glomeruli would cause increased β2M in blood and decreased β2M in urea, in contrast, the disorder of kidney's tubules would cause increased β2M in urea and decreased β2M in blood. In lupus nephritis and neonates, the index of serum β2M/cystatin C is suggested to indicate the renal function. Moreover, serum β2M levels at discharge would predict the long-term mortality and graft loss in kidney transplantation recipients. A large nationally representative cohort exhibited serum β2M concentration was associated with a significantly increased risk of cardiovascular and all-cause mortality. Recently, the concentration of β2M was also deemed as a marker of frailty in older people. Thus, the β2M test could indicate how advanced the disease is and the likely prognosis for the patient at the time of diagnosis.

MATERIALS AND METHODS

5.1 Subjects and Schemes:

Study conducted in Government Rajaji Hospital, Madurai for the duration of 12 months (May 2021 to June 2022).

Study Design **:** Prospective observational study of 50 patients within the period of one year.

Study centre **:** In ward patients of Department of general Medicine, Government Rajaji Hospital, Madurai

Study Population : All patients admitted to medical wards with AKI due to hemotoxic snake envenomation.

5.2 Inclusion Criteria

- Patients who undergone hemodialysis for AKI due to hemotoxic snake envenomation
- Age >or= 13yrs to 60 yrs

5.3 Exclusion Criteria

- Patients with previous history of CKD were excluded.
- Patients with comorbidities like DM, Hypertension, heart failure, CLD, CAD, Tuberculosis and CVA

- Patients with USG abdomen shows abnormalities in kidneys like bipolar length of kidneys , cysts, stones, or any other structural abnormalities of kidneys.
- Patients on Nephrotoxic medications.
- Patient with septic shock, MODS
- Patient with hypotension
- Patient with obstructive uropathy, glomerular diseases
- Patient with previous history of AKI due to any cause
- Patient with rheumatological systemic disorders
- Patient with snakebite AKI not undergone hemodialysis
- Patient with neurotoxic snake bite
- Patient on mechanical ventilator support
- Patient with renal artery stenosis
- Patient who developed anaphylactic shock to ASV

5.4 Methodology

- All patients admitted to medical wards with AKI (defined as per KDIGO 2012) due to hemotoxic snake envenomation were screened. Hemotoxic envenomation was defined as the presence of whole blood clotting time >20 minutes in patients who sustained a snake bite.
- Demographic, clinical, laboratory details and periodic assessment of eGFR, BP, urine albumin excretion, Sr.urea, Sr.creatinine and urine β2m

are collected at the time of enrollment and periodically at end of 2 weeks , 12 weeks and 24 weeks from diagnosis of AKI respectively.

- An ultrasound examination of the abdomen to assess kidney size was performed before recruitment.
- Urine b2m will be estimated by CLIA method, serum Creatinine by modified jaffe's method, eGFR by CKD-EPI formula, urine albumin by nephelometry.

5.5 Parameters observed

- Complete blood count
- Complete hemogram
- RBS
- RFT, LFT ,Sr. Electrolytes, sr.LDH
- Urine routine and albumin
- Urine beta 2 microglobulin
- eGFR
- Whole blood clotting time
- USG abdomen and pelvis
- Sr.CPK

STATISTICAL ANALYSIS

6.1 TERMINOLOGY

6.1.1 Mean

The mean is the average of the data points, it is denoted $\bar{x}$. There are three types of data for which we would like to compute the mean, ungrouped of frequency 1, ungrouped with a frequency distribution, and grouped.

Starting with the first type, ungrouped of frequency 1, is when data is given to you as a list and it is not organized into a frequency distribution. When this happens, we compute the average as we have always done, add up all of the data points, and divide by the number of data points.

Mean $\bar{x} = \frac{\sum x}{N}$

6.1.2 Standard Deviation and its Interpretation

Standard deviation is a measure of the average distance between the values of the data in the set and the mean. Standard deviation shows how much variation or dispersion exists from the average (mean), or expected value. More precisely, it is a measure of the average distance between the values of the data in the set and the mean. A low standard deviation indicates that the data points tend to be very close to the mean; a high

standard deviation indicates that the data points are spread out over a large range of values.

Data with frequency distribution, $sd = \sqrt{\frac{\sum f(x-\bar{x})^2}{n-1}}$

The sample standard deviation is a statistic known as an estimator. In cases where the standard deviation of an entire population cannot be found, it is estimated by examining a random sample taken from the population and computing a statistic of the sample. As mentioned above, most often the standard deviation is estimated using the corrected sample standard deviation (using N−1).

6.1.3 P value and its significance

The P value, or calculated probability, is the probability of finding the observed. The level of statistical significance is often expressed as a p-value between 0 and 1. The smaller the p-value, the stronger the evidence of rejecting the null hypothesis.

- A p-value less than 0.05 (typically ≤ 0.05) is statistically significant. It indicates strong evidence against the null hypothesis, as there is less than a 5% probability the null is correct (and the results are random). Therefore, we reject the null hypothesis, and accept the alternative hypothesis.
- A p-value higher than 0.05 (> 0.05) is not statistically significant and indicates strong evidence for the null hypothesis. This means we retain the

null hypothesis and reject the alternative hypothesis. You should note that you cannot accept the null hypothesis; we can only reject the null or fail to reject it.

6.2 Statistical Method Used

- Statistical analyses of the data are conducted using the software IBM SPSS 21.0 version standard deviations are derived for all parametrical variables.
- P value of <0.05 was considered statistically significant and all the tests were two tailed.

RESULTS AND DISCUSSION

7.1 Age distribution of the patients

Table 7.1 Age distribution of the patients and their percentages

Age Distribution	**No. of Patients**	**%**
10-20 years	4	8
21-30 years	10	20
31-40 years	18	36
41-50 years	7	14
51-60 years	11	22
Mean Age	37.72±13.07	

The age distribution of the patients and their percentages were calculated and tabulated in Table 7.1 and it is graphically represented in Figure 7.1. 8 % of the patients in the age group of 10-20 years, 20 % of the patients in the age group of 21-30 years, 36 % of the patients in the age group of 31-40 years, 14 % of the patients in the age group of 41-50 years and 22 % of the patients in the age group of 51-60 years were observed. The mean age distribution of the patients is 37.72±13.07 years were observed.

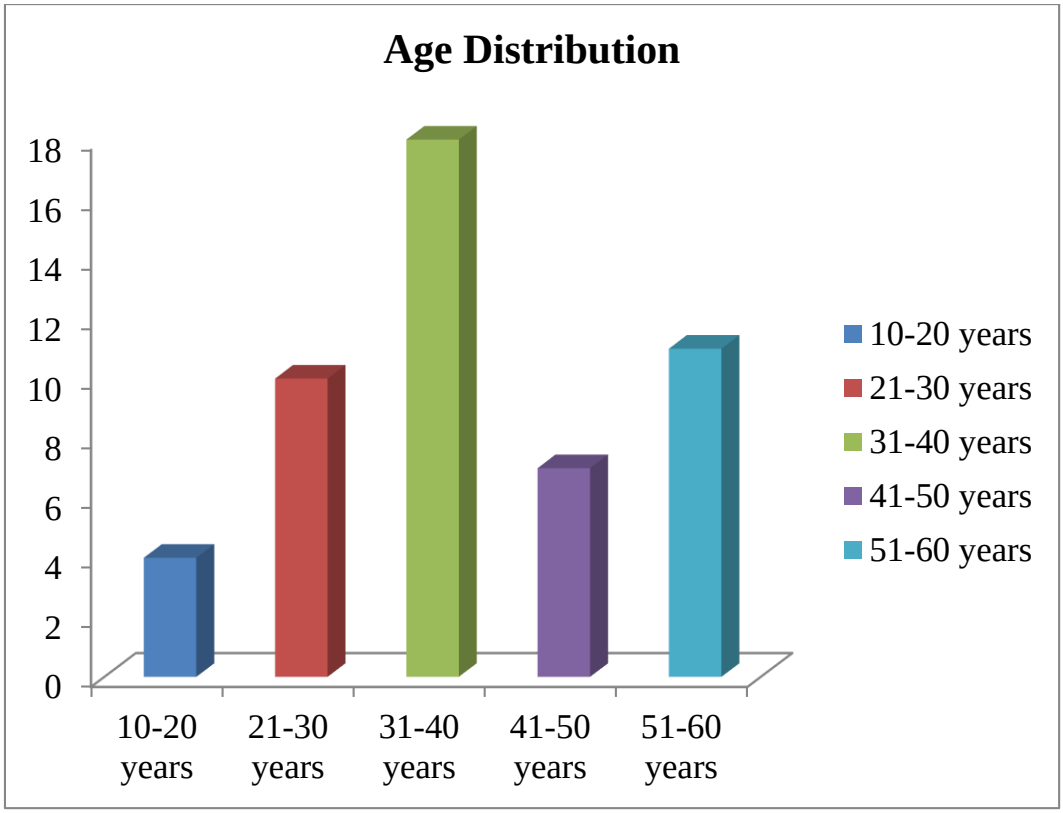


Figure 7.1 Graphical representation of the age distribution of the patients and their percentages

7.2 Gender distribution of the patients

Gender distribution of the patients and their percentages were observed and given in Table 7.2. 54 % of the male patients and 46 % of the female patients were observed and it is graphically represented in Figure 7.1.

Table 7.2 Gender distribution of the patients and their percentages

Gender Distribution	No. of Patients	%
Male	27	54
Female	23	46

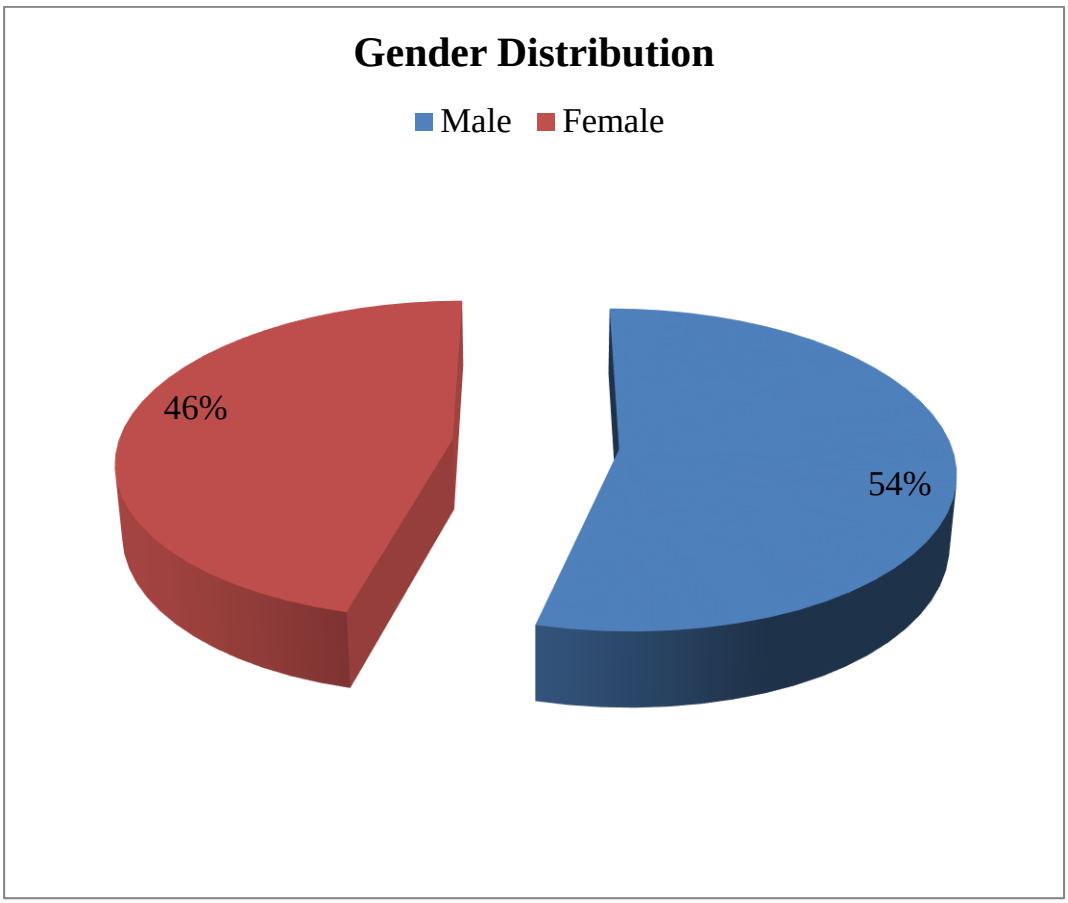


Figure 7.2 Graphical representation of the gender distribution of the patients and their percentages

7.3 Urine albumin of the patients

The urine albumin of the patients and their ranges were observed and given in Table 7.3. In both weeks, the higher no. of the patients were observed in the value of 1+ and lower no. of the patients were observed in the value of 3+; the higher no. of normal level of urine albumin patients were observed and it is graphically represented in Figure 7.3. The p-value of urine albumin is 0.1776 > 0.05 statistically insignificant.

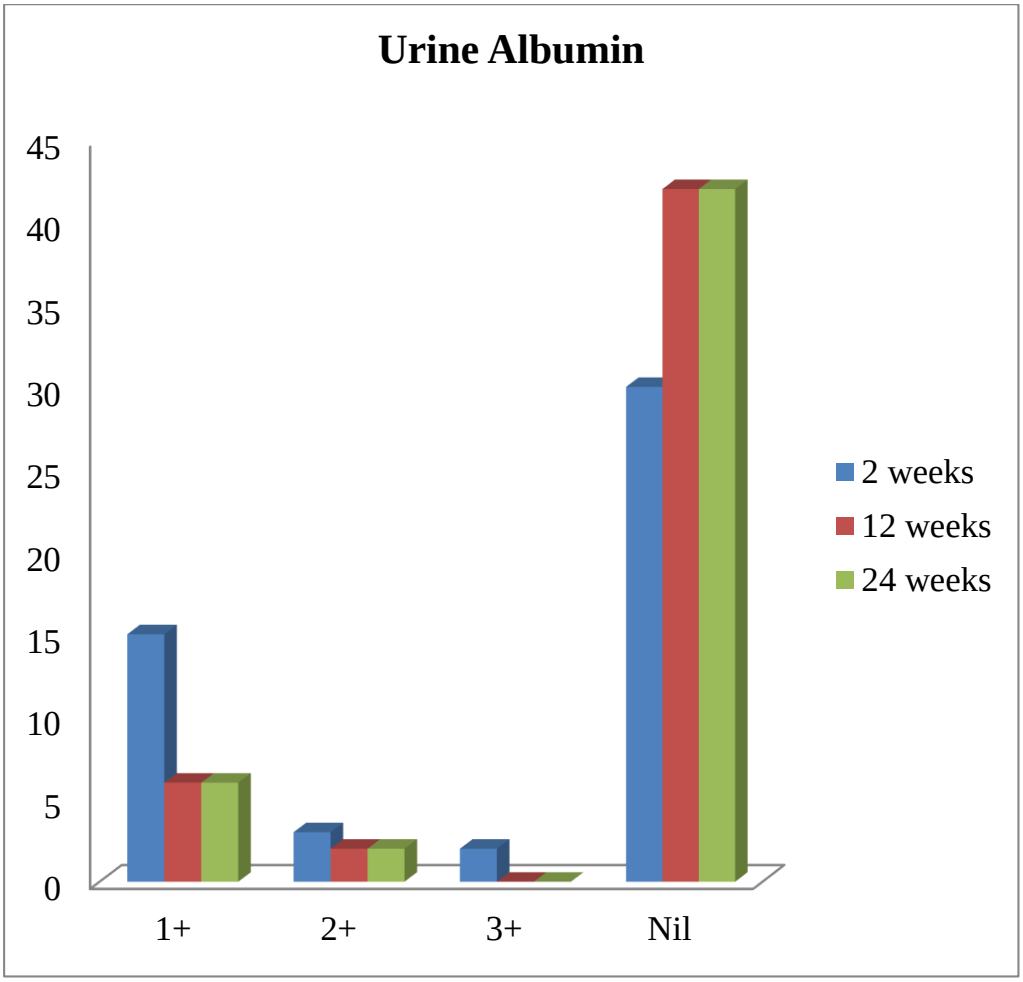


Figure 7.3 Graphical representation of the comparison between the mean urine albumin of the patients and weeks

Table 7.3 Comparison between the urine albumin of the patients and weeks

Urine Albumin	2 weeks	12 weeks	24 weeks
1+	15	6	6
2+	3	2	2
3+	2	0	0
Nil	30	42	42
P value	0.1776		

7.4 Mean Sr. Urea of the patients

Mean Sr. urea of the patients between the weeks were observed and given in Table 7.4. The Sr. Urea mean value of the 2 weeks is 46.34±9.55 mg/dL, 12 weeks of 38.42±8.52mg/dL and 24 weeks of 34.84±9.22 mg/dL were observed and it's illustrated in Figure 7.4. The p-value Sr. urea is 0.00001 < 0.05 statistically significant.

Table 7.4 Comparison between the mean Sr. Urea of the patients and weeks

Sr. Urea	2 weeks	12 weeks	24 weeks
Mean	46.34	38.42	34.84
SD	9.55	8.52	9.22
P value	0.00001		

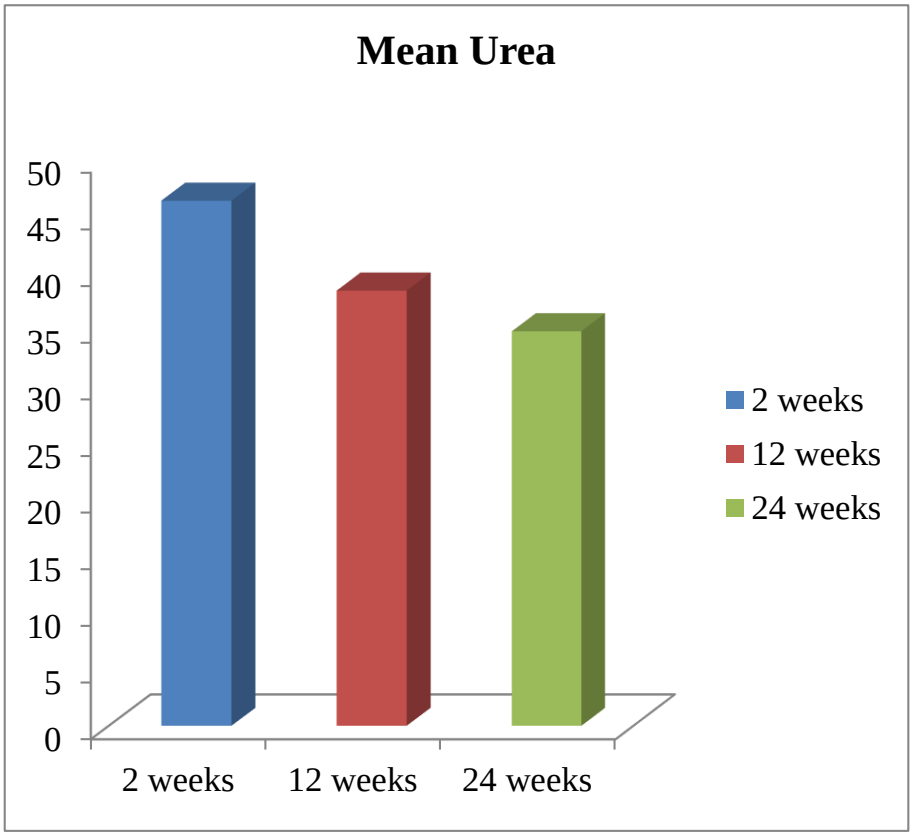


Figure 7.4 Graphical representation of the comparison between the mean Sr. urea of the patients and weeks

7.5 Mean Sr. Creatinine of the patients

Mean Sr. Creatinine of the patients between the weeks were observed and given in Table 7.5. The Sr. Creatinine mean value of the 2 weeks is 1.418 ± 0.32mg/dL, 12 weeks of 1.278 ± 0.31mg/dL and 24 weeks of 1.168 ± 0.43mg/dL were observed and it's illustrated in Figure 7.5. The p-value Sr. Creatinine is 0.00001 < 0.05 statistically significant.

Table 7.5 Comparison between the mean Sr. Creatinine of the patients and weeks

Sr. Creatinine	**2 weeks**	**12 weeks**	**24 weeks**
Mean	1.418	1.278	1.168
SD	0.32	0.31	0.43
P value	0.00001		

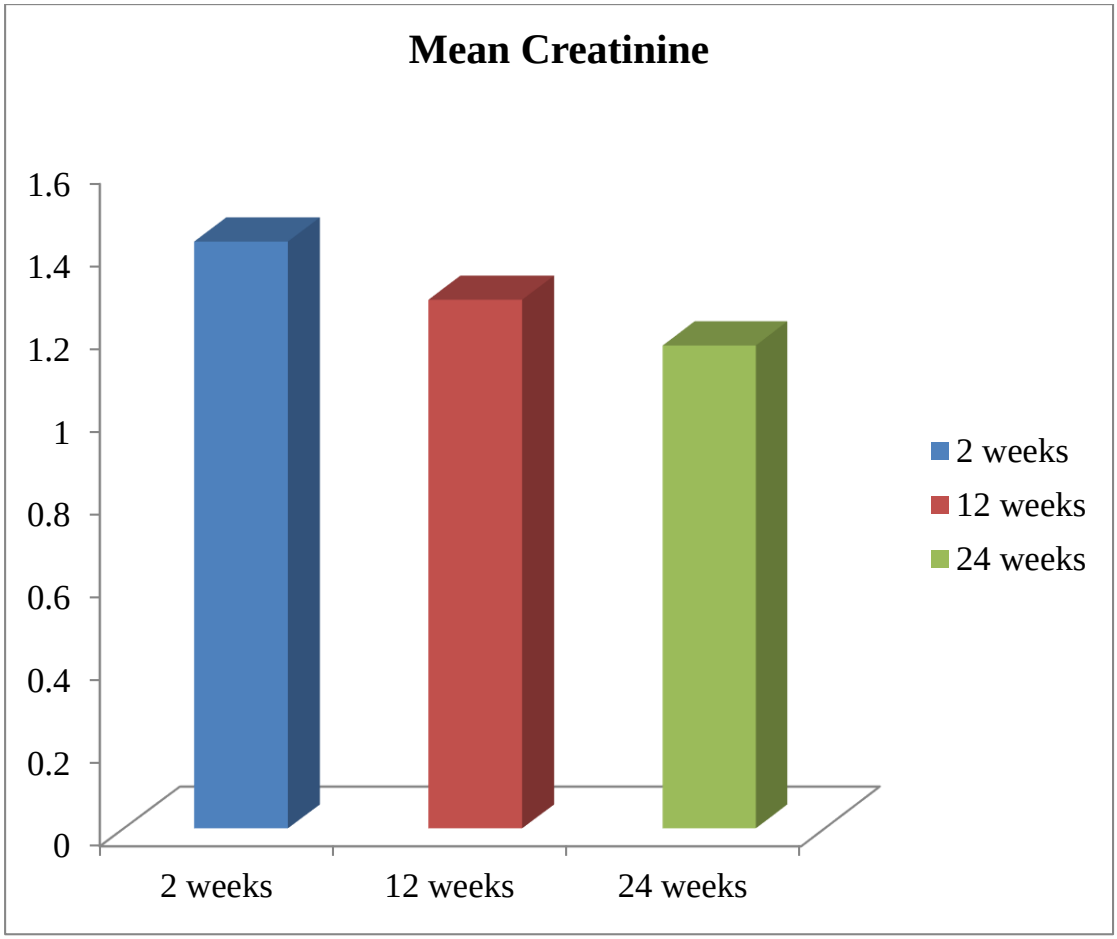


Figure 7.5 Graphical representation of the comparison between the Sr. Creatinine of the patients and weeks

7.6 Mean eGFR of the patients

Table 7.6 comparison between the mean eGFR of the patients and weeks

eGFR	2 weeks	12 weeks	24 weeks
Mean	57.38	61.38	65.7
SD	7.004	8.85	9.78
P value	0.00001		

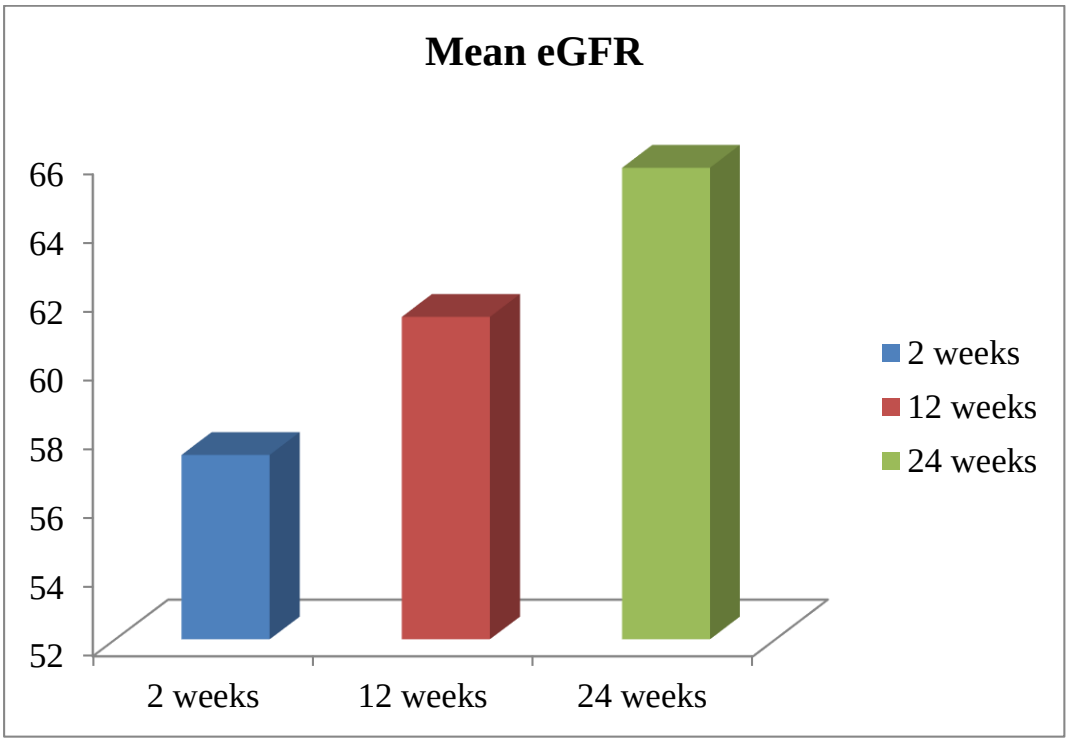


Figure 7.6 Graphical representation of the comparison between the mean eGFR of the patients and weeks

Mean eGFR of the patients between the weeks were observed and given in 7.6. Mean eGFR value was increased between 2 weeks to 24 weeks and it's graphically represented in Figure 7.6; p-value of mean eGFR is 0.00001 < 0.05 statistically significant.

7.7 Comparison between mean urine Beta 2 Microglobulin of the patients

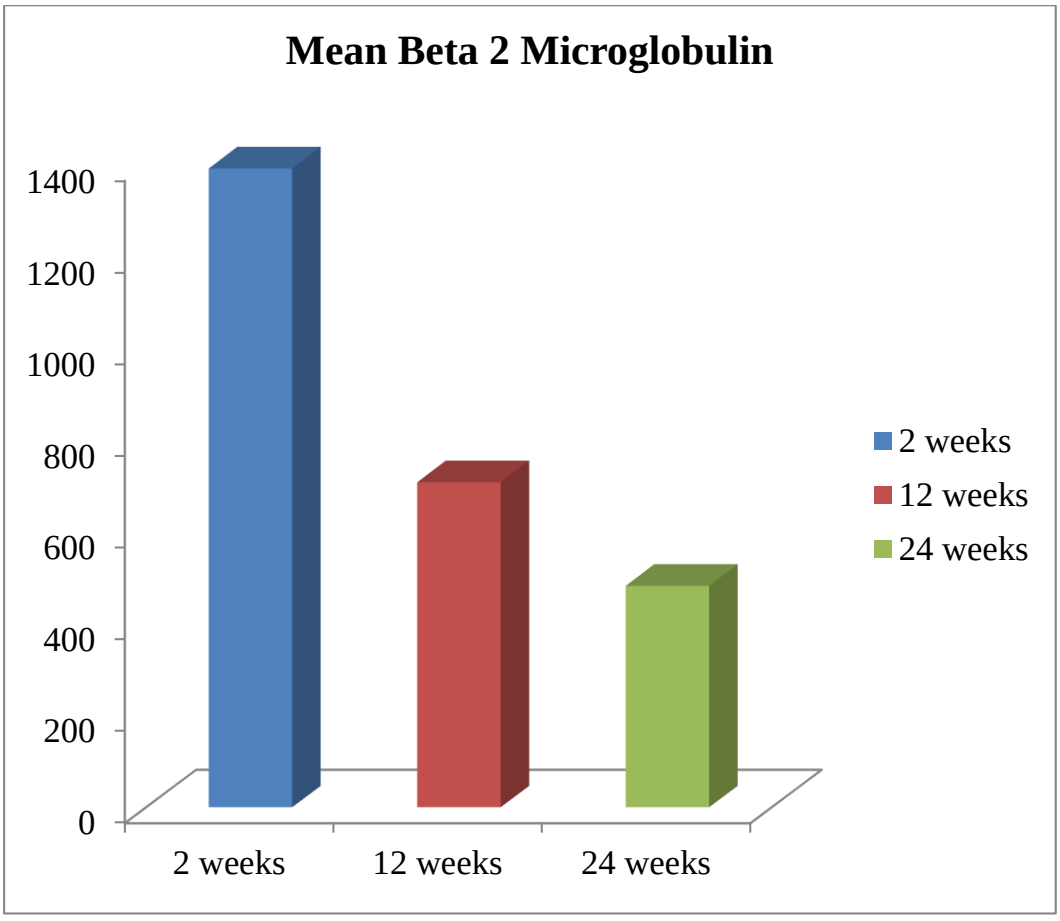


Figure 7.7 Graphical representation of the comparison between mean urine Beta 2 Microglobulin of the patients and weeks

Table 7.7 Comparison between mean urine Beta 2 Microglobulin of the patients and weeks

Urine Beta 2 Microglobulin	2 weeks	12 weeks	24 weeks
Mean	1391.36	710.52	485.78
SD	7025.63	2792.15	1663.62
P value	0.00001		

Comparison between mean urine Beta 2 Microglobulin of the patients and weeks were observed and represented in Table 7.7. Mean urine Beta 2 Microglobulin value was decreased between 2 weeks to 24 weeks and it's graphically represented in Figure 7.7; p-value of mean urine Beta 2 Microglobulin is 0.00001 < 0.05 statistically significant.

7.8 Comparison between the Kidney injury patients'

Comparison between the Kidney injury patients' clinical parameters and weeks were observed and represented in Table 7.8. There was significant difference between 2 weeks and 12 weeks were observed.

Table 7.8 Comparison between the Kidney injury patients' clinical parameters and weeks

Parameters	2 weeks	12 weeks	24 weeks	P value
Urea	61.85±6.17	53.5±10.14	53.6±5.87	0.02
Creatinine	1.96±0.55	1.85±0.35	1.98±0.51	0.01
eGFR	48.87±6.33	45.62±3.5	47.87±4.73	0.001
Beta 2 globulin	7333.25±17243	3226.37±6779	1784.37±4092	0.032

7.9 Correlation between Creatinine and urine Beta 2 Microglobulin

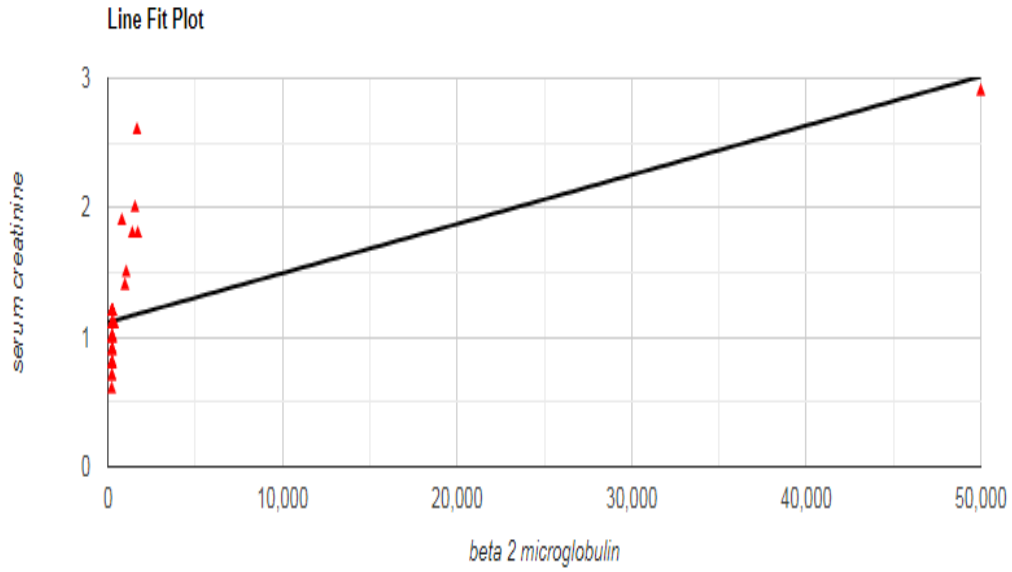


Figure 7.9 illustration of the correlation between Creatinine and urine Beta 2 Microglobulin

Results of the pearson correlation indicated that there is a significant large positive relationship between beta 2 microglobulin and serum creatinine, (r (48) = .612, p < .001).

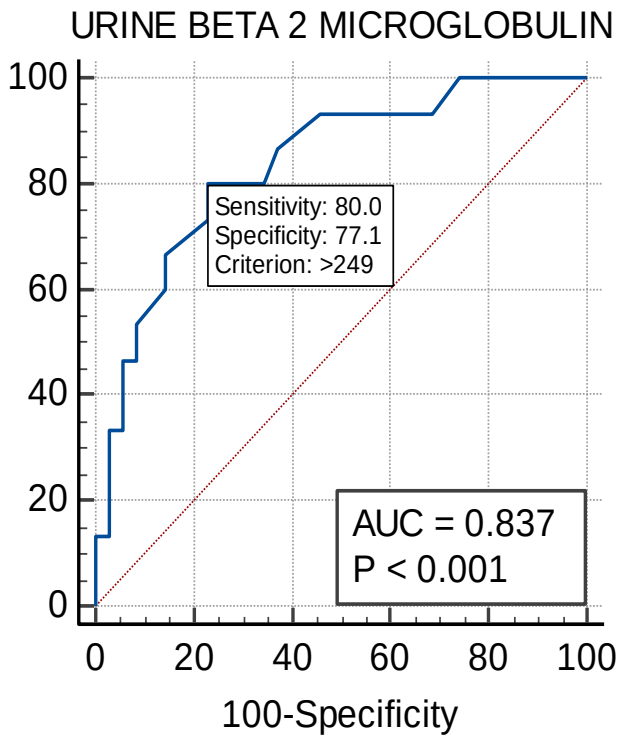


Figure 7.9a Area under the curve

The sensitivity and specificity of urine Beta 2 Microglobulin were calculated kidney injury patients and it is represented in Table 7.9 and Figure 7.9. p-value of the urine Beta 2 Microglobulin is 0.001 < 0.05 statistically significant.

Table 7.9 Statistic value and their CI value

Statistic	Value	95% CI
Sensitivity	80.00%	40.78% to 84.61%
Specificity	77.1%	73.47% to 97.89%
Disease prevalence	40.00%	26.41% to 54.82%
Positive Predictive Value	81.25%	58.56% to 93.00%
Negative Predictive Value	79.41%	67.72% to 87.64%
Accuracy	80.00%	66.28% to 89.97%

DISCUSSION

Acute kidney injury (AKI) is a well-known life-threatening systemic effect of snake envenomation which commonly happens secondary to snake bites from families of Viperidae and Elapidae. Enzymatic toxins in snake venom result in injuries to all kidney cell types including glomerular, tubulo-interstitial and kidney vasculature. Pathogenesis of kidney injury due to snake envenomation includes ischaemia secondary to decreased kidney blood flow caused by systemic bleeding and vascular leakage, proteolytic degradation of the glomerular basement membrane by snake venom metalloproteinases (SVMPs), deposition of microthrombi in the kidney microvasculature (thrombotic microangiopathy), direct cytotoxic action of venom, systemic myotoxicity (rhabdomyolysis) and accumulation of large amounts of myoglobin in kidney tubules.

Beta-2 microglobulin (B2M) is a low-molecular-weight polypeptide (11800 Da), which is present on the surface of all nucleated cells, expressing the major histocompatibility class I. Under physiologic conditions, B2M is produced at a constant rate and is eliminated from circulation by kidneys. In patients with a range of inflammatory, hematologic, immunodeficiency, and renal diseases, plasma B2M levels are elevated.

In patients with chronic kidney disease (CKD), plasma B2M levels are elevated, especially in patients on hemodialysis (HD) in whom glomerular filtration rate (GFR) is almost completely absent. B2M is also a surrogate marker of middle-molecular-weight uremic toxins in patients on HD, which is cleared only by high-flux membrane. In some studies, predialysis serum B2M level predicted mortality and increase of B2M clearance during HD was associated with improved outcomes. In addition, elevated plasma B2M level is a potential risk factor for the development of dialysis-related amyloidosis .

In this study majority of patient's age were between 31-40 years (36%), 22% were between 51-60 years, 20% were between 21-30 years, 14% between 41-50years and 8% were between the age of 10-20years.

Mean age in this study group was37.72±13.07years. In Jaswanth et al., study mean age was 41.83 years. In our study majority of the patients were male (54%) and 46% were females.

Urine Albumin 1+ in 2 week (n=15), 12 weeks (n=6) and in 24 weeks (n=6). 2+ in 2 week (n=3), 12 weeks (n=2) and in 24 weeks (n=2). 3+ in 2 week (n=2), 12 weeks (n=0) and in 24 weeks (n=0). In Jaswanth et al., study number of patients having urine albumin >1+ was 28, in our study urine albumin >1+ was 20 patients.

Mean serum Urea level in 2 weeks 46.34±9.55 mg/dl, 12 weeks 38.42±8.52 mg/dl, in 24weeks 34.84±9.22mg/dl. There was decrease in serum urea level noted in followup weeks.

Mean serum creatinine level in 2 weeks 1.418±0.32mg/dl, in 12 weeks 1.278±0.31mg/dl, in 24 weeks 1.168±0.43mg/dl. Creatinine level was decreasing constantly in following weeks.

Mean eGFR in 2weeks was 57.38±7.004, in 12 weeks was 61.38±8.85, in 24weeks was 65.7±9.78. Mean urine beta 2 microglobulin in 2 weeks was 1391.36, in 12 weeks 710.52, in 24 weeks 485.78.

The serum creatinine, urea, eGFR improved and beta 2 microglobulin level declined significantly between 12 weeks and 24 weeks.

Urine beta 2 microglobulin level at 2weeks were correlated with serum creatinine level in 24 weeks, there was a strong correlation between microglobulin level and development of kidney disease.

In our study urine bête 2 microglobulin have 80.00%sensitivity and 77.1% specificity in predicting kidney injury.

CONCLUSION

Urine beta 2 microglobulin is one of the validated marker for acute kidney injury detection. From this study, we conclude that urine beta 2 Microglobulin level may be a potential, reliable early marker for detecting acute kidney injury to chronic kidney disease progression in hemotoxic snake bite patients suffered from acute kidney injury. Long term follow up is further needed to confirm the exact correlation between urine beta 2 microglobulin and Chronic kidney disease progression.

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LIST OF ABBREVIATIONS

CKD	-	Chronic Kidney Disease
GFR	-	Glomerular Filtration Rate
AKI	-	Acute Kidney injury
ESRD	-	End stage renal disease
B2M	-	Beta 2 microglobulin
KIM	-	Kidney injury molecule
NGAL	-	Neutrophil gelatinase associated lipocalin
KDIGO	-	Kidney disease improving global outcomes
PCI	-	Percutaneous coronary intervention
BUN	-	Blood urea creatinine
NTD	-	Neglected tropical disease

ஆராய்ச்சிஒப்புதல்படிவம்

பெயர்:

வயது:

தேதி:

நோயாளி எண்:

ஆராய்ச்சி சேர்க்கை எண்:

இந்த ஆராய்ச்சியின் விவரங்களும் அதன் நோக்கங்களும் முழுமையாக எனக்கு விளக்கபட்டது.

எனக்கு விளக்கபட்ட விஷயங்களை நான் புரிந்து கொண்டு எனது முழுமனதுடன் சம்மதிக்கிறேன்.

இந்த ஆராய்ச்சியில் பிறரின் நிர்பந்தமின்றி என் சொந்த விருப்பத்தின் பேரில்தான் பங்கு பெறுகிறேன் மற்றும் நான் இந்த ஆராய்ச்சியில் இருந்து எந்த நேரமும் பின்வாங்கலாம் என்றும் அதனால் எந்த பாதிப்பும் எனக்கு ஏற்படாது என்பதையும் புரிந்துகொண்டேன்.

நான் என்னுடைய சுயநினைவுடன் மற்றும் முழுசுதந்திரத்துடன் இந்த மருத்துவ ஆராய்ச்சியில் பங்கு கொள்ள சம்மதிக்கிறேன்.

PROFORMA

Case No:

Name: Age: Sex: IP No:

Address: Occupation:

Phone number:

Rural /urban:

Income / socioeconomic status :

marital status :

CHIEF COMPLAINTS:

Neurological complaints -

Hematological complaints –

Local symptoms -

PAST HISTORY:

Comorbidities- HT/ DM/ TB/ BA/ EPILEPSY/ HIV/bleeding disorder/nephrotoxic drug intake /Renal/ hepatic/ cardiac illness / transplant patients/ malignancy.

PERSONAL HISTORY:

H/O alcoholism / smoking:

GENERAL EXAMINATION: Built -

General condition- Consiousness /Orientation / Febrile or afebrile

/Pallor / Icterus / Cyaonsis / Clubbing / Pedal edema

/Generalized lymphadenopathy

ANTHROPOMETRY – Weight- Height - BMI –

VITALS BP / PR / SP02/RR

SYSTEMIC EXAMINATION : CVS-

RS -

ABDOMEN-

CNS-

LOCALISED EXAMINATION:

LAB INVESTIGATIONS:

1. complete haemogram
2. Blood sugar
3. RFT
4. LFT
5. Serum electrolytes
6. Serum creatinine
7. GFR
8. Urine routine
9. Urine Albumin Excretion
10. Urine Beta 2 Microglubulin
11. Whole blood Clotting time

INSTITUTIONAL ETHICS COMMITTEE
MADURAI MEDICAL COLLEGE &GOVT.RAJAJI HOSPITAL, MADURAI
CDSCO: Reg. No. ECR/1365Inst/TN/2020 &DHR Reg.No.EC/NEW/INST/2020/484

Study Title	:	Urine Beta 2 microglobulin as a predictive biomarker for acute kidney injury to chronic kidney disease progression in patients with acute renal failure following hemotoxic snake bite
Principal Investigator	:	**Dr.A.Vignesh**
Designation	:	PG in MD., General Medicine
Guide	:	Dr.David pradeep kumar,MD., Professor of General Medicine
Department	:	Department of General Medicine Government Rajaji Hospital & Madurai Medical College, Madurai

The request for an approval from the Institutional Ethics Committee (IEC) was considered on the IEC meeting held on **16.11.2021** at GRH Auditorium, Govt. Rajaji Hospital, Madurai at 10.00 A.M

The Members of the committee, the Secretary and the Chairman are pleased to inform you that your proposed project mentioned above is **Approved.**

You should inform the IEC in case of any changes in study procedure, methodology, sample size investigation, Investigator or guide or any other changes.

1. You should not deviate from the area of work for which you had applied for ethical clearance.
2. You should inform the IEC immediately, in case of any adverse events or serious adverse reactions. If encountered during from study.
3. You should abide to the rules and regulations of the institution(s)
4. You should complete the work within the specific period and if any extension is required, you should apply for the permission again for extension period.
5. You should submit the summary of the work to the ethical committee on completion of the study.
6. Serious Adverse Events occurred in the study should be intimated to the IEC within 24 hours of occurrence of the event.

MEMBER SECRETARY,
IEC, Madurai Medical College,
Madurai
Dr.K.RAADHIKA, M.D(Pharm)
Associate Professor
Member Secretary
IEC - Madurai Medical College
Madurai.

03 JAN 2022

CHAIRMAN,
IEC, Madurai Medical College,
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CERTIFICATE – II

This is to certify that this dissertation work titled **"URINE BETAMICROGLOBULIN AS A PREDICTIVE BIOMARKER FORACUTE KIDNEY INJURY TO CHRONIC KIDNEY DISEASEPROGRESSION IN PATIENTS WITH ACUTE RENAL FAILUREFOLLOWING HEMOTOXIC SNAKE BITE"** of the candidate **Dr. A.VIGNESH** with registration Number **200120101525** for the award of **M.D.,** in the branch of **GENERAL MEDICINE.**I personally verified the urkund.com website for the purpose of plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **16** percentage of plagiarism in the dissertation.

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