Serum Creatinine and Cystatin C provide conflicting evidence of Acute Kidney Injury following acute ingestion of Potassium permanganate and Oxalic Acid

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Abstract

Acute Kidney Injury (AKI) is common following deliberate self-poisoning with a combination washing powder containing oxalic acid ($H_2C_2O_4$) and Potassium permanganate ($KMnO_4$). Early and rapid increases in serum creatinine (sCr) follow severe poisoning.

We investigated the relationship of these increases with direct nephrotoxicity in an ongoing multicenter prospective cohort study in Sri Lanka exploring AKI following poisoning. Multiple measures of change in kidney function were evaluated in 48 consenting patients who had serial sCr and serum cystatinC (sCysC) data available.

Thirty eight (38/48, 79%) patients developed AKI (AKIN criteria). Twenty eight (58%) had AKIN stage 2 or 3. Initial increases in urine creatinine (uCr) excretion were followed by a substantial loss of renal function. The AKIN stage 2 and 3 (AKIN2/3) group had very rapid rises in sCr (a median of 118% at 24 hours and by 400% at 72 hours post ingestion). We excluded the possibility that the rapid rise resulted from the assay used or muscle damage. In contrast, the average sCysC increase was 65% by 72 hours.

In most AKI, sCysC increases to the same extent but more rapidly than sCr, as sCysC has a shorter half-life. This suggests either a reduction in Cystatin C production or, conversely, that the rapid early rise of sCr results from increased production of creatine and creatinine to meet energy demands following severe oxidative stress mediated by oxalic acid and $KMnO_4$. Increased early creatinine excretion supports the latter explanation, since creatinine excretion usually decreases transiently in AKIN2/3 from other causes.

Introduction

Deliberate self- poisoning with household products is uncommon globally. However, it is an important problem in some rural areas of Sri Lanka, where household products such as bleaches, disinfectants, paints and washing powders account for 15% of self-poisoning incidents each year [1]. In particular, deliberate ingestion of combination washing powder sachets consisting of 12.5g of oxalic acid and 1.2g of potassium permanganate has been a substantial public health problem in Southern Sri Lanka since 2006 [2].

Data on systemic toxicity of combination washing powder poisoning is limited to one large case series reporting on 115 patients admitted to 2 referral hospitals in Southern Sri Lanka. There were 18 deaths. Of those patients (n = 20) who had serial biochemistry data; 28% developed renal failure [raised serum creatinine (sCr) over 1.3mg/dl] [2].

We noted a remarkably steep and rapid rise of sCr in some cases. A similar phenomenon occurs independently of AKI in paraquat poisoning [3]. We explored the extent to which a rise in sCr in oxalic acid/$KMnO_4$ poisoning should be attributed to AKI or other mechanisms.

Materials and Methods

Study design

We studied a subset of patients (n = 48) with combination washing powder poisoning recruited prospectively to a multicenter cohort study of AKI from poisoning in Sri Lanka.

The patients presented to two general hospitals in Southern province of Sri Lanka between October 2010 and February 2014. Exclusion criteria for the cohort were age < 15 years, confirmed pregnancy, mixed overdoses and late presenters (more than 24 hours post ingestion). The substance ingested was based on patient's history and/or by inspection of the sachet/label or by perusing transfer notes from peripheral hospitals. Exposure was confirmed by assays for oxalic acid and Potassium permanganate on urine samples. Demographic and clinical data were collected during the hospital stay. Patients meeting inclusion criteria, providing consent and providing with ≥4 blood and urine samples (n=102) were considered for detailed biomarker assays as described in Figure 1. However, comprehensive biomarker assays were carried out in only 48 randomly selected patients due to funding limitations

Written informed consent was obtained from patients or an accompanying relative. The multicenter toxic-AKI cohort study was approved by the Human Research Ethics Committees of the Faculty of Medicine, University of Peradeniya, Sri Lanka and University of New South Wales, Australia.

Specimen collection and laboratory assays

Serial urine and blood samples were collected at 4, 8, 16 and 24 hours post-ingestion then daily until discharge or death. Further samples were collected at one month and/or three months after discharge. All specimens were stored briefly at -20°C after immediate processing and then at -80°C. Urine creatinine and sCr (Jaffe method) were measured according to Biolabo S.A.S recommendations on a Roche Hitachi 912 Chemistry Analyzer. Enzymatic creatinine assay was conducted on Indiko^TMb Clinical and Specialty Chemistry System (Thermo Scientific). Creatine kinase (CK) assay (Vitros products/Ortho Clinical Diagnostics) was conducted on the same samples using a dry chemistry analyzer. Serum cystatin C was assayed using microparticle

enhanced immunoturbidometry on a clinical chemistry analyzer (Konelab^{TM}, Thermo Fishers Waltham, MA). Bio-PlexPro^{TM}RBM human kidney toxicity assay panel 2 kit was used on the Bio-Plex 200 systems (BIO-RAD, USA) to quantify urinary cystatin C (uCysC). All assays were conducted as per manufacturer's recommendation and inter and intra-assay precision was < 10%. Oxalate in urine (Thermo scientific oxalate assay kit) was measured (Indiko^{TM} Clinical and Speciality chemistry System) as per manufacturers recommendations and urine manganese ion (Mn^{2+}) was measured using atomic absorption spectrophotometry (AAS) method as described elsewhere [4].

AKI was defined and categorized based on Acute Kidney Injury Network (AKIN) criteria [AKIN stage I: increase in sCr to > 150% of baseline or absolute increase of ≥0.3 mg/dl from baseline; AKIN stage II: increase of > 200% of sCr from baseline and AKIN stage III: increase in sCr to > 300% of baseline or increase of >4 mg/dl and absolute sCr increment of > 0.5 mg/dl] [5]. The AKIN definition requires a baseline sCr measurement 3 months prior to injury, a variable not available in the study participants. Therefore the lowest sCr value identified between hospital admission and three months post discharge was used as the baseline as used previously in similar cohort [3].

We estimated GFR (mL/min) using the Jelliffe equation; this has previously been validated in patients with non steady state kidney function [6, 7]. GFR was also calculated using the non-steady state sCysC based Larson formula [8]. Traditional formula for creatinine clearance CrCl were used [7] to calculate CrCl in AKIN stage 2 and 3 (AKIN2/3) patients whom urine output data were available (n=15). Creatinine clearance was calculated with following formula: CrCl = uCr (mg/dL) × urine volume (ml) / mid-point estimate of sCr (mg/dL) × time (min).

We compared sCr estimation by the Jaffe method and enzymatic method to ensure the former had not been affected by chromogens [9, 10]. Further sCr levels were correlated with CK to determine if direct muscle injury might explain sCr rise.

Statistical analysis

All non-normally distributed continuous variables were reported as medians, inter quartile ranges (IQRs) and categorical variables were reported as percentages. Continuous data were analysed using the Mann Whitney-U test and categorical data were analysed using Fisher's exact test. Serum creatinine and sCysC data are presented as absolute levels as well as relative change from the baseline. Urinary cystatin C was normalized to uCr [11]. All statistical analysis were conducted between no AKI (no evidence of AKI), AKIN1 and AKIN2/3 groups and analysis was performed using GraphPadPrism version 6 (Graph Pad Software, San Diego, USA).

Results

Of the forty-eight patients, 10 (21%) did not develop AKI (no AKI) and 38 (79%) developed varying degrees of AKI (Figure 1).

Baseline characteristics

Baseline, demographic and clinical variables of included patients are presented in Table 1. Baseline and demographic variables such as age, gender, body weight, estimated amount ingested and time to admission from ingestion were similar in patients with AKIN2/3 and No AKI/AKIN1 groups. However, concentrations of serum creatinine (mg/dl) and sCysC (mg/l) at 4 hrs post ingestion were significantly higher in AKIN2/3 ($p<0.01$) (Table 1). There were no

important differences between the patients we studied and other patients presenting with this poisoning during this time (Supplementary Table 1).

Absolute and relative changes in serum creatinine and cystatin C

Absolute sCr concentration increased by up to 3 fold within 16 hours following ingestion. In contrast, sCysC only started to increase around 24 hours and then slowly rose until day 3 (Figure 2). The median relative change of sCr from baseline in AKIN2/3 group was 118% at 24 hours and 400% by 72 hours (Figure 3). The median increase in sCysC only reached 65% at 72 hours (Figure 3). In contrast patients who did not develop AKI or who had AKIN1 only showed a mild increase in both markers. As expected the non-steady state eGFR for the two markers had very poor agreement. [(eGFR sCr Vs eGFR sCysC (limits of agreement = -64.5 to 4.0, bias = -30.-3)] (Supplementary Figure 1a).

Changes in urinary cystatin C

Urinary cystatin C data were available in 39 patients and serial concentrations were normalized to uCr [11]. A significant rise of uCysC in AKIN2/3 group was observed after 24 hours and no AKI/AKIN1 group had only mild increases (Supplementary Figure 2a & 2b).

Changes in other kidney functional indices in sub-group with complete urine collection

Median urine output among these 15 patients with AKIN2/3 fell initially to <500ml at 24 hours but increased to 1530ml at 72 hours (Supplementary figure 3b). The calculated CrCl showed a rapid and sustained reduction in CrCl from 24 to 72 hours (Figure 4). The calculated CrCl did not agree closely with the non-steady state eGFR based on either sCr or sCysC but was closer to the sCr eGFR [(CrCl vs eGFR_sCr (limits of agreement = -43.6 to 26.4, bias = -8.6)), (CrCl vs

eGFR_sCysC (limits of agreement = -83.0to 15.0, bias = -34.0))] (Supplementary Figure 1b & 1c).

Influence of measurement technique, muscle damage and poison dose on sCr

Serum creatinine measurement by the Jaffe and enzymatic methods were highly correlated (bias = 0.01, r = 0.93). Serum CK levels were generally low and there was no correlation between Jaffe sCr and CK (r = 0.008) (Supplementary Figure 4 & 5). There was a modest correlation between severity of AKI (changes in serum creatinine as per AKIN stages) and oxalate concentration in initial urine samples (r = 0.42), but none for Mn^{2+} ions (r = 0.02) (Supplementary Figure 6). Correlations between sCysC (initial sample points) levels and poison levels were poor (oxalate r = 0.2, Mn^{2+} ions r = - 0.07).

Discussion

Using the AKIN definition, AKI developed in 79% of patients; and 58% had AKIN stage 2 or 3. The median rise of sCr at 24 hours in AKIN2/3 patients was 2.37 mg/dL which exceeds the maximum predicted sCr rise used for non-steady state sCr kinetics [12]. Compared to calculated CrCl, sCr levels over-estimates and sCysC grossly under estimates true AKI following oxalic acid/$KMnO_4$ ingestion.

In our study, the sCysC increment was small and relatively delayed despite increases in several other measures indicating severe nephrotoxicity. Serum cystatin C is produced at a constant rate [13], filtered freely by glomeruli and completely reabsorbed and catabolized by renal epithelia cells [14, 15]. It is widely used surrogate marker of GFR [16, 17]. Serum cystatin C changes more rapidly in response to changes in renal function as a consequence of its shorter elimination half-life (2 hours compared with 6 hours for sCr with normal renal function) [16, 18]. Some

studies have shown the superiority of sCysC as an early biomarker of AKI, others have shown that sCysC performs similarly or worse than sCr [19-22].

The observed rapid increases in sCr suggest a contribution of non-renal mechanisms following oxalic acid/KMnO$_4$ poisoning. Conversely, the apparent underestimation of true kidney damage by sCysC may be due to inhibition of sCysC production by non-renal mechanisms. These mechanisms need to be explored further, but one possibility is that increases in intracellular reactive oxygen species (ROS) might induce formation of sCysC dimers. Dimerization occurs co-translationally in the endoplasmic reticulum (ER) and is regulated by the levels of ROS derived from mitochondria in mice [23]. Increased ROS levels also promote the intracellular retention of dimerized cystatin C and this may reduce cystatin C secretion [24, 25]. Ideally, this should be validated by confirming an increased presence of cystatin C dimers in tissues via western blot [26], something we were unable to do in this study. This would explain underestimation of true kidney damage by sCysC; a toxic mechanism leading to profound inhibition of cystatin C production coinciding with reduced elimination in AKI.

We explored various explanations for the very rapid rise in creatinine. Interference with the Jaffe method can be mediated by antibiotics like cephalosporin and many biomolecules like bilirubin, glucose and acetone [27-30]. Enzymatic methods are not affected. Since the levels measured by the two methods are strongly correlated, the early rise of sCr is unlikely to be due to an assay based error (Supplementary Figure 4). Rhabdomyolysis releases cellular contents such as creatine, creatinine and CK [31]. However the early rise of sCr seems unlikely to be due to overt muscle damage as there was minimal rise in CK (Supplementary Figure 5). Rhabdomyolysis is also not generally reported following these toxic agents (Unpublished data).

Patient demographic data demonstrate a uniformly distributed male: female population with young age and similar weight range (Table 1). Thus, individual factors explaining a more rapid increment of sCr (age, gender, weight) can be excluded. Furthermore diet-based influences (cooked meat and high protein) are unlikely as patients were provided with meals offered by the hospital [32, 33].

Oxalic acid (CAS 6153–56–6) is a colourless crystalline material with reducing ability. Potassium permanganate (CAS 7722-64-7) is a purple crystalline powder with powerful oxidizing effect [2]. Systemic toxicity of $KMnO_4$ is postulated to be due to oxidative injury from free radicals generated by the absorbed MnO_4^- ion, although detailed mechanisms are unclear [34]. Acute kidney injury correlated much better with urine oxalate concentrations (than Mn^{2+}) (Supplementary Figure 6) and it is likely that oxalic acid toxicity is responsible for most of the observed AKI.

Oxalic acid reacts with calcium inside renal tubules and produces calcium oxalate monohydrate crystals which are deposited in the tubular epithelium and renal tubular lumen leading to tubular obstruction and cell dysfunction [35] from oxidative stress and mitochondrial toxicity [36, 37]. Nephrotoxicity due to oxalic acid poisoning is probably similar to AKI after ethylene glycol poisoning, as the latter is metabolised to oxalic acid [38-40].

Isolated renal mitochondria respond to calcium oxalate monohydrate by increasing ROS, lipid peroxidation [41, 42] and oxidation of thiol proteins [43]. Oxalate toxicity is further mediated by activation of Phospholipase A2 (PLA2) [43, 44] in response to oxidative stress [37, 45-47].

Mitochondrial toxicity may also occur via activation of the mitochondrial permeability transition (MPT) [48]. This leads to rapid and progressive osmotic swelling and dissipation of transmembrane gradient and release of cytochrome C under severe oxidative stress [40]. Patients

poisoned with oxalic acid and KMnO$_4$ demonstrated elevated levels of cytochrome C (SACTRC unpublished data), which supports the presence mitochondrial oxidative injury following oxalic/KMnO$_4$ poisoning.

Opening of MPT pore in the inner mitochondrial membrane allows solutes to diffuse across the inner membrane leading to depolarization, inhibition of oxidative phosphorylation and ATP depletion [40]. Creatine, creatol and methylguanidine act as a homeostatic antioxidant to reduce circulating free radicals [49]. Generation of free radicals leads to extra production of sCr [50, 51] to meet the higher energy demand under oxidative stress as noted following several paraquat poisoning [3]. Therefore we propose that the observed rapid and large rise of sCr is contributed by increased production and conversion of creatine to creatinine.

Metabolic acidosis has been recorded in oxalic acid poisoning [35, 38]. Increased conversion of creatine to creatinine with acidosis may also make a minor contribution to the sCr rise [3, 52].

Glomerular filtration accounts for the majority of creatinine elimination, but creatinine is actively secreted by number of transporters expressed in proximal tubules [53, 54]. Specially, human organic cation transporters (hOCT2) [55] and anionic transporters secrete creatinine [53, 56-59]. A small increment of sCr due to competitive or non-competitive inhibition of renal creatinine transport by oxalic acid, MnO_4^- and Mn^{2+} ions is plausible.

Strengths and Limitations

The major strengths of this study are inclusion of varying degrees of AKI patients, with the laboratory confirmation of the poison and with good collection of data and samples including many within 24 hours post ingestion. There are several important limitations. In the most severely poisoned patients' death occurs very rapidly due to hypocalcaemia [2, 60]. This study

had none of these fatal poisonings, although accurate diagnosis of AKI may not be relevant to these patients.

Previous studies have demonstrated that the name of the poison ingested by patients is generally accurate and reflected in subsequent assay confirmations [61, 62]. However, the amount ingested is less accurate. Generally people reported that they ingested one sachet of each from the combination washing powder. This may explain why estimated amount ingested was similar in the No AKI/AKIN1 and AKIN2/3 groups despite differences in the measured urinary concentrations (Table 1.).

Serum Mn^{2+} and oxalate levels were not quantified; we relied on spot urine concentrations to examine the relationship between dose and AKI. Urine data confirms exposure, but because of variation in urine output and renal impairment, are only a very rough measure of dose. Since these studies were carried out in two hospitals where laboratory facilities are limited, we were not able to examine for oxalate crystals in the urine samples immediately after collection. It is not valid to examine crystals in thawed stored samples [63].

Good urine output data was available in only 15 AKIN2/3 patients for practical reasons in this poor-resource setting and thus we have limited data on directly measured creatinine clearance.

Conclusions

Oxalic acid/$KMnO_4$ ingestion causes toxic AKI but the rapid and large increment of sCr cannot be entirely explained by direct nephrotoxicity. The best rationale for the excessive increase is oxidative stress increasing production of creatine and creatinine to meet intensified energy demand. The rise did not result from assay interference or gross muscle damage. Inhibition of sCr transporter systems and acidosis also may make minor contributions. Serum cystatin C

severely under-estimated the AKI. Other methods to monitor GFR and AKI in oxalic /KMnO$_4$ poisoning such as isotopes and urinary AKI biomarkers are desirable. Until these are widely available, sCr should continue to be used but clinicians should be aware that the rise in sCr may overestimate the loss of renal function and the extent of injury.

Conflict of interest

We declare that none of the authors have a conflict of interest

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Tables

Table 1: Baseline and demographic and clinical characteristics

| Baseline Data	No AKI / AKIN1	AKIN2/3	p
Age (years)	22 (20 - 29)	25 (20 – 32)	0.28
Male (%)	36%	46 %	0.04
Weight (kg)	50 (45 – 61)	52 (42 – 65)	0.97
Estimated amount ingested (g)	13.7 (12.5 – 13.7)	13.7 (11.9– 13.9)	0.92
Time to admission from ingestion (hours)	2 (1-4)	4 (2-5)	0.06
Pulse rate (beats/minute)†	74 (76 – 80)	80 (76 – 84)	0.26
Blood pressure – systolic (mmHg)†	110 (110 – 120)	120 (115 -140)	1.00
Blood pressure – diastolic (mmHg)†	70 (70 – 80)	80 (72 – 90)	1.00
sCr at 4 hours post ingestion (mg/dl)	0.79 (0.66 – 0.89)	1.48 (0.89 – 1.82)	0.0001
sCys C at 4 hours post ingestion (mg/l)	0.77 (0.70– 0.83)	0.98 (0.87 – 1.22)	0.001

Data expressed as medians and inter quartile ranges (IQRs) or percentages (%). Comparisons were based on NoAKI/AKIN1 Vs AKIN 2/3. P values were calculated using Mann Whitney-U test (continuous data) and Fisher's exact test (categorical data). † refers to the admission measurements.
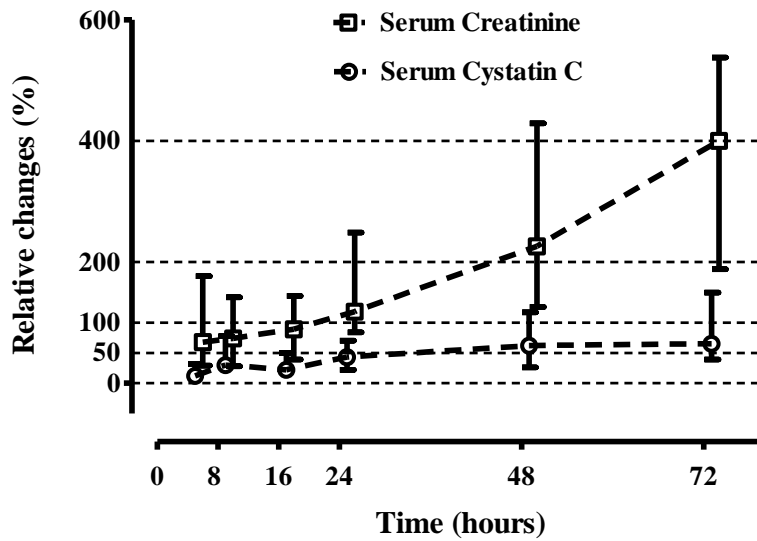
Figures
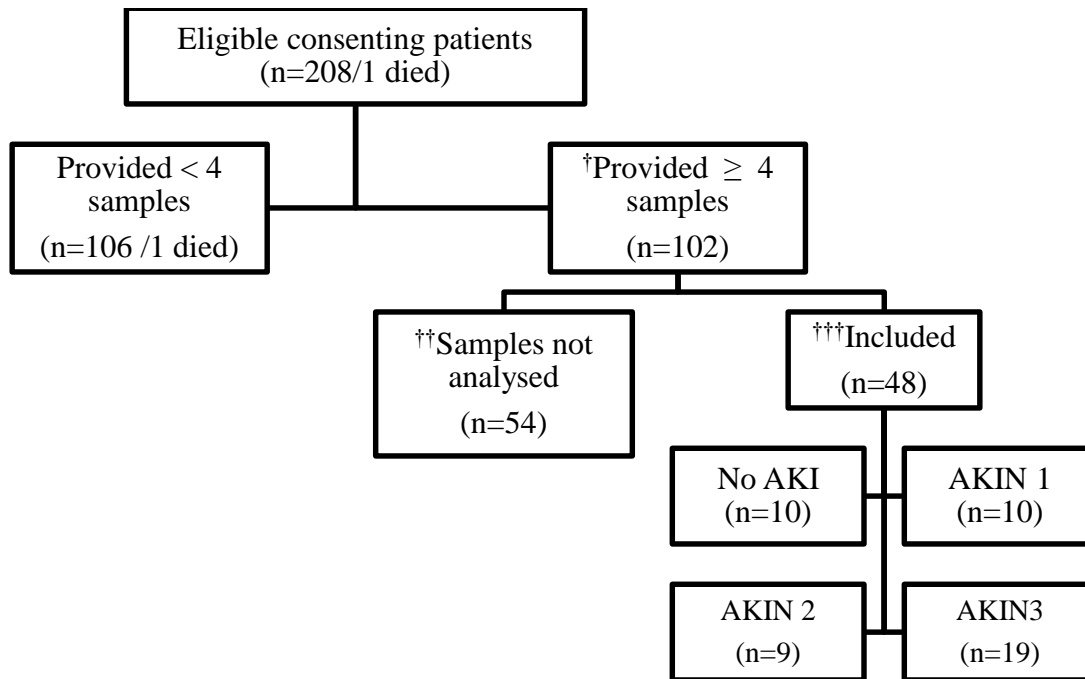
Figure 1: Patient recruitment profile.

Data expressed as number of patients (n). [†] Patients provided ≥ 4 samples (n=102) were considered for detailed biomarker assay cohort. [††] Patients who provided ≥ 4 samples (n=54) but did not have biomarkers measured due to funding limitations. [†††] Randomly selected patients for biomarker assays (n = 48).

AKIN0/1

AKIN2/3

a

b

c

d
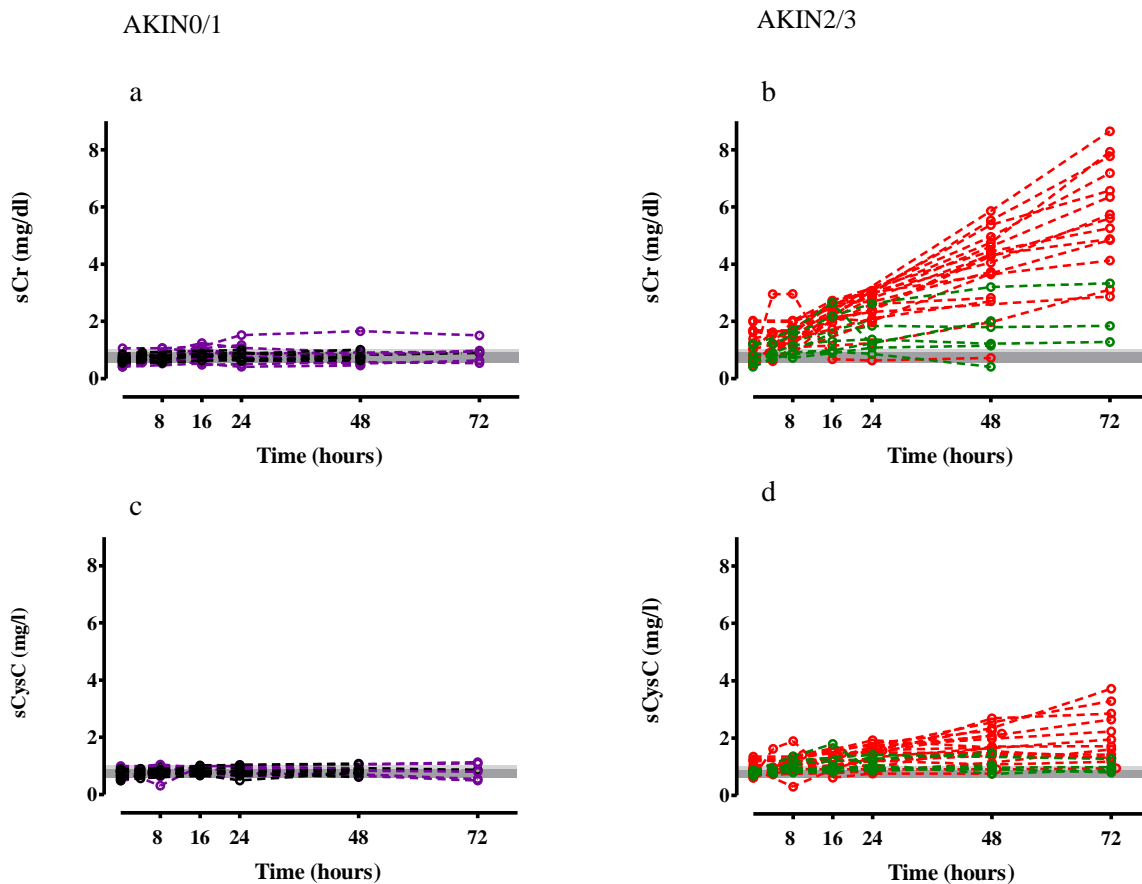
Figure 2: Serial serum biomarker concentration profiles following oxalic acid /KMnO$_4$ poisoning.

Serial serum concentration of absolute sCr (a & b) and sCysC (c & d) over first 72 hours are depicted [Black dotted line – NoAKI, purple dotted line – AKIN 1, green dotted line – AKIN 2 red dotted line – AKIN 3]. The gray shaded area illustrates the normal range based on respective biomarkers measured in healthy individuals (dark gray area - 5th – 75th percentile; light gray area - 75th percentile – 95th percentile).

Figure 3: Relative changes (%) in both creatinine and cystatin C in patients with AKIN2/3.

Data on 28 AKIN 2 & 3 patients are shown here. Baseline levels were defined as lowest biomarker concentration during follow up or hospital stay for plotting the relative changes in biomarkers as depicted in the figure
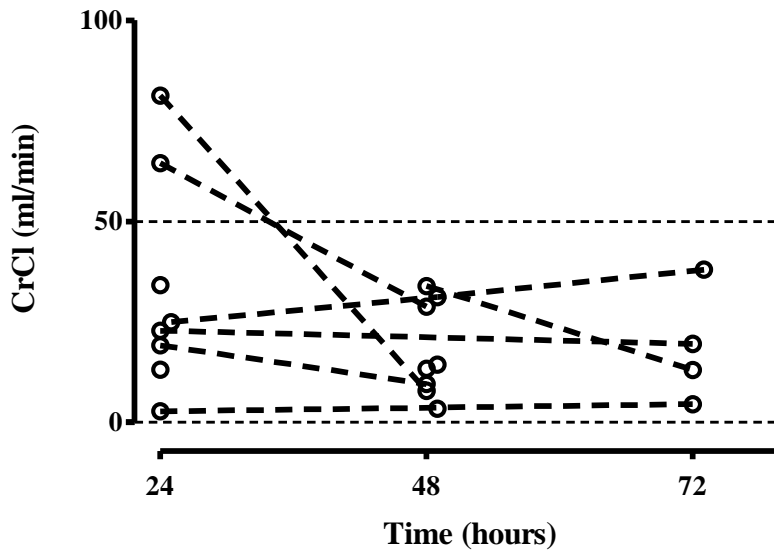
Figure 4: Calculated creatinine clearances (mL/min) of 15 AKIN2/3 patients.

Creatinine Clearance was estimated based on following formula; CrCl = urine creatinine (mg/dl) × urine volume (ml) / midpoint creatinine (mg/dl) × time (min).