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Kidney damage biomarkers detect acute kidney injury but only functional markers predict mortality after paraquat ingestion

Fahim Mohamed[a,b,c,d,e], Nicholas A. Buckley[a,b,e], Shaluka Jayamanne[a], John W. Pickering[f,g], Philip Peake[d], Chathura Palangasinghe[a], Thilini Wijerathna[a], Indira Ratnayake[a], Fathima Shihana[a], Zoltan H. Endre[d,f,*]

[a] South Asian Clinical Toxicology Research Collaboration, University of Peradeniya, Peradeniya, Sri Lanka
[b] Clinical Pharmacology and Toxicology Group, Professorial Medicine Unit, The Prince of Wales Clinical School, University of New South Wales, New South Wales, Australia
[c] Department of Pharmacy, Faculty of Allied Health Sciences, University of Peradeniya, Sri Lanka
[d] Department of Nephrology, Prince Of Wales Hospital and Clinical School, University of New South Wales, Sydney, Australia
[e] Department of Pharmacology, SOMS, Sydney Medical School, University of Sydney, New South Wales, Australia
[f] Department of Medicine, University of Otago, Christchurch, New Zealand
[g] Emergency Department, Christchurch Hospital, Christchurch, New Zealand

HIGHLIGHTS

- Only functional biomarkers (sCr and sCysC) predicted death following paraquat poisoning.
- Urinary CysC and clusterin were useful early biomarkers (at 8 and 16 h) in diagnosing the later onset of functional AKI.
- Use of urinary CysC and clusterin within the first day after ingestion may guide early intervention for reno-protection.
- The increase in specific renal injury biomarkers was consistent with the mechanistic pathways of paraquat induced-nephrotoxicity.
- Point-of-care biomarker detection would be required to enable early intervention in selected patients in rural Asia.

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ABSTRACT

Acute kidney injury (AKI) is common following paraquat ingestion. The diagnostic performance of injury biomarkers was investigated in serial blood and urine samples from patients from 5 Sri Lankan hospitals. Functional AKI was diagnosed using serum creatinine (sCr) or serum cystatin C (sCysC). The 95th centile in healthy subjects defined the urinary biomarker cutoffs for diagnosing structural AKI. 50 poisoned patients provided 2 or more specimens, 76% developed functional AKI [AKIN stage 1 (n = 12), 2 (n = 7) or 3 (n = 19)]; 19/26 patients with AKIN stage 2/3 also had functional AKI by sCysC criteria (≥50% increase). Urinary cystatin C (uCysC), clusterin (uClu) and NGAL (uNGAL) increased within 24 h of ingestion compared with NoAKI patients and healthy controls. Each biomarker demonstrated moderate diagnostic utility [AUC–ROC: uCysC 0.79, uNGAL 0.79, uClu 0.68] for diagnosis of functional AKI at 16 h. Death occurred only in subjects with functional AKI. Structural biomarker-based definitions detected more AKI than did sCr or sCysC, but did not independently predict death. Renal injury biomarkers did not add clinical value to patients who died rapidly due to multi-organ failure. Use of injury biomarkers within 16–24 h may guide early intervention for reno-protection in less severe paraquat poisoning.

* Corresponding author at: Department of Nephrology, Prince of Wales Hospital, High Street, Randwick, Sydney, New South Wales 2031, Australia. Fax: +61 293824409.
E-mail addresses: fahim.cader@gmail.com (F. Mohamed), nicholas.buckley@sydney.edu.au (N.A. Buckley), shalukajaya@yahoo.com (S. Jayamanne), john.pickering@icloud.com (J.W. Pickering), phyllip.peake@sesiahs.health.nsw.gov.au (P. Peake), chathura7@yahoo.com (C. Palangasinghe), thilini.wijerathna@gmail.com (T. Wijerathna), rmiu.ratnayake@yahoo.com (I. Ratnayake), fathimashihana@gmail.com (F. Shihana), z.endre@unsw.edu.au (Z.H. Endre).

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1. Introduction

Deliberate self-poisoning with paraquat herbicide is common and has an estimated case fatality of more than 50% (Dawson et al., 2010), particularly when followed by acute kidney injury (AKI) (Kim et al., 2009; Lee et al., 2002). Paraquat-induced oxidative stress in the acute phase leads to toxicity in many organs particularly lungs and kidneys (Dinis-Oliveira et al., 2008; Gawarammana and Buckley, 2011) while paraquat induced AKI may aggravate toxicity to other organs by decreasing paraquat clearance (Beebeejaun et al., 1971).

Several studies have shown that the rapid increase in sCr following paraquat poisoning (Gil et al., 2009; Roberts et al., 2011) cannot be solely driven by the AKI-mediated decrease in glomerular filtration rate (GFR) and hence over-estimates true renal functional loss (Mohamed et al., 2015). Therefore, alternative approaches would be useful for early diagnosis or confirmation of paraquat-induced nephrotoxicity. A panel of seven biomarkers proposed by the Predictive Safety Testing Consortium (PSTC) was qualified by the Food and Drug Administration (FDA) and European Medicines Agency (EMEA) for safety assessment in pre-clinical drug development studies (Dieterle et al., 2010; Ferguson et al., 2008). These diagnose AKI early with high specificity and sensitivity depending upon site and mode of renal injury (Bonventre et al., 2010).

The clinical utility of most novel urinary biomarkers in detecting AKI has not been explored after paraquat poisoning. A few small clinical studies have utilised serum cystatin C (sCysC), urinary kidney injury molecule-1 (uKIM-1), plasma (pNGAL) (neutrophil gelatinase-associated lipocalin) and urinary NGAL (uNGAL) to predict death (Roberts et al., 2011) and in one study to diagnose AKI, where increases in sCr preceded increases in uNGAL and uKIM-1 (Gil et al., 2009). In contrast, urinary KIM-1, urinary cystatin C (uCysC) and albumin (uAlb) were sensitive biomarkers in predicting paraquat-induced AKI within 16–24 h in a nephrotoxic rat model as defined by histopathological change (Wunnapuk et al., 2013). In order to determine the clinical utility of injury biomarkers, we performed frequent serial biomarker measurements in a prospective patient cohort following paraquat poisoning, utilising the FDA/EMEA qualified panel of biomarkers plus additional selected novel urinary damage biomarkers.

We hypothesised that a panel of novel urinary structural damage biomarkers are superior to serum creatinine in independently detecting paraquat-induced nephrotoxic AKI (ToxAKI) and correlate with specific pathways of renal injury. The main objective of this study is to evaluate the utility of PSTC biomarkers panel and additional selected urinary biomarkers in early diagnosis of paraquat-induced ToxAKI and to explore whether increase in specific biomarker relate to mechanism-specific injury pathways. The other aim of this study is to evaluate whether pre-clinical paraquat ToxAKI rodent model findings translate into clinical practice.

2. Methods

2.1. Study design and data collection

This nested cohort study within an ongoing multi-centre observational study on self-poisoning in Sri Lanka was approved by the human research ethics committees of both the University of New South Wales (Sydney), Australia and the University of Peradeniya (Peradeniya, Sri Lanka). Between October 2010 and March 2013, patients admitted to study hospitals within 24 h of paraquat ingestion were consented after initial resuscitation and clinical management using written informed consent from each patient or a relative. Patients who were <15 years, pregnant, had co-ingested other toxins, or unable to provide samples were excluded. Paraquat ingestion was confirmed by a positive urine dithionate test. Demographic and clinical data were collected from consenting patients until discharge.

2.2. Sample collection and biomarker assays

Blood and urine samples were collected at 4, 8, 16 and 24 h after ingestion where possible, then daily until discharge or death and at follow-up at one and three months. Blood and urine samples were also collected from consenting healthy volunteers to establish normal baseline biomarker concentrations. All samples were processed within 30 min of collection. Blood samples were spun at 2000–3000 rpm and serum samples were transferred in to small cryotubes. Urine samples were immediately centrifuged at 1500–2000 rpm and the supernatant stored. Both serum and urine aliquots were stored at −20 °C for up to 3 months and then −70 °C until batch analysis within 6 months.

Biomarker assays were conducted batch-wise on samples collected from both patients and healthy controls. Serum and urine creatinine were measured using the Jaffe method (kinetic method, rate blank and compensated) on a Hitachi 912 automatic analyzer (Roche, Japan). Serum CysC was quantified using microparticle enhanced immunoturbidimetry on a clinical chemistry analyzer (Konelab™, Thermo Fisher, Waltham, MA) following the manufacturer's recommendations.

DuoSet ELISA kits (R&D systems®) were used to assay uKIM-1, and uClu. Urinary IL-18 was measured using the platinum enzyme-linked immunosorbent assay (Bender MedSystems, Vienna, Austria). Intra- and inter-assay precision for ELISA was <10%. Six AKI biomarkers [uCysC, uAlb, urinary trefoil factor 3 (uTFF3), osteopontin (uOstP), beta-2-microglobulin (uβ2M) and uNGAL] were quantified simultaneously using Bio-Plex Pro™ RBM Human Kidney Toxicity Assays panel 2 on the Bio-Plex 200 system (BIO-RAD, USA). Inter- and intra-assay precision was <15% and <5%, respectively. Serum and urinary paraquat levels were measured at the Therapeutic Research Centre, University of Queensland, Brisbane, Australia, using LC–MS/MS (Wunnapuk et al., 2011). Biomarker concentrations were reported as the absolute concentration or normalised to uCr excretion (Ralib et al., 2012; Westhuyzen et al., 2003). The area under the concentration curve (24 h AUC, a measure of the biomarker concentration integrated over time) at 24 h for each biomarker was calculated using the trapezoidal rule. Apparent creatinine clearance was calculated in ml/min from: [urine flow rate (ml/min) × uCr (mg/dl)/sCr].

2.3. Outcome measurement

Functional AKI was defined by two approaches based either on an increase in sCr or sCysC. Diagnostic performance of each urinary biomarker was assessed using both these definitions and compared in sensitivity analysis. Acute kidney injury network (AKIN) criteria were used to define functional AKI based on sCr (Mehta et al., 2007) and to categorise patients into severity stages. Despite noted limitations of sCr, AKIN definition was used in this study since it is widely used definition to assess the performance of novel injury biomarkers in clinical studies (Siew et al., 2011; Waikar et al., 2012). Further, clinical biomarker studies have used different percentage change in sCr or sCysC to define functional AKI (Briguori et al., 2010; Nejat et al., 2010; Pickering and Endre, 2014; Siew et al., 2011; Waikar et al., 2012). Therefore, development of moderate or severe AKI (stage 2 or 3, an increase in sCr of ≥200% or 300%, respectively) was selected as the primary outcome definition (Basu et al., 2014) of functional AKI in this cohort since rapid increases in serum creatinine within 24 h of paraquat ingestion