Original Article

Amino Acids from Urine as Possible Biomarkers for Early Detection of Vancomycin Nephrotoxicity

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ABSTRACT

Drug-induced nephrotoxicity is an important therapeutic concern, as many endogenous compounds are filtered through the kidneys for excretion into urine. Vancomycin is a drug of last resort used to treat multiple drug-resistant infections, and is primarily used in paediatrics to treat infections caused by gram-positive organisms resistant to beta-lactam antibiotics. Vancomycin is primarily (80–90%) excreted through the kidney. To identify biochemical markers useful for the early diagnosis of nephrotoxicity, amino acid profiling was performed in young Wistar rats treated with vancomycin. A liquid chromatography-mass spectrometry-based method was developed for targeted amino acid analysis from urine samples collected after dosing with vancomycin (300 mg/kg). Alterations in amino acids levels were observed in urine immediately after the first dosing, and increased in prominence during the course of treatment. Nephrotoxicity was confirmed using established methods such as histopathological evaluation and clinical chemistry analysis. Of note, a significant change in amino acid levels in urine was observed well before any noticeable increase in traditional markers. This suggests that quantification of amino acids from urine could be a good alternative to blood-based analysis in neonates and children as a strategy for the detection of kidney injury at an earlier stage than any existing methods.

Keywords: Amino acid profiling, LC-MS, Method validation, Nephrotoxicity, Targeted analysis, Vancomycin.

Submission Date: 26-06-2021;
Revision Date: 01-01-2022;
Accepted Date: 16-05-2022.

DOI: 10.5530/ijper.56.3.130

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INTRODUCTION

Vancomycin was the first glycopeptide antibiotic to be approved by the Food and Drug Administration (FDA) in 1958, and has a unique mechanism of action in treating *Staphylococcal* and *Streptococcal* infections in paediatric patients.[1] The incidence rate of vancomycin-associated acute kidney injury ranges from 7.2–27.2% in children and 12–42.5% in adults. In a regression analysis study of 175 children, the likelihood of nephrotoxicity increased by 16% with each 5 mg/kg vancomycin dose and by 11% with every additional day of treatment.[2-4] Concomitant use of vancomycin with Tazobactam/Piperacillin combination is also reported to cause acute kidney injury (AKI).[5] While, monitoring blood levels of vancomycin in adults are reported as a way of preventing acute kidney injury (AKI) in high-risk adult population, this is not a useful method when it comes to paediatric population because of ethical concerns in collecting blood samples (1-2 mL) by vein puncture frequently for monitoring purposes.[6-7] There is a need for alternative analytical technique to estimate biomarkers

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from easily available biological matrices such as urine, where the sample collection is non-invasive.

Conventional parameters such as creatinine and blood urea nitrogen (BUN) represent established biomarkers of nephrotoxicity. However, the lack of regional specificity and the significant increase in these markers following the loss of two-thirds or more of renal functional efficiency limit their sensitivity and specificity in the detection of early nephrotoxicity. In light of these limitations, toxicology researchers have focused their attention towards metabolomics to detect injury prior to pathological damage. Many workers have reported increased levels of amino acid as a pathological manifestation of acute kidney injury.[8-10]

In the current study, urine amino acids were evaluated for their potential as candidate markers for vancomycin-induced nephrotoxicity using an LC-MS based targeted amino acid profiling. Glycine, serine, glutamine, tryptophan, alanine, and threonine were evaluated since these are the associated products of proteinuria in AKI. In order to correlate the observed change in the levels of urine amino acids with nephrotoxicity, histopathology of kidney was carried out along with an evaluation of known clinical chemistry parameters such as urine creatinine and blood urea.

MATERIALS AND METHODS

Chemicals, Reagents and Instruments

Standards for glycine, serine, glutamine, tryptophan, alanine, threonine, and norleucine were a gift from the Biochemistry department, KMC, Manipal. All reference materials had a purity ≥ 98%. Creatinine reagent, and urea (BUN) reagent kits were obtained from Aspen, India. Acetonitrile (LC-MS grade) was purchased from Biosolve, France. Formic acid was from Merck, USA. Chromatographic column ZIC-HILIC (SeQuant) (250 × 4.6 mm, 3 µm) was from Merck.

Amino acid analysis were performed on a LC-MS (Thermo Scientific) consisting of a Dionex Ultimate LC system 30000 interfaced with a linear ion-trap analyser and an electrospray ionisation. Chromatographic and mass spectrometric method development were performed using Chromeleon and LTQ XL softwares respectively. Batch analyses were carried out using XCalibur software and quantification was performed using LC Quan. Serum creatinine and BUN were measured using a Merck semi-autoanalyzer.

Preparation of stock solutions, calibrators and quality controls

Stock solutions (1000 µg/mL) of glycine, serine, glutamine, tryptophan, alanine, threonine, and norleucine (internal standard) were prepared using Milli Q water. Working stocks of 100 µg/mL of each analyte were prepared in the mobile phase. Calibration levels of 0.3, 0.5, 0.7, 1.0, 2.0, 4.0, 6.0, 8.0, and 10.0 µg/mL were prepared daily from working stock solutions of standards by spiking pooled healthy rat urine. Prior to preparation of quality controls, basal level of analytes in pooled healthy urine samples were determined by multiple injections at the optimized conditions. Preparation of median quality control (MQC) and high-quality control (HQC) was achieved by spiking urine with working stocks of all analytes to a final level of 2 µg/mL and 8 µg/mL, respectively. Low quality control (LQC) was prepared from a nine times dilution of HQC. Lower limit of quantification (LLOQ) of glycine and other analytes were prepared by twenty-and thirty-three times dilutions, respectively, of a 10 µg/mL solution with the mobile phase.

Optimization of sample extraction from urine

Methanol and acetonitrile were evaluated for protein precipitation, but this technique was not considered suitable because of a matrix effect and also due to a possible loss of significant amounts of amino acids. The 'dilute and shoot' approach was therefore attempted. A 100 times dilution of the sample in the mobile phase was done and 5 µL of diluted sample was injected for LC-MS.[11-12]

LC-MS Method Conditions

Optimized liquid chromatographic conditions were as follows: Column: SeQuant ZIC-HILIC (250 × 4.6 mm, 3 µm); Temperature of column oven and auto sampler: 35°C and 4°C, respectively; Mobile phase composition: acetonitrile:water (75:25) with 0.1% v/v/v formic acid. Separation was carried out isocratically at a flow rate of 0.8 mL/min, and an injection volume of 5 µL was used. The use of HILIC column technology resulted in better retention of these ionized basic groups of amino acids because of ionic interactions with the sulfoalkylbetaine of the stationary phase.[13-15] The retention time of target amino acids ranged from 5–20 min. Efforts to decrease the retention time resulted in a decreased MS response and a high background noise. Acetonitrile was used as the organic modifier because of its weak hydrogen-bonding ability and its established use with HILIC platforms.[16]

Optimized mass spectrometry (MS) conditions: Vaporizer temperature was kept at 300°C and spray voltage was at 2.5 kV. Sheath gas, auxillary gas and sweep gas flows were optimized at 55, 15 and 1 arbitrary units respectively. The ion transfer capillary was maintained at a voltage of 20 V and at 300°C. SRM transition

and collision energy (CE) for each amino acid were as follows: (m/z 76→30, CE: 82) for glycine, (m/z 106→60, CE:33) for serine, (m/z 147→130, CE:44) for glutamine, (m/z 205→188, CE:41) for tryptophan, (m/z 90→44, CE:37) for alanine, (m/z 120→74, CE:28) for threonine, and (m/z 132→86, CE:35) for norleucine.

Validation of the LC-MS method

Validation was performed as per FDA guidelines and as per Lee *et al.*, to establish the precision, accuracy, linearity, recovery, matrix effect and stability.[17-18]

A set of six samples spiked with the analytes and internal standard were injected prior to each sequence to determine system suitability. A minimum of six healthy urine samples from individual rats with the internal standard were processed to assess the selectivity. Carryover effect was evaluated by injecting six blank samples after each LLOQ and ULOQ samples.

Calibration curves of concentrations 0.3–10 µg/mL were constructed for alanine, serine, glutamine, tryptophan, and threonine, and of concentration 0.5–10 µg/mL for glycine, with five replicates at each concentration. To determine linearity, analyte to IS (internal standard) area ratio was plotted against concentration and studied the linear regression. Accuracy was studied from six replicates of quality control (QC) samples. Mean calculated values were compared with the nominal values. Inter-day precision was determined from six replicates of QC samples over three consecutive days. The relative standard deviation (% RSD) was used for characterising precision.

To evaluate the extraction efficacy and total recovery of amino acids from urine, six replicates of QC samples (LQC, MQC and HQC) were prepared by spiking blank urine with respective working standard solution of amino acids and processed. Post extraction spike method was used to evaluate matrix effect.

Stability of stock solution was determined at 2–8°C for 30 days on MQC samples. Matrix stability was evaluated at LQC and HQC at room temperature for 8 hr, freeze-thaw (3 cycles, −80°C) and at -70°C was also evaluated for a period of up to 60 days. Stability samples were compared with a set of freshly prepared comparison samples at each QC levels.

Animal Study Design

All animal experimental protocols were approved prior to experiments by the Institutional Animal Ethics Committee, Manipal University (IAEC/KMC/62/2015), and experiments were carried out according to the guidelines of the Committee for the Control and Supervision of Experiments of Animals, India. Twenty-five female rats (Wistar) of 5–6 weeks age and 80–120 g, were obtained from the central animal research facility of Manipal Academy of Higher Education. The animals were housed in sterile polypropylene cages containing sterile husk, at a maximum of three animals per cage, and at conditions of 18–29°C, 30–70% relative humidity, and a 12 hr dark/12 hr light phase. The animals were allowed access to feed and water *ad libitum*.

Prior to experimentation, animals were allowed to acclimatize to their surroundings for 7 days. Animals were divided into five groups with five animals in each group. Treatment groups were given 300mg/kg/day Vancomycin *intra-peritonially* (*ip*) for 7 days and then once weekly for up to 28 days, while the control group were given distilled water (*ip*). Blood and urine samples were collected terminally on days 4, 8, 15, and day 29 before euthanasia from the designated treatment groups. Blood and urine samples were also collected from the control group animals on similar days and were euthanized on 29th day. All treatments were given in the evening and samples were collected the following morning. A day prior to urine collection, animals were housed in individual metabolic cages for 18 hr (fasted with free access to water). Before termination, blood was collected from the retro orbital plexus after anaesthesia for a brief period of time. Animals were euthanized using excess thiopentone sodium (*i.p.*) and both kidneys were perfused using 20 mL of normal saline, extracted, washed with normal saline, and stored in 10% neutral buffered formalin at room temperature.[19]

Urine was centrifuged at 15,000 rpm for 10 min at 4°C, aliquoted, and stored at −80°C until sample analysis. Blood samples were collected in micro centrifuge tubes and centrifuged (4°C, 8000×*g*, 10 min) to separate serum, which was frozen at −80°C until further analysis. All urine samples were analysed for the selected amino acids, and creatinine. Serum samples were analysed for urea (BUN). Isolated kidneys were evaluated for nephrotoxicity by histopathological analysis. In all situations, adequate measures were undertaken to minimize pain and discomfort to the animals.

Amino Acid Profiling

Aliquots of urine samples (10 µL) were thawed and added 10 µL of internal standard (Norleucine) and 980 µL of mobile phase (100-fold dilution). Samples were vortexed for 1 min and centrifuged at 15,000 rpm for 10 min at 4°C. The resulting supernatant was transferred into auto-sampler vials and 5 µL was injected for LC-MS analysis using the optimized method. The final urine concentration of the amino acids were

normalised to 0.25mg/dL creatinine and the percent change in the levels of amino acids in comparison with the untreated control was determined.

Clinical Chemistry Evaluation

On the day of analysis, aliquots of urine sample were thawed at room temperature, and *in vitro* quantitative determination of creatinine in urine was performed using a modified Jaffe's method.[20-21] Blood Urea Nitrogen (BUN) was determined using the urease/GLDH method.[22-23]

Histopathology Examination

Segments of kidney were fixed in 10% neutral buffered formalin, routinely processed and embedded in paraffin, sectioned at 5 µm, stained with haematoxylin and eosin, and examined under light microscopy. Histological observations were scored based on severity of necrosis (score 0: No effect or no injury; 1: Minimal or < 10%; 2: Mild or 10–25%; 3: Moderate or 25–40%; 4: Marked or 40–50%; 5: Severe or > 50%).

Statistical Analysis

All values were represented as mean ± SD, and values with $p<0.05$ were considered as statistically significant. ANOVA was used for statistical analysis. Tukey's multiple comparison post hoc test using Graph Pad Prism version 5.03 (GraphPad Software Inc., USA) was also performed.

RESULTS AND DISCUSSION

In this study, we assessed the potential of selected amino acids in urine to act as candidate markers for vancomycin nephrotoxicity. Urine was chosen as the matrix of choice as it is a waste product which can be collected without invasive procedures and contains metabolites that can mirror an individual's health status.[24] Under nephrotoxic conditions, the excretion of amino acids such as glycine, alanine, tryptophan, serine, glutamine, and threonine is habitually altered because of impaired reabsorption by the renal tubules, increased cellular turnover, or increased permeability of the glomerular membranes.

Amino acids are zwitterionic in nature with a log p of −1.06 to −3.21, making them excellent candidates for electron spray ionisation technique in MS analysis. In acetonitrile:water (50:50% v/v) mixtures, all α-amino acids form protonated ions that fragment via a common fragmentation pattern to form an immonium ion by successive loss of water and carbon monoxide. In the case of tryptophan, owing to the functional group on the side chain, the formation of the immonium ion is in competition with the loss of ammonia to form

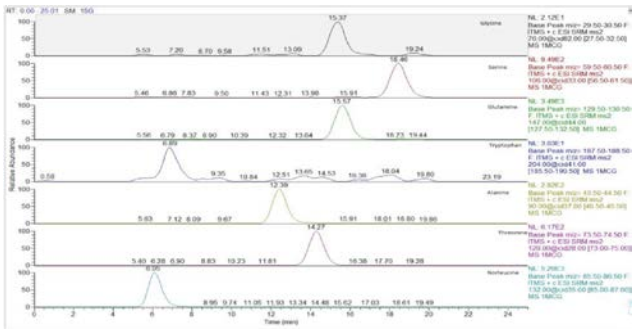

Figure 1: LC-MS chromatogram of a vancomycin treated rat urine sample in the SRM mode under optimized conditions for targeted amino acids.

fragments.[25-26] This helps in adapting a selective reaction-monitoring (SRM) technique to give added specificity to the analysis.

Chromatographic conditions were optimized to achieve a short run time with good peak shape and selectivity for all analytes and the internal standard. The chromatograms obtained with this optimized method are presented in Figure 1.

For the extraction of markers from urine, the 'dilute and shoot' approach was found to be successful, with a reduced matrix effect. 100 times dilution of urine in the mobile phase yielded recoveries > 77%, with minimum matrix interference. Sample extraction from urine using protein precipitation was found to be unsuitable due to an observed matrix effect.

Bioanalytical Method Validation

The method was successfully validated and the results were within the limits set by relevant guideline. System suitability was evaluated using % coefficient of variation (CV) of retention time and peak area ratio, which were ≤ 0.50 and ≤ 19.62, respectively. No significant endogenous interference was observed at the retention times of analytes and internal standard in rat urine, indicating that the method is selective. The regression coefficient (R^2) values obtained were at or above 0.9835 for glycine, 0.9838 for serine, 0.9837 for glutamine, 0.9839 for tryptophan, 0.9836 for alanine, and 0.9839 for threonine. The validation results also showed consistent values for accuracy, precision, matrix effect, and extraction recovery, which are shown in Table 1. The inter-batch accuracy of the method was satisfactory, with the % nominal concentration well within the acceptance limits of 85–115%. The inter-batch precision results were also within the acceptance limits, as evidenced by % CV values < 15%. The matrix effect was within the acceptance level, evidenced by % CV of internal standard normalized matrix

Table 1: Key performance parameters of the developed LC-MS method for amino acid profiling.

		QC level	Gly	Ser	Glu	Tryp	Ala	Thr	Nor
Inter-batch accuracy	% NC	LLOQ	95.25	104.74	100.02	104.44	107.54	104.70	-
		LQC	105.51	103.52	101.83	102.34	107.60	103.14	-
		MQC	98.25	104.81	105.14	101.59	105.33	102.10	-
		HQC	107.68	106.87	102.14	98.33	92.18	97.29	-
Inter-batch precision	% CV	LLOQ	1.59	11.45	8.13	0.97	6.43	4.13	-
		LQC	2.08	8.46	6.97	4.73	0.81	5.96	-
		MQC	2.38	8.20	1.54	3.73	2.53	9.39	-
		HQC	2.91	6.66	5.20	4.32	3.13	6.86	-
Matrix effect	% CV	LQC	14.12	8.33	5.9	13.80	12.85	10.96	4.93
		HQC	13.62	13.74	13.85	14.19	14.93	12.98	5.88
Extraction recovery	% Recovery	LQC	123.37	102.84	70.63	109.18	100.46	99.77	109.90
		MQC	86.79	110.66	77.19	103.49	110.84	107.80	110.63
		HQC	108.66	110.75	95.02	104.89	96.63	107.98	108.33

Gly: glycine; Ser: serine; Glu: glutamine; Try: tryptophan; Ala: alanine; Thr: threonine; Nor: norleucine; QC, quality control; LLOQ, lower limit of quantification; LQC, lower quality control; MQC, median quality control; HQC, higher quality control; CV, coefficient of variation; NC, nominal concentration.

factor (MF) < 15%. The % recovery observed in the extraction recovery study was consistent, precise, and reproducible, and the % CV across the QC level was found to be < 20%, which was within the acceptable limits. For calibration curve optimisation, endogenous concentrations in control (untreated) samples were determined by multiple injections of a pooled matrix. The upper limit of the calibration curve was set at 10 times the normal level and the lower limit was set below the endogenous measured concentration.

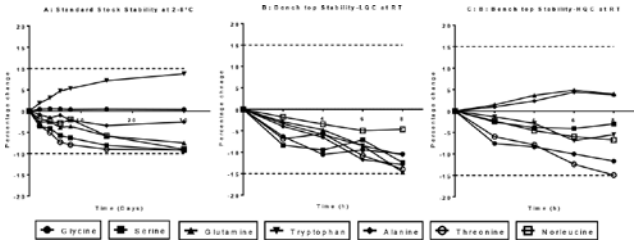

Figure 2: Graphical representation of stability of selected amino acids. A) Stock solution stability B) Bench top Stability (LQC) and C) Bench top stability (HQC).

Stability of Amino Acids in the Experimental Conditions

Results of the stability study showed that all amino acids were stable for 30 days at 2–8°C. Stock solution stability of all analytes and the internal standard was found to be within the acceptance limit of ±10%. The stability of the markers in matrix analysed in bench top and freeze-thaw studies showed that all amino acids were stable for 8 hr at room temperature and after three freeze-thaw cycles in matrix. The calculated concentrations of LQC and HQC were found to be within the acceptance criteria of 85–115% of the nominal concentration, and mean % change of analytes was within ± 15%. The stability study results are presented in Figure 2. Long term stability evaluation in matrix proved that the selected markers are stable in plasma for the studied of up to 60 days at -70°C.

Targeted Amino Acid profiling of Urine

The results of the amino acid profiling are shown as percentage changes in levels of amino acids during treatment at various time points with respect to day zero (Table 2). Increased levels of Glycine, Tryptophan and Alanine were observed, starting from day 3 of the treatment, and the % increase was highest on day 14. Percentage increase in the levels of these amino acids were very pronounced, and found to be more than 75% from as early as from day 3 and were statistically significant. Urinary excretion of other amino acids such as Serine, Glutamine and Threonine also showed an increase in their levels but their % increase was not significant. Changes in the levels of these markers were relatively unaffected through days 14-28.

Evaluation of Traditional Markers of Nephrotoxicity

Although Creatinine and BUN levels increased with treatment, it was only on day 14, a significant increase of more than 50% was seen (Table 2). A comparison of the % changes in levels of traditional markers of nephrotoxicity (Creatinine, BUN) with the levels of amino acids on Day 3, Day 7 and Day 14 is presented in Figure 3, which clearly indicate that there is a significant

Table 2: Percentage increase in levels of amino acids in comparison with traditional markers from day 0 to day 28 in rats after treatment with vancomycin.

	Markers (µg/mL)	†Percent change (Mean ± SD)			
		Day 3	Day 7	Day 14	Day 28
Amino acids	Glycine	98.17±26.66*	120.1±15.86 *	136.86±27.72*	145.61±24.91*
	Serine	35.65±35.80	63.12±25.49*	75.45±8.97*	69.10±14.54*
	Glutamine	26.81±23.81	55.04±18.55*	46.44±8.85*	67.28±12.98*
	Tryptophan	109.40±19.40	147.80±22.20	169.50±31.05	166.30±47.04
	Alanine	76.40±34.31*	103.95±36.26*	139.76±22.98*	146.04±13.00*
	Threonine	27.02±14.00	46.53±28.17	60.21±4.63*	67.08±26.54*
Traditional markers	Creatinine	10.25±3.72	15.17±7.62	76.38±11.08*	85.16±68.90*
	BUN	7.94±3.23	38.67±22.99	56.57±53.32	57.95±34.54

† Percentage change with respect to day 0. BUN: Blood Urea Nitrogen, N=5 for each group. One-way ANOVA and Tukey's multiple comparison post hoc test were used to determine statistical significance. *p<0.05 compared with day 0; Percent change = (treated–healthy)/healthy*100.

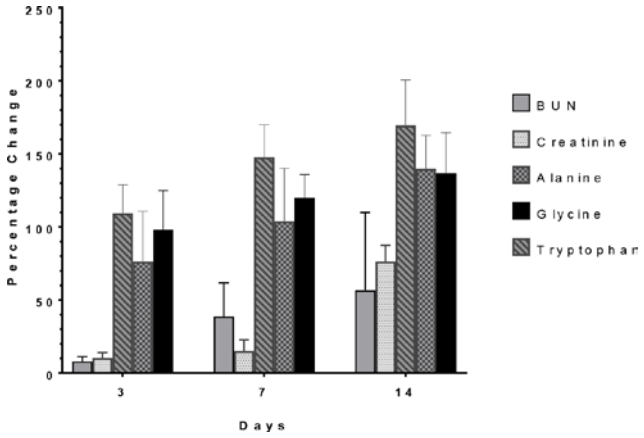

Figure 3: Representation of % changes in levels of traditional markers of nephrotoxicity (Creatinine, BUN) and the levels of amino acids on Day 3, Day 7 and Day 14.

change in levels of amino acids from day 3 of treatment in comparison to that of traditional markers.

We also correlated the levels of amino acids in urine and creatinine clearance at various time points. There was no correlation observed at Day 4 and Day 8. A significant correlation between the two variables was observed on Day 15 and Day 29 (Table 3).

Histopathology of Kidney

Results of histopathology evaluation are presented in Table 4 and Figure 4. The control group showed normal renal histology (Figure 4A). The vancomycin-treated groups presented with various features of nephrotoxicity such as congested blood vessels (Figure 4B), hyaline cast (Figure 4C), tubular dilation (Figure 4D), tubular degeneration and inflammatory cell infiltrates (Figure 4E). The observations were scored according to severity of necrosis (Table 4). Histologic sections of kidney

Table 3: Correlation data between amino acids in urine and creatinine clearance at various time points.

Markers	*P* value (correlation coefficient)			
	Day 4	Day 8	Day 15	Day 29
Glycine	0.855 (0.233)	0.567 (0.567)	<0.001 (0.877)	<0.001 (0.986)
Serine	0.987 (0.378)	0.345 (0.567)	0.001 (0.899)	<0.001 (0.879)
Glutamine	0.657 (0.155)	0.657 (0.345)	0.001 (0.799)	<0.001 (0.789)
Tryptophan	0.078 (0.235)	0.076 (0.467)	<0.001 (0.987)	<0.001 (0.899)
Alanine	0.045 (0.456)	0.023 (0.678)	<0.001 (0.879)	<0.001 (0.989)
Threonine	0.456 (0.479)	0.034 (0.347)	<0.001 (0.789)	<0.001 (0.789)

Correlation between the two variables was assessed by Pearson's correlation coefficient and a p<0.05 was considered statistically significant

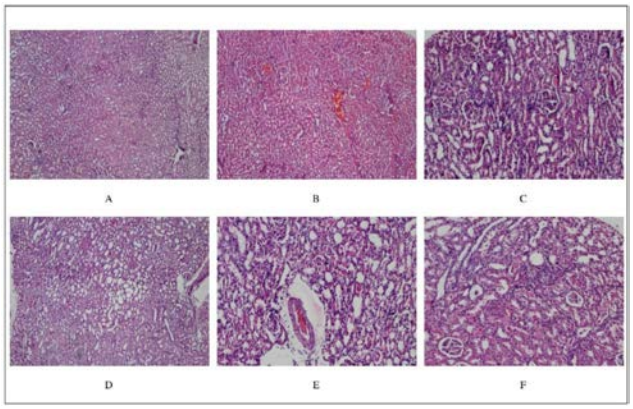

Figure 4: Features of renal histology in vancomycin-treated rat kidney at various time points.

(A) Healthy renal histology, (B) Congested blood vessels, 4x, (Day 3), (C) Hyaline cast, Tubular degeneration, 10x, (Day 7),(D)Tubular dilation, Hyaline cast, 4x, (day 14), (E) Tubular degeneration, Inflammatory infiltrates, 10x, (Day 14), (F) Inflammatory cell infiltrates, Tubular degeneration, Glomerular degeneration, 10x, (day 28).

Table 4: Vancomycin treatment-related kidney histopathology observation.

Histopathological observation scoring (out of 5)				
Time	**Tubular degeneration**	**Tubular dilation**	**Eosinophilic cast**	**Inflammation**
Day 3	/ (1/5) + (3/5) ++ (1/5)	/ (3/5) + (2/5)	/ (3/5) + (2/5)	+ (3/5) ++(2/5)
Day 7	+++ (1/5) ++++ (4/5)	/ (2/5) + (2/5) ++ (1/5)	/ (3/5) + (2/5)	++(2/5) +++ (3/5)
Day 14	++++ (2/5) +++++ (3/5)	++ (1/5) +++ (3/5) ++++ (1/5)	/ (1/5) ++ (3/5) +++ (1/5)	++++ (4/5) +++++ (1/5)
Day 28	+ (3/5) ++ (2/5)	/ (1/5) + (2/5) ++ (2/5)	/ (3/5) + (2/5)	++ (1/5) +++ (2/5) ++++ (2/5)

Histopathologic assessments were scored as follows: /≙ no injury, + ≙ minimal, ++ ≙ mild, +++ ≙ moderate, ++++ ≙ marked, and +++++ ≙ severe injury. The number of animals out of 5 presenting with kidney injury is represented in parenthesis, i.e. (1/5) denotes one out of 5 animals.

in treated groups showed varying degrees of tubular degeneration, with epithelial sloughing into the lumen and tubular necrosis. Vascular degeneration of tubules was also observed. Increased tubular dilation was seen from day 3 to day 14 and reduction of tubular dilation and tubular degeneration was observed on day 28. Most of the vancomycin-treated animals showed glomerular degeneration on days 3 and 7, with increased Bowman's space. Almost all treated animals showed a wide variation of inflammatory cell infiltrates, including numerous neutrophils, eosinophils, and plasma cells. Congested capillaries and haemorrhage were also observed in some histologic sections. Eosinophilic casts, such as hyaline casts, were observed on days 3 and 7 of the treatment, and were increased at day 14. Correlation of the change in amino acid level to kidney injury was supported by the histopathological examination results, where kidney injury was observed to start at day 3 and become severe by Day 14.

CONCLUSION

A validated analytical method was developed for targeted amino acid analysis from urine using LC-MS as a possible alternative to predict nephrotoxicity. The method was applied to study the nephrotoxicity associated with vancomycin usage in animal model. Glycine, tryptophan and alanine were found to be very promising markers for an early detection of nephrotoxicity. Significant increase in the excretion of these amino acids was evident from as early as day 3, which was corroborated with histopathological evaluation of the kidneys of treated animals. It is worth noting here that the traditional markers detected the nephrotoxicity only after 14 days. This suggests that an amino acid profiling approach can be used to detect nephrotoxicity at early stages of treatment, thus preventing further nephrotoxicity. Future studies to validate the potential of these amino acids to serve as biomarkers of nephrotoxicity are warranted.

ACKNOWLEDGEMENT

Authors acknowledge Department of Biochemistry, Kasturba Medical College, Manipal for providing amino acid samples.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AKI: Acute Kidney Injury, **BUN:** Blood Urea Nitrogen, **HQC:** High Quality Control, **LQC:** Low Quality Control, **MQC:** Median Quality Control, **LCMS:** Liquid Chromatography Mass Spectrometry.

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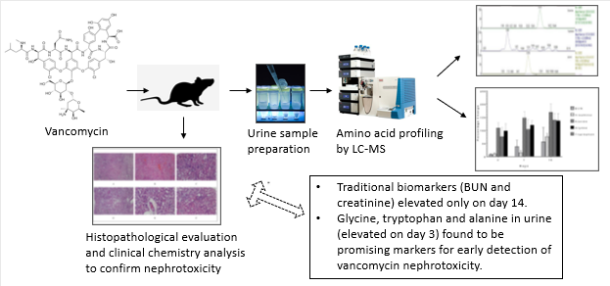
PICTORIAL ABSTRACT



SUMMARY

An analytical method based on LCMS was developed and validated for early detection of nephrotoxicity by Vancomycin. This method is of paramount importance for pediatric population considering that the sample matrix used for analysis is urine, which does not require any invasive procedure for sample collection. The method was able to detect a possible kidney injury as early as from 3 days of treatment.

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Cite this article: Nanaware HR, Moorkoth S, Kumar N, Jasphin S, Raveendran A, Chaudhari BB. Amino Acids from Urine as Possible Biomarkers for Early Detection of Vancomycin Nephrotoxicity. Indian J of Pharmaceutical Education and Research. 2022;56(3):795-803.