MEDICAL SCIENCE MONITOR

CLINICAL RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2018; 24: 1397-1407
DOI: 10.12659/MSM.905843

Received: 2017.06.19
Accepted: 2017.09.15
Published: 2018.03.07

Metabolic Profiling of Amino Acids Associated with Mortality in Patients with Acute Paraquat Poisoning

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEF 1,2 Xiuxian Wan
BC 3 Chunyu Zhou
F 3 Xin Kang
F 3 Dayong Hu
B 3 Wen Xue
B 3 Xinhua Li
ABCDEG 1,3 Hui Bao
AG 1,3 Ai Peng

1 Department of Nephrology and Rheumatology, Affiliated Shanghai Tenth Clinical Medical College of Nanjing Medical University, Nanjing, Jiangsu, P.R. China
2 Department of Nephrology, Lianyungang Oriental Hospital, Lianyungang, Jiangsu, P.R. China
3 Center for Nephrology and Clinical Metabolomics, Tongji University School of Medicine, Shanghai, P.R. China

Corresponding Authors: Ai Peng, e-mail: pengai@tongji.edu.cn; Hui Bao, e-mail: nbspela@hotmail.com
Source of support: This work was supported by the Natural Science Foundation of China No: 81270136, 81671897 (to A.P.), No: 81500508 (to H.B.), Shanghai Pujiang Program No: 15PJ1406800 (to H.B.), Shanghai international cooperation program No: 16410724200 (to H.B.)

Background: Paraquat is a major cause of fatal poisoning after ingestion in many parts of Asia and the Pacific nations. However, optimal prognostic indicators to evaluate patient mortality have not been unequivocally established. Following acute paraquat poisoning, a number of amino acids (AA), are abnormally expressed in metabolic pathways. However, the alterations in AA metabolite levels after paraquat poisoning remain unknown in humans.

Material/Methods: In the present study, 40 patients were enrolled, of whom 16 survived and 24 died. A metabolomics approach was used to assess changes in AA metabolites in plasma and its potential prognostic value following paraquat poisoning. Mass spectrometry (MS) based on metabolite identification was conducted.

Results: Twenty-five AA levels in plasma were abnormally expressed in non-survivor patients. Among them, creatinine, indolelactate, and 3-(4-hydroxyphenyl)lactate were found to be highly correlated with paraquat death prediction. It was noted that the intensity levels of these 3 AA metabolites in the non-survivor group were substantially higher than in the survivor group. Furthermore, we examined receiver operating characteristic (ROC) curves for clinical validation. ROC results showed that 3-(4-hydroxyphenyl)lactate had the highest AUC of 0.84, while indolelactate and creatinine had AUCs of 0.75 and 0.83, respectively, suggesting that they can be used to predict the clinical outcome (although this methodology is expensive to implement).

Conclusions: Metabolic profiling of AA levels could be a reliable tool to identify effective indicators for the early high precision prognosis of paraquat poisoning.

MeSH Keywords: Amino Acids, Acidic • Metabolomics • Mortality • Paraquat

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/905843

3156 3 6 37

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Background

Paraquat (N, N -dimethyl-4,4-lipoprotein dichloride; PQ), a highly effective herbicide, is a major cause of fatal poisoning after ingestion in many parts of Asia and the Pacific nations. It is very toxic to humans and there is no antidote to its effects or any effective therapy, producing a mortality rate of 50–90% in affected individuals [1]. Hence, the early diagnosis and prognosis of PQ poisoning is of great importance. Currently, many potential prognostic indicators have been studied to evaluate the severity of poisoning, such as the concentration of PQ in plasma, acute physiology and chronic health evaluation (APACHE II) and sequential organ failure assessment (SOFA), pancreatic enzymes, uric acid and a neutrophil counts [2–4]. However, the optimal markers remain to be established.

Amino acids (AA) are used for protein biosynthesis and also function as precursors for the synthesis of proteins and many signaling molecules that are involved in the growth, renewal and repair of cells [5]. They include 22 proteinogenic AA that are natural components of polypeptides. Many AA that are non-proteinogenic also have important roles as metabolic intermediates. For instance, gamma-aminobutyric acid and glutamate act as inhibitory and excitatory neurotransmitters, respectively [6]. Glycine is a biosynthetic precursor to porphyrins used in red blood cells [7]. Hence, altered AA metabolism likely leads to many human diseases. There are significant differences in d-AA concentrations between healthy people and Alzheimer disease (AD) patients [8]. A number of studies have found marked changes in muscle and plasma AA status in chronic obstructive pulmonary disease [9]. Also, patients with liver disease often have impaired AA metabolism [10].

However, the alterations in AA metabolites after PQ poisoning remain unknown. We therefore prospectively studied AA metabolites to identify altered metabolism specific to PQ poisoning that may be used to evaluate the degree of PQ poisoning, and uncover favorable indicators for early detection and outcome predictions.

Material and Methods

Population studied

Our study was conducted at the Shanghai Tenth People's Hospital of Nanjing Medical University between January 2012 and September 2014. The control group (n=10) included individuals without any previous disease, aged 20–55 years and no recent history of exposure to heavy metals or drugs. Patients with acute oral PQ poisoning (n=40) were enrolled in the study according to the following criteria: Suffered acute PQ poisoning by ingestion within 48 hours; urine or plasma PQ levels were >0.1 mg/L; age range 15–75 years; informed consent obtained from the patient or their family. Patients were excluded from the study according to the following criteria: the poison was not taken orally; admitted to hospital >48 hours after ingestion; ingestion of PQ with other toxins; pregnancy; a previous history of heart, kidney, liver, pancreas, or central nervous system disease, or failed to give consent. All patients who took part in the study were monitored for ≥90 days.

Treatment

The guidelines published by the Chinese Physician Association (2013 version) were carefully followed to ensure standardized treatment protocols for all patients enrolled in the study [11]. Thus, each patient underwent gastric lavage and was then prescribed 1 g/kg of activated charcoal and IV furosemide (40 mg/day). Hemoperfusion with charcoal was instigated if the dithionite test in urine was positive. Continuous renal replacement therapy was initiated if the patients had symptoms of hepatitis, respiratory insufficiency, and/or acute renal failure. The following support treatments were also initiated if required: vitamin C, glutathione and/or GSH as antioxidant therapy; measures to prevent infection and antibiotics if required; maintenance of acid-base status and appropriate electrolyte and fluid levels; pulse therapy with methylprednisolone; measures to protect vital organs. Oxygen therapy was only administered as a palliative measure in patients who were terminally ill.

Data collection

Data, collected by experienced physicians, including the general condition of patients, laboratory tests, and treatments, as previously described [4]. The levels of PQ in samples were measured using high-performance liquid chromatography (HPLC) (Agilent HPLC 1260; Palo Alto, CA, USA). The likely quantities of PQ taken when patients or family members provided this information were carefully documented; otherwise, an estimate of the quantity of PQ swallowed was made according to the following criteria: a small sip, 5 mL or a mouthful, 20 mL. Blood samples were taken for routine blood parameter measurements on day 1, 3, 5, 7, and 14 after hospital admission. The blood samples for metabolomics were taken on day 1, 7, 14, and 28 after hospital admission. APACHE II and SOFA scores were recorded for all patients after admission.

Metabolic profiling

Mass spectrometry (MS), based on metabolite identification, was conducted as previously described. The concentration of PQ in plasma was measured using HPLC (Agilent HPLC 1260; Palo Alto, CA, USA), using the modified method of Whitehead et al. [12]. Metabolon Inc. (Durham, USA) measured the metabolomics data as previously described [13,14].

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

In brief, proteins were precipitated and various metabolites isolated from samples by agitation in methanol for two minutes and centrifugation. The concentration of each metabolite was measured in plasma samples using an untargeted Liquid Chromatography/Mass Spectrometry (LC/MS) and Gas Chromatography/Mass Spectrometry(GC/MS) analyzer. The identity of each metabolite was determined automatically using software developed at Metabolon, by comparing their ionic features to a reference library of chemical standards that included molecular weights, retention times, in-source fragments and adducts, as well as relevant mass spectrometry spectra. The area under the curve (AUC) of the peak of a metabolite was used to quantify each one [15]. After mass filtering, alignment, and internal standard normalization, the data were further quantified.

Heat maps were constructed using "R" (*http://cran.r-project.org/*), which is a data matrix that by using color gradients can visualize cell values and also provide a picture of the smallest and largest values in the matrix [16]. Unsupervised clusters of samples were used to generate heat maps for the control, survivor, and non-survivor groups, with a fixed metabolite in order to visualize metabolic patterns. Data were median-centered; for each metabolite, the data were centered about the median value and appropriate color scales used to refer to a decrease or increase in the relative level of the metabolite. We also carried out, where appropriate, a heat map assessment of the Spearman correlation matrix [17].

SIMCA-P+ 13.0.3.0 Software (Umetrics, Sweden) was used to instruct a multivariate model, with variables being scaled to unit variance and centered about the mean prior to model construction. To analyze the data obtained by GC/MS and LC/MS, an unsupervised principal component analysis (PCA) was utilized in conjunction with analysis based on orthogonal partial least squares discrimination (OPLS-DA) [18,19]. The OPLS-DA method was used to determine predictive mortality involving two main classes, namely the survivor group versus non-survivor group. After taking into account score plots for OPLS and VIP calculations, the analytic program chose significant metabolites that were clearly responsible for PQ mortality. Variables were selected as targets when their VIP values were >1.0 and their characteristics were analyzed subjectively using a *t*-test (two-tailed), assuming asymmetrical variance, with a threshold value $p<0.001$ and a correlation >0.75.

Ethical statement

The Institutional Review Board of the Shanghai Tenth People's Hospital, Nanjing Medical University, approved the study ((IRB: 2010RES017) with all patients providing written informed consent for participation, or if not possible, consent was provided by appropriate family members.

Table 1. Patient demographics.

Characteristics	Value	
Age (years, median IQR)	31	(23–41)
Male (%)	21	(52.5%)
Ingestion volume (mL, median IQR)	20	(15–50)
APACHE	5	(2–9)
SOFA	3	(1–6)
Plasma PQ concentration (mg/L, median IQR)	0.5	(0.3–4.5)
Urine PQ concentration (mg/L, median IQR)	2.9	(1.0–20.4)
Delay from ingestion to admission (h, median IQR)	10	(4–12)

IQR – interquartile range.

Statistical analyses

All data are presented as percentages or medians (25 or 75 quantiles). The *p*-values are quoted as two tailed with a *p*-value <0.05 considered to be statistically significant. Prior to analysis, data were subjected to tests for a normal distribution and for equality of standard deviations. To determine if there were differences between the groups, Fisher exact tests for categorical variables; chi-squared tests and *t*-tests for continuous variables were employed. To determine the rate of fatality in patients poisoned with PQ, receiver operating characteristic (ROC) curves were constructed. SPSS version 22 and GraphPad Prism version 5 were used for all statistical analyses.

Results

Characteristics of the patients enrolled in the study

A total of 40 patients who ingested PQ within 48 hours of admission and 10 healthy control subjects were enrolled in the study. As shown in Table 1, the median interquartile range (IQR) of PQ-poisoned patients was 31 years (range 23–41). Approximately, the median of ingested PQ was 20 mL (15–50). The median plasma and urine concentration was 0.5 mg/L (0.3–4.5) and 2.9 mg/L (1.0–20.4), respectively. Approximately 37% of the patients underwent hemoperfusion therapy and 0.05% received treatment in the form of gastric lavage. Mortality was seen in 40% of the PQ-poisoned patients after treatment.

Changes in AA metabolites in patients who were acutely poisoned by PQ

AA metabolism is complex since a huge number of potential metabolites are involved. Metabolic profiling is an invaluable

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

method to study toxicity as it provides an inimitable insight into the biological reactions that produce toxicological insults [20].

Metabolic profiling, based on GC/MS and LC/MS, was used to assess the effects of PQ poisoning. We investigated 102 AA metabolites by MS analysis. The patterns of metabolites were visualized by constructing a heat map from data obtained from the control, survivor, and non-survivor groups. A red/blue color scheme was used to refer to an increase or decrease in the relative levels of identified metabolites (Figure 1 and Supplementary Figure 1). We found twenty-five AA, shown in a red color, that were strongly associated with PQ poisoning mortality, including N-acetylalanine, N-acetylaspartate, glutamine, 1-methylimidazoleacetate, N-acetyl-1-methylhistidine, glutarylcarnitine, phenyllactate, p-cresol sulfate, 3-(4-hydroxyphenyl)lactate, 4-hydroxyphenylacetate, 5-hydroxymethyl-2-furoic acid, kynurenate, kynurenine, indolelactate, serotonin, C-glycosyltryptophan, tiglyl carnitine, 3-methylglutarylcarnitine, citramalate, homocysteine, dimethylarginine, urea, creatinine, 4-acetamidobutanoate and guanidinosuccinate ($p<0.05$, Table 2). Among them, the intensity of glutamine and p-cresol sulfate was decreased, while other metabolite levels were significantly increased.

To further analyze the data, we applied an unsupervised PCA and supervised OPLS-DA between the survivor group and the non-survivor group. The PCA score scatter plot (Figure 2A) showed reasonable separation between the survivor group and the non-survivor group. We found eight AA that were strongly associated with PQ poisoning mortality, including creatinine, indolelactate, 3-(4-hydroxyphenyl)lactate, kynurenate, 3-methylglutarylcarnitine, C-glycosyltryptophan, phenyllactate and N-acetylserine ($p<0.05$, Figure 2B). Among these AA metabolites, creatinine, indolelactate and 3-(4-hydroxyphenyl)lactate, were found to be most closely associated with PQ poisoning mortality (RS >0.75, Figure 2B, 2**C**), consistent with the findings of the heat map analysis.

Amino acid metabolites may be associated with the PQ poisoning mortality

Following PQ poisoning, a number of AA were abnormally expressed in metabolic pathways. It was noted that PQ poisoning resulted in a remarkable elevation in creatinine, indolelactate and 3-(4-hydroxyphenyl)lactate levels that peaked on the day of admission (Figure 3A), remained significantly high for about two weeks after injury, and then slowly decreased over 28 days, congruent with the injury phase of PQ poisoning ($p<0.05$, Figure 3B–3D), suggesting these three AA metabolites were involved in PQ poisoning.

To validate the PCA and OPLS-DA data, the changes of these three AA metabolites intensity levels are shown in the control, survivor, and non-survivor groups (Figure 4). When the control and survivor groups were compared, it was found that levels of creatinine, indolelactate and 3-(4-hydroxyphenyl)lactate in the non-survivor group were all markedly upregulated on the day of admission (Figure 4A, 4C, 4E). Consistent with the findings in PCA and OPLS-DA assays, the three AA metabolite levels slowly decreased during the time course of the investigation. Indeed, the level of the three AA metabolites in the non-survivor group remained substantially higher than in the survivor group after two weeks ($p<0.05$, Figure 4B, 4D, 4F), as revealed by the relative intensity, suggesting that PQ-induced enhanced expression of these three AA metabolites may be positively correlated with PQ poisoning mortality.

Creatinine, indolelactate and 3-(4-hydroxyphenyl)lactate are useful indicators for predicting mortality

To understand the possible relationship between the AA metabolites and PQ mortality, we further examined these three AA metabolites for clinical validation. Table 3 presents the detailed characteristics of the survival group and the non-survival group. For the survival group and the on-survival group, the mean plasma PQ concentration was 0.3±0.2 mg/L (n=24) and 7.5±6.3 mg/L (n=16), respectively, which was a statistically significant difference ($p<0.001$). SOFA and APACHE II scores were dramatically higher in the non-survivor group ($p<0.001$). Furthermore, to identify whether these three AA metabolites were associated with the resultant outcome (survival or non-survival), ROC curves were constructed. As shown in Figures 5A and 5B, it is clear that 3-(4-hydroxyphenyl)lactate had the best AUC of 0.84, while indolelactate and creatinine had an AUC of 0.75 and 0.83, respectively. These findings were statistically significant, suggesting that they may be useful in predicting clinical outcomes.

It is well known that creatinine is involved in proline and arginine metabolism, being vital pathways involved in the biosynthesis of these AA from glutamate. It generated from creatinine phosphate breakdown in various muscle types, being produced at a regular rate [21]. Indolelactate and 3-(4-hydroxyphenyl)lactate belong to tryptophan and tyrosine metabolic pathway, respectively (*http://www.hmdb.ca/* and *http://www.kegg.jp/*, Figure 5C). In PQ poisoning conditions, it is indicated to evaluate the prognosis of PQ poisoning.

Discussion

The early prognosis of PQ poisoning remains a clinical challenge. Additionally, it seems obvious that early identification of factors that influence the outcome in PQ poisoning may improve the early diagnosis and rapid medical intervention for patients who are at the highest risk of death. Therefore, our research aimed to identify effective indicators for PQ poisoning.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Figure 1. 102 amino acid metabolites were investigated by GC-MS/LC-MS based metabolomics analysis. Metabolic patterns were visualized using heat maps generated from the control, survivor, and non-survivor groups. A blue/red color scheme was used to denote a decrease/increase in a metabolite relative level. C1-C10, Control; S1-S24, Survivor group; N1-16, Non-survivor group.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 2. Twenty-five amino acids were strongly associated with PQ poisoning mortality.

Metabolites	Non-survivor *vs.* survivor (Fold change)	*P*-value
N-acetylalanine	1.56	0.029
N-acetylaspartate	1.70	0.001
Glutamine	0.73	0.004
1-methylimidazoleacetate	2.79	0.040
N-acetyl-1-methylhistidine	3.76	0.032
Glutarylcarnitine	1.57	0.011
Phenyllactate	2.15	0.001
p-Cresol sulfate	0.37	0.040
3-(4-hydroxyphenyl)lactate	2.50	0.040
4-hydroxyphenylacetate	2.00	0.040
5-hydroxymethyl-2-furoic acid	5.64	0.002
Kynurenate	4.83	0.010

Metabolites	Non-survivor *vs.* survivor (Fold change)	*P*-value
Kynurenine	2.05	0.009
Indolelactate	2.68	0.011
Serotonin	2.07	0.040
C-glycosyltryptophan	2.15	0.013
Tiglyl carnitine	2.15	0.034
3-methylglutarylcarnitine	2.92	0.001
Citramalate	2.50	0.026
Homocysteine	2.64	0.001
Dimethylarginine	1.69	0.014
Urea	1.45	0.040
Creatinine	1.92	0.003
4-acetamidobutanoate	2.19	0.030
Guanidinosuccinate	4.78	0.017

PQ – Paraquat.

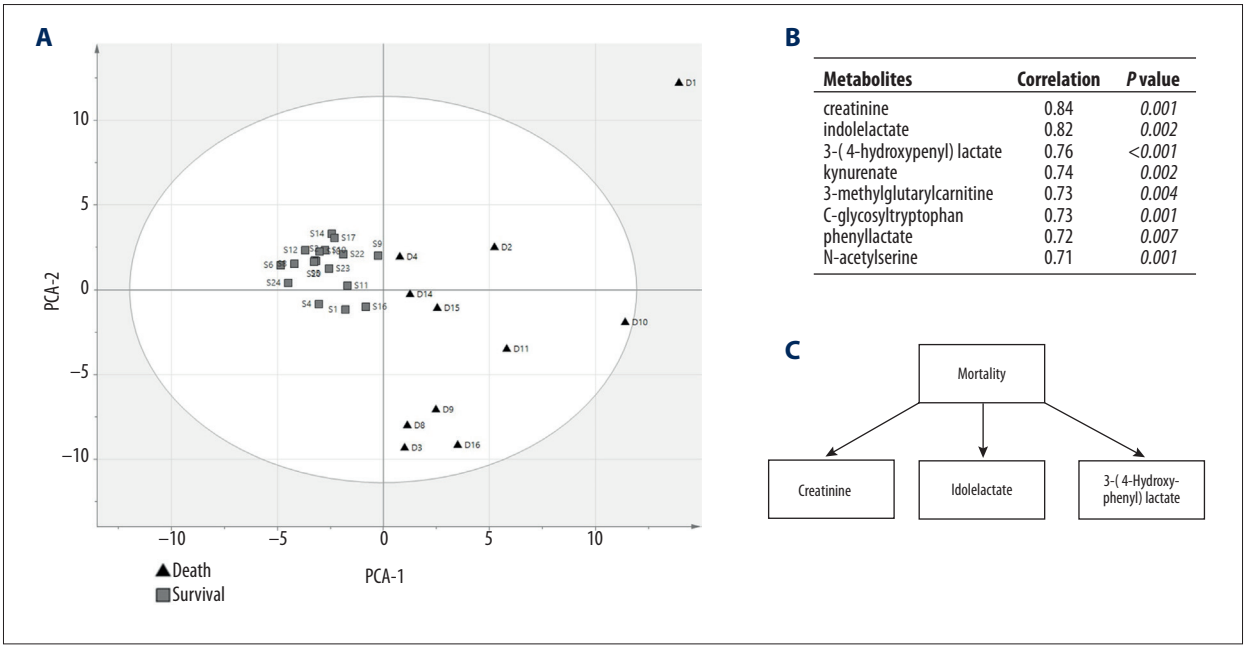


Figure 2. Alterations in amino acid (AA) metabolites in acute PQ poisoned patients. (**A**) The PCA score scatter plot showed reasonable separation between the survivor and non-survivor groups. D – Death group (n=16), gray triangles; S – Survivor group (n=24), diamonds. PCA – Principal component analysis. (**B**) SIMCA-P+ 13.0.3.0 analysis showed eight AA metabolites that were closely associated with PQ mortality. (**C**) Consistent with the findings in the heat map analysis, creatinine, indolelactate and 3-(4-hydroxyphenyl)lactate were found to be most commonly associated with PQ poisoning mortality. PQ – Paraquat.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

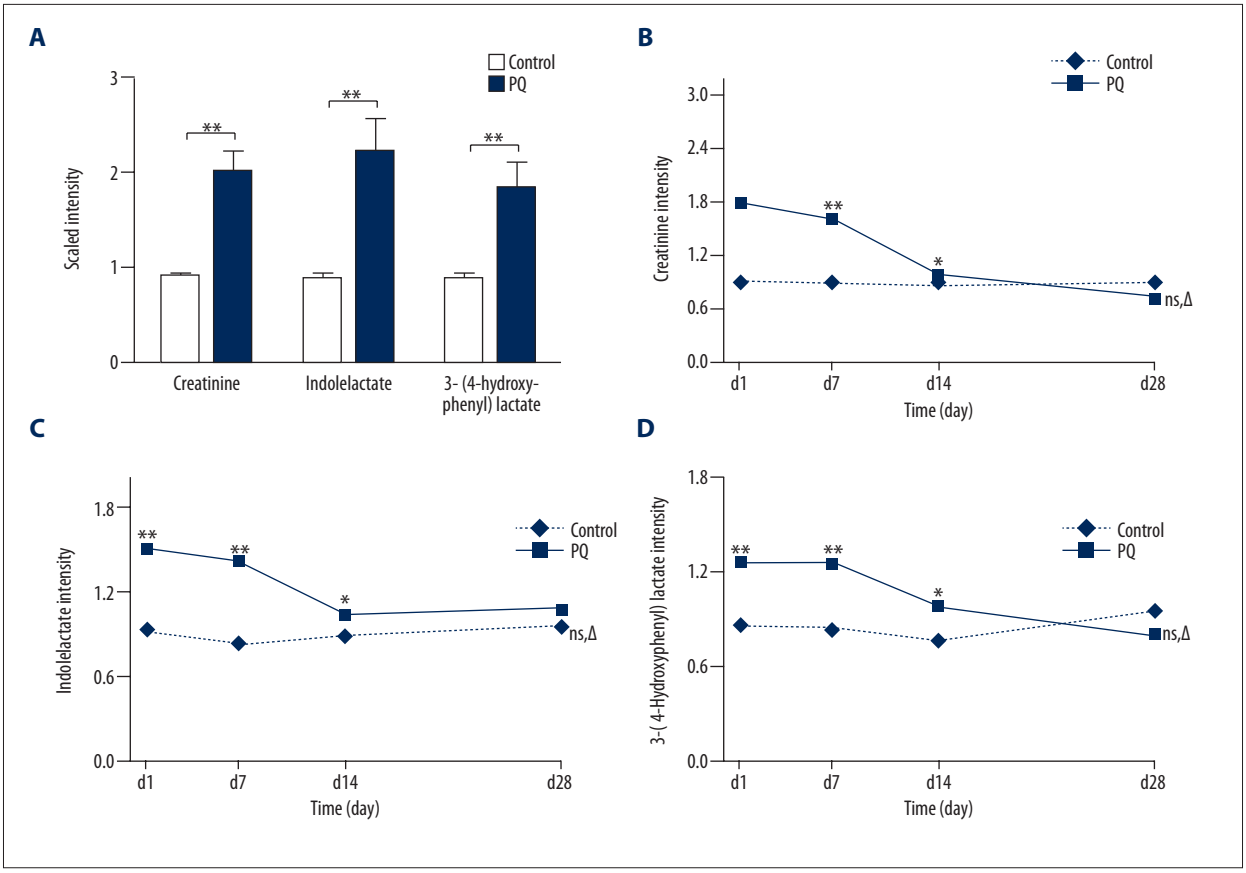


Figure 3. Creatinine, indolelactate and 3-(4-hydroxyphenyl)lactate were involved in PQ poisoning. (**A**) PQ poisoning resulted in a remarkable elevation in creatinine, indolelactate, and 3-(4-hydroxyphenyl)lactate levels on the first day of hospital admission. * $p<0.05$ and ** $p<0.001$ versus control group. ns – not significant. (**B–D**) Changes in creatinine, indolelactate and 3-(4-hydroxyphenyl)lactate levels over 28 days. The levels of three AA metabolites remained high for almost two weeks, and then slowly declined over 28 days. Control group (n=10), PQ group (n=40). * $p<0.05$ and ** $p<0.001$ versus control group. ns – not significant. Δ $p<0.05$ versus PQ group on the day of admission. PQ – Paraquat.

All life depends on AA as they are the fundamental building blocks of proteins as well as many other biological molecules [22,23]. Based on the balance of nitrogen and growth, traditionally AA are classified as being nutritionally essential AA (EAA) or non-essential AA (NEAA) [24]. EAA play important roles in the expression of genes, i.e., the transcription of encoded information in a gene into ribonucleases and proteins [25]. In contrast, NEAA are intimately involved in many cell signaling pathways that regulate proteolysis, the expression of genes, DNA and the synthesis of proteins, lipid, glucose and endogenous metabolite metabolism, neurotransmission and immunity, among other functions [26].

Recently, altered AA metabolism has been recognized to be an important and underestimated cause of many human diseases. For example, changes of the levels in several important AA have been reported in the pathogenesis of AD [27]. Urea cycle function may be induced in endothelial cells of AD patient brains, possibly to remove excess ammonia produced from increased AA catabolism [28]. Also raised plasma branched-chain AA (BCAA) levels in insulin resistance has been shown to be relevant in predicting the development of type 2 diabetes mellitus [29]. However, there is a paucity of information available about the changes of AA metabolites after PQ poisoning. Studying PQ poisoning from an AA metabolic perspective provides new insights into PQ pathogenesis and may lead to the discovery of important indicators that can partially aid in early detection and outcome prediction.

To the best of our knowledge, our study is the first that used serum AA metabolomics to evaluate prognosis in PQ poisoning in humans. Metabolic profiling of low molecular weight metabolites in tissue constituents has shown promising results in uncovering biomarkers and metabolic fingerprint in clinical toxicology and has previously been suggested as a potential technique for early diagnosis of PQ poisoning [30].

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

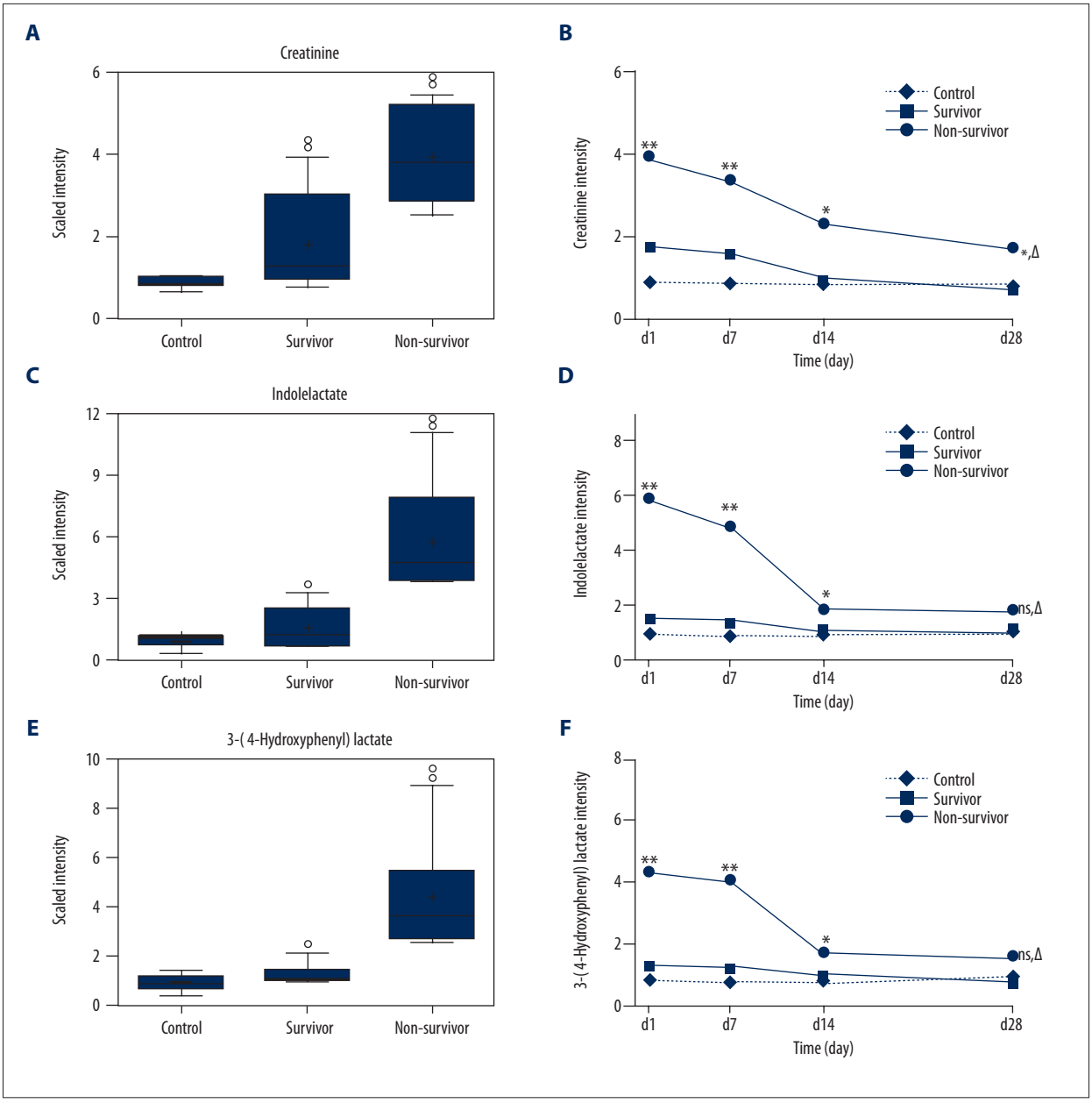


Figure 4. PQ-induced enhanced expression of three amino acid metabolites may be associated with PQ poisoning mortality. (**A, C, E**) Compared to the control and survivor groups, the levels of creatinine, indolelactate and 3-(4-hydroxyphenyl)lactate in the non-survivor group was markedly increased on the day of hospital admission. Box plots depict the median (line) and range (edges of box plots). The *p*-values are based on Wilcoxon's test. (**B, D, F**). Changes in three AA metabolites levels are shown in the control, survivor, and non-survivor groups over 28 days. The three AA metabolite levels slowly decreased during the time course of the investigation. Indeed, the level of the three AA metabolites in the non-survivor group remained substantially higher than in the survivor group. Control group (n=10), Survivor group (n=24), Non-survivor group (n=16). * $p<0.05$ and ** $p<0.001$ versus Survivor group. ns, not significant. Δ $p<0.05$ versus. Non-survivor group on the day of admission. PQ – Paraquat.

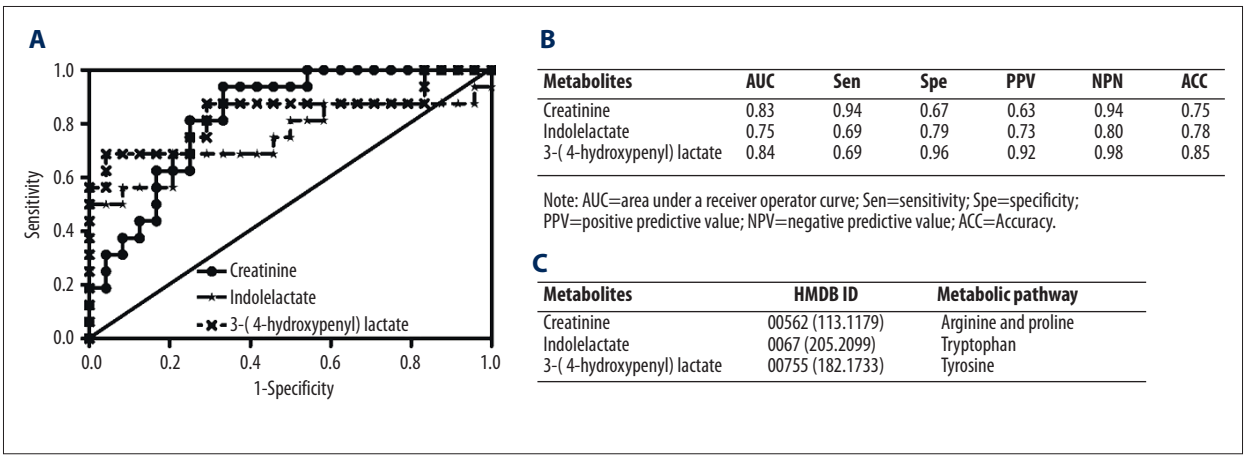
The metabolomics results in our study are yielded a number of particularly interesting findings. First, following acute PQ poisoning, 25 AA molecules were abnormally expressed in metabolic pathways ($p<0.05$, Table 2). They belong to different metabolisms, like alanine and aspartate metabolism, glutamate metabolism, lysine metabolism, tryptophan metabolism, taurine metabolism, arginine and proline metabolism, etc. Among them, 4-acetamidobutanoate and guanidinosuccinate are NEAA, while others are EAA. Secondly, we found the intensity of glutamine and p-cresol sulfate was decreased, while

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 3. Characteristics of the survivor and non-survivor groups in PQ poisoning.

	Survivors (n=24)	Non-survivors (n=16)	*P*-value
No. of participants (women/men)	10/14	10/6	0.097
Age (years)	32 (14–67)	31 (16–58)	0.689
Plasma PQ concentration (mg/L)	0.3±0.2	7.5±6.3	<0.001
Urine PQ concentration (mg/L)	5.8±11.1	104.0±158.2	0.001
Scoring			
APACHE II	3.0±2.0	10.0±4.0	<0.001
SOFA	2.0±1.0	6.0±2.0	<0.001

Plasma PQ concentration and urine PQ concentration were collected on admission; other parameters were the peak values within 5 days following intake of paraquat. PQ – Paraquat.



B

Metabolites	AUC	Sen	Spe	PPV	NPN	ACC
Creatinine	0.83	0.94	0.67	0.63	0.94	0.75
Indolelactate	0.75	0.69	0.79	0.73	0.80	0.78
3-(4-hydroxypenyl) lactate	0.84	0.69	0.96	0.92	0.98	0.85

Note: AUC=area under a receiver operator curve; Sen=sensitivity; Spe=specificity; PPV=positive predictive value; NPV=negative predictive value; ACC=Accuracy.

C

Metabolites	HMDB ID	Metabolic pathway
Creatinine	00562 (113.1179)	Arginine and proline
Indolelactate	0067 (205.2099)	Tryptophan
3-(4-hydroxypenyl) lactate	00755 (182.1733)	Tyrosine

Figure 5. Creatinine, indolelactate and 3-(4-hydroxyphenyl)lactate are useful indicators for predicting PQ poisoning mortality. (**A, B**) Evaluating the prognostic clinical value of creatinine, indolelactate, and 3-(4-hydroxyphenyl)lactate in PQ poisoning based on ROC curves. 3-(4-hydroxyphenyl)lactate had the highest AUC of 0.84, while indolelactate and creatinine had AUCs of 0.75 and 0.83, respectively. The values can be used to predict clinical outcomes. ROC – Receiver Operating Characteristic. (**C**) The metabolic pathway of creatinine, indolelactate and 3-(4-hydroxyphenyl)lactate. PQ – Paraquat.

others were significantly increased. Mechanisms for this finding merit future validation. Also, we applied an unsupervised PCA and supervised OPLS-DA analysis between the survivor group and the non-survivor group. Consistent with the findings in the heat map analysis, creatinine, indolelactate, and 3-(4-hydroxyphenyl)lactate were suggested to be the most closely related AA metabolites with PQ mortality (RS >0.75, Figure 2B, 2C). Hence, we further studied these three AA for clinical validation. It was noted that the level of these three AA metabolites in patients who died was substantially higher than in the survivor group (Figure 4).

Creatinine (molecular weight 113 daltons), is involved in arginine and proline metabolism, and is a commonly used marker to indicate renal damage in PQ poisoning [31]. Yamaguchi et al. assessed laboratory test data of 160 patients with acute PQ poisoning on hospital admission and found that the prognosis of PQ poisoning depends on acid-base status and the renal functions of the patients [32]. In agreement with previous findings [33,34], our research has shown that creatinine levels can be used to predict death, which was associated with an AUC of 0.83 (Figure 5A, 5B), indicating an elevated creatinine was a useful prognostic indicator of death. Arginine is an essential AA which increases the production of NO, ROS, Ca^{2+} release, as well as the expression of inducible nitric oxide synthase [35]. It has been reported that creatinine levels rise more rapidly in patients with severe PQ poisoning, but the mechanism(s) remain unclear. The increase might be due to severe free radical injury induced by PQ toxicity [36].

For example, it is known that PQ poisoning triggers an inflammatory response, accompanied by raised levels of the cytokine TNF-α, which is associated with systemic inflammation. TNF-α is known to increase the expression of iNOS in aortic endothelial cells, leading to an increased production of NO compared to basal levels, which impairs the vascular responses of smooth

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

muscle [37]. Therefore, detecting an early increase in creatinine levels may be an accurate prognostic indicator for death.

Indolelactate and 3-(4-hydroxyphenyl)lactate belong to tryptophan and tyrosine metabolic pathway, respectively, which act as a natural anti-oxidant. It has been shown that it decreases the generation of ROS by neutrophils and mitochondria, thereby moderating oxidative stress in sepsis. In our study, the relative intensity of indolelactate and 3-(4-hydroxyphenyl)lactate showed a significant increase after PQ poisoning (Figure 3C, 3D). Mechanisms are unknown. It may be due to the PQ-induced enhanced level of oxidative stress. In addition, ROC curve analysis in this study revealed that 3-(4-hydroxyphenyl)lactate had the best AUC of 0.84, while indolelactate had the AUC of 0.75, for the prediction of prognosis in patients with PQ poisoning (Figure 5A, 5B). These observations may validate the hypothesis that indolelactate and 3-(4-hydroxyphenyl)lactate is a good predictor of death. However, mechanisms underlying this finding remain are unclear, but warrant further investigation in future studies.

Conclusions

Taken together, this study applied a GC-MS/LC-MS based metabolomics approach to assess the AA metabolite changes and its prognostic values in human plasma following PQ poisoning. Three AA metabolites, creatinine, indolelactate, and 3-(4-hydroxyphenyl)lactate, were demonstrated to be associated with PQ poisoning fatality. Therefore, metabolic profiling of AA could be a reliable tool for identifying effective indicators for early prognosis of PQ poisoning.

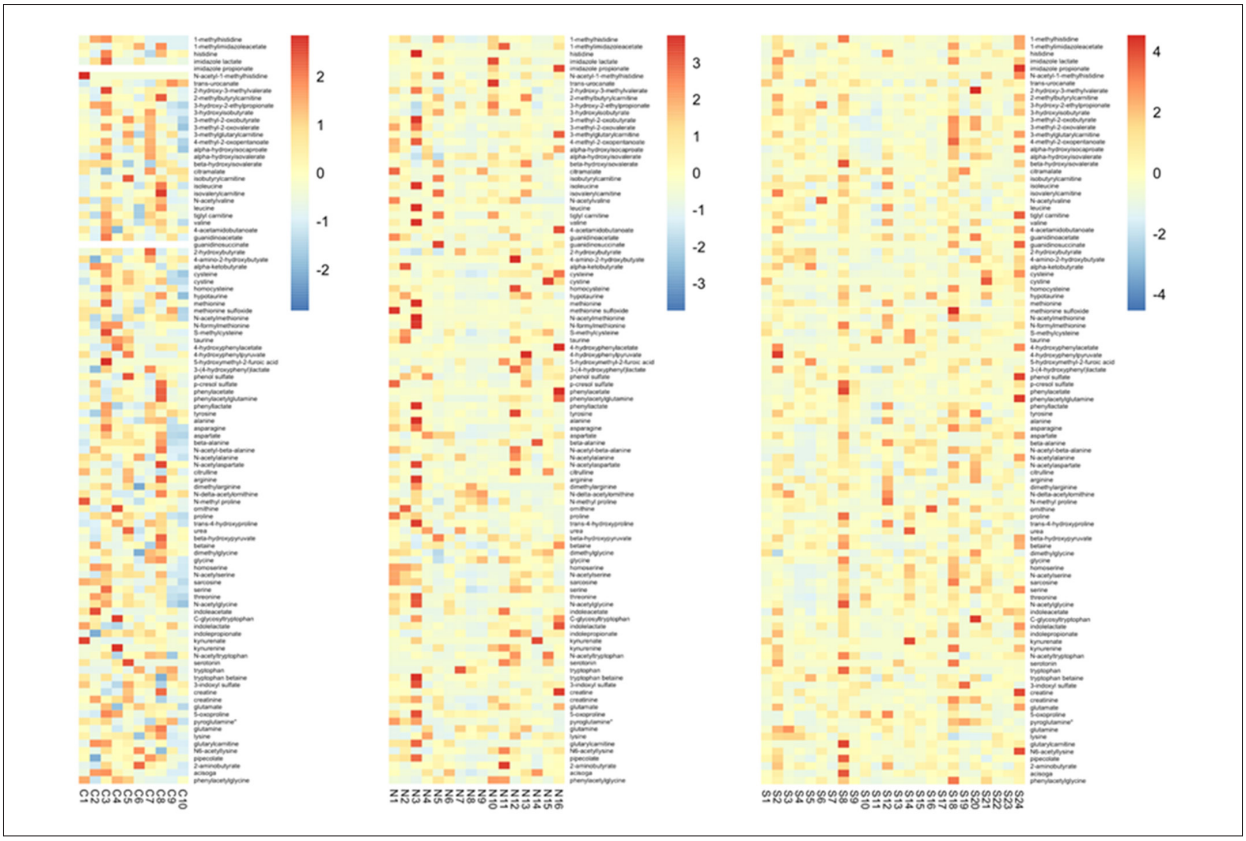
Acknowledgements

The authors thank study participants for their collaboration and all staff of the Nephrology Department of Shanghai Tenth People's Hospital for their dedication to the study.

Conflicts of interest

None.

Supplementary Figure



Supplementary Figure 1. 102 amino acid metabolites were investigated by GC-MS/LC-MS based metabolomics analysis. Metabolic patterns were visualized using heat map generated from the control, survivor, and non-survivor group. A blue/red color scheme was used to refer to a decrease/increase in a metabolite relative level. C1–C10 – Control; S1–S24 – Survivor group; N1–16 – Non-survivor group.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

References:

1. Weng CH, Hu CC, Lin JL et al: Sequential organ failure assessment score can predict mortality in patients with paraquat intoxication. PLoS One, 2012; 7(12): e51743
2. Lee JH, Hwang SY, Kim HR et al: Effectiveness of the sequential organ failure assessment, acute physiology and chronic health evaluation II, and simplified acute physiology score II prognostic scoring systems in paraquat-poisoned patients in the intensive care unit. Hum Exp Toxicol, 2017; 36(5): 431–37
3. Li Y, Wang M, Gao Y et al: Abnormal pancreatic enzymes and their prognostic role after acute paraquat poisoning. Sci Rep, 2015; 5: 17299
4. Zhou CY, Kang X, Li CB et al: Pneumomediastinum predicts early mortality in acute paraquat poisoning. Clin Toxicol (Phila), 2015; 53(6): 551–56
5. Wu G, Bazer FW, Davis TA et al: Arginine metabolism and nutrition in growth, health and disease. Amino Acids, 2009; 37(1): 153–68
6. Wu SF, Mu XC, Dong YX et al: Expression pattern and pharmacological characterisation of two novel alternative splice variants of the glutamate-gated chloride channel in the small brown planthopper *Laodelphax striatellus*. Pest Manag Sci, 2017; 73(3): 590–97
7. Lemeshko VV: Electrical potentiation of the membrane permeabilization by new peptides with anticancer properties. Biochim Biophys Acta, 2013; 1828(3): 1047–56
8. Kumar J, Sim V: D-amino acid-based peptide inhibitors as early or preventative therapy in Alzheimer disease. Prion, 2014; 8(1): 119–24
9. Jonker R, Deutz NE, Erbland ML et al: Effectiveness of essential amino acid supplementation in stimulating whole body net protein anabolism is comparable between COPD patients and healthy older adults. Metabolism, 2017; 69: 120–29
10. Kinny-Koster B, Bartels M, Becker S et al: Plasma amino acid concentrations predict mortality in patients with end-stage liver disease. PLoS One, 2016; 11(7): e0159205
11. Treatm CSoTPa. guideline for the diagnosis and treatment of acute paraquat poisoning(2013). Chinese Journal of Critical Care Medicine, 2013; 33(6): 484–89
12. Whitehead RD Jr., Montesano MA, Jayatilaka NK et al: Method for measurement of the quaternary amine compounds paraquat and diquat in human urine using high-performance liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci, 2010; 878(27): 2548–53
13. Seymour CW, Yende S, Scott MJ et al: Metabolomics in pneumonia and sepsis: An analysis of the GenIMS cohort study. Intensive Care Med, 2013; 39(8): 1423–34
14. Wunnapuk K, Medley GA, Liu X et al: Simple and sensitive liquid chromatography-tandem mass spectrometry methods for quantification of paraquat in plasma and urine: application to experimental and clinical toxicological studies. J Chromatogr B Analyt Technol Biomed Life Sci, 2011; 879(28): 3047–52
15. Dehaven CD, Evans AM, Dai H, Lawton KA: Organization of GC/MS and LC/MS metabolomics data into chemical libraries. J Cheminform, 2010; 2(1): 9
16. Labbi A, Vlachos M, Boutsidis C: InventorsMatrix re-ordering and visualization in the presence of data hierarchies patent 8,943,106. 2015
17. Hastie T TR, Friedman J: The elements of statistical learning, data mining inference and prediction. Springer-Verlag, 2001: 453–80
18. Mickiewicz B, Duggan GE, Winston BW et al: Metabolic profiling of serum samples by 1H nuclear magnetic resonance spectroscopy as a potential diagnostic approach for septic shock. Crit Care Med, 2014; 42(5): 1140–49
19. Mickiewicz B, Vogel HJ, Wong HR, Winston BW: Metabolomics as a novel approach for early diagnosis of pediatric septic shock and its mortality. Am J Respir Crit Care Med, 2013; 187(9): 967–76
20. Korashy HM, Attafi IM, Famulski KS et al: Gene expression profiling to identify the toxicities and potentially relevant human disease outcomes associated with environmental heavy metal exposure. Environ Pollut, 2017; 221: 64–74
21. See HH, Schmidt-Marzinkowski J, Pormsila W et al: Determination of creatine and phosphocreatine in muscle biopsy samples by capillary electrophoresis with contactless conductivity detection. Anal Chim Acta, 2012; 727: 78–82
22. Brasselagnel C, Lavoinne A, Husson A: Control of mammalian gene expression by amino acids, especially glutamine. FEBS J, 2009; 276(7): 1826–44
23. Ruggeri F, Zosel F, Mutter N et al: Single-molecule electrometry. Nat Nanotechnol, 2017; 12: 488–95
24. Le Plenier S, Walrand S, Noirt R et al: Effects of leucine and citrulline versus non-essential amino acids on muscle protein synthesis in fasted rat: A common activation pathway? Amino Acids, 2012; 43(3): 1171–78
25. Wang J, Wu Z, Li D et al: Nutrition, epigenetics, and metabolic syndrome. Antioxid Redox Signal, 2012; 17(2): 282–301
26. Hou Y, Yin Y, Wu G: Dietary essentiality of "nutritionally non-essential amino acids" for animals and humans. Exp Biol Med (Maywood), 2015; 240(8): 997–1007
27. Pan X, Nasaruddin MB, Elliott CT et al: Alzheimer's disease-like pathology has transient effects on the brain and blood metabolome. Neurobiol Aging, 2016; 38: 151–63
28. Griffin JW, Bradshaw PC: Amino acid catabolism in Alzheimer's disease brain: Friend or foe? Oxid Med Cell Longev, 2017; 2017: 5472792
29. Yoon MS: The emerging role of branched-chain amino acids in insulin resistance and metabolism. Nutrients. 2016; 8(7): pii: E405
30. Dunn WB, Goodacre R, Neyses L, Mamas M: Integration of metabolomics in heart disease and diabetes research: Current achievements and future outlook. Bioanalysis, 2011; 3(19): 2205–22
31. Mohamed F, Buckley NA, Jayamanne S et al: Kidney damage biomarkers detect acute kidney injury but only functional markers predict mortality after paraquat ingestion. Toxicol Lett, 2015; 237(2): 140–50
32. Yamaguchi H, Sato S, Watanabe S, Naito H: Pre-embarkment prognostication for acute paraquat poisoning. Hum Exp Toxicol, 1990; 9(6): 381–84
33. Kim SJ, Gil HW, Yang JO et al: The clinical features of acute kidney injury in patients with acute paraquat intoxication. Nephrol Dial Transplant, 2009; 24(4): 1226–32
34. Gil HW, Hong JR, Jang SH, Hong SY: Diagnostic and therapeutic approach for acute paraquat intoxication. J Korean Med Sci, 2014; 29(11): 1441–49
35. Pekarova M, Kubala L, Martiskova H et al: The unique role of dietary L-arginine in the acceleration of peritoneal macrophage sensitivity to bacterial endotoxin. Immunol Res, 2013; 56(1): 73–84
36. Mohamed F, Endre Z, Jayamanne S et al: Mechanisms underlying early rapid increases in creatinine in paraquat poisoning. PLoS One, 2015; 10(3): e0122357
37. Aires RD, Capettini LS, Silva JF et al: Paraquat poisoning induces TNF-alpha-dependent iNOS/NO mediated hyporesponsiveness of the aorta to vasoconstrictors in rats. PLoS One, 2013; 8(9): e73562

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]