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ORIGINAL ARTICLE

Untargeted urinary metabolomic profile of isolated chest trauma patients

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ABSTRACT

Aim To explore the untargeted urinary metabolomic biomarkers in isolated chest injury patients.
Methods It was a prospective study for 1 year period that included patients of isolated chest trauma as per inclusion criteria. Day 0, 3, and 7 urine samples from patients and day 0 sample from age matched healthy relatives were collected. Samples were prepared and gas chromatography mass spectrometry analysis was done using Agilent Technologies**.** Multivariate analyses were done using the 'Biomarker Analysis' module of the MetaboAnalyst 6.0 to identify potential biomarkers associated with chest injuries. To identify the promising biomarkers with high sensitivity and specificity, exploratory multivariate receiver operating characteristic (ROC) curve analysis was used.
Results A total of 31 patients and 31 healthy controls were enrolled. A total of 101 metabolites were annotated. Based on ROC analyses, the top 15 potential markers (azelaic acid, glycerol, anthranilic acid, 2-palmitoylglycerol, 2-hydroxyhippuric acid, 4-hydroxybenzeneacetic acid, arachidic acid, 3-hydroxyhippuric acid, adenine, sucrose, butanedioic acid, 4-hydroxyphenyllactic acid, butanoic acid, 4-hydroxybenzoic acid and 2-hydroxybenzeneacetic acid) were identified. Selected frequencies of >0.6% led to the selection of 9 biomarkers. Predictive accuracy of 66.5% of the most accurate 50-feature panel of model with the AUC of 0.708 with 95% confidence interval was found.
Conclusion Nine metabolites (azelaic acid, glycerol, anthranilic acid, 2-palmitoylglycerol, 2-hydroxyhippuric acid, 4-hydroxybenzeneacetic acid, arachidic acid, 3-hydroxyhippuric acid and adenine) based on their selected frequencies of more than 0.6% can be considered as biomarkers in isolated chest trauma patients.
Keywords: gas chromatography-mass spectrometry, injury severity score, metabolism, thoracic injuries

INTRODUCTION

Trauma induces significant changes in cellular metabolism in different organs and tissues, in terms of production of metabolites and bioenergetics (1,2). These metabolic signatures differ between individuals and represent the final integrated response to the pathophysiological insult (3,4). The risk of deterioration in trauma patients is assessed and monitored by close clinical and laboratory monitoring (5). Despite advancements in trauma care, some patients deteriorate despite adequate treatment, with conventional tests sometimes lagging in detecting early biochemical changes. As the metabolome of an organism serves as the closest link to its phenotype and provides an idea of early biological processes that have occurred in an individual (6), it might be helpful as an important adjuvant in the management of trauma patients. The techniques of liquid chromatography-mass spectrometry (LC-MS), and gas chromatography-mass spectrometry (GC-MS) have been employed for metabolomic evaluation of many disorders, including trauma (7-14).

The term 'metabolome' was coined in 1998 by Oliver et al. (15). It consists of the complete set of small molecules and metabolites found within a biological sample. It is common for disease conditions to cause metabolic reprogramming in patients. As a result, a patient's biofluid may undergo metabolic reprogramming. Biofluids containing altered metabolites that correlate well with disease conditions can be used as biomarkers. Metabolomics can help in the identification and determination of metabolites in a biological sample under normal conditions or in altered state, thus allowing to profile a larger number of molecules than covered in standard clinical laboratory tests. It can also provide valuable insights into the dynamic changes occurring at the molecular level and their association with physiologic response (16).

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Different metabolites have been reported to be dysregulated in patients after trauma (1-3, 6-8, 17,) with a significant number of studies involving traumatic brain injury patients (7,8, 18-20). There have been no such studies on isolated chest trauma patients. Parent et al. (17) reported that trauma patients showed oxidative stress and lower nucleotide synthesis on day 1 as compared to healthy individuals.

The aim of this study was to explore the untargeted urinary metabolomic profile of patients with isolated chest injuries and their age-matched healthy relatives to assess the changes in metabolomic profile and to find out potential biomarkers of chest injury.

PATIENTS AND METHODS

Patients and study design

This prospective, exploratory study was conducted over 1 year period between July 2022 and June 2023. It involved all patients of isolated chest trauma who were admitted in the Department of Trauma Surgery and Critical Care at All India Institute of Medical Sciences, Rishikesh, India.

Inclusion criteria were age between 18 and 65 years, directly admitted or referred from another hospital within 48 hours of injury, and age-matched healthy relatives of the patient for the control group. Exclusion criteria were patients with age >65 years or <18 years, pregnant females, obese (BMI >30), patients with prior organ dysfunction, cancer, diabetes mellitus, cardio-pulmonary ailments, or metabolic disorders and patients with haematuria or anuria at initial presentation.

All patients were treated as per Advanced Trauma Life Support (ATLS) protocol upon arrival to the trauma emergency. After initial assessment and management, participants were informed about the study (including information regarding voluntary withdrawal) and written informed consent was taken from the participants.

The research received ethical approvals from the Institutional Ethics Committee of All India Institute of Medical Sciences Rishikesh (AIIMS/IEC/22/413).

Methods

On day zero, a 5 mL urine sample was collected from patients and their age-matched healthy relatives. Routine urine analysis of the same urine sample was also done. Serial urine samples on day 3 and 7 were collected from patients only. The samples were stored at temperatures below minus -30 °C in cryogenic vials and were transported in liquid nitrogen containers to IIT Roorkee for analysis with proper temperature maintenance. The samples were prepared by adding 12N KOH to 1mL of urine for hydrolysis at 60 °C for 15 minutes followed by cooling the samples, and adding acetic acid to achieve a pH of 4-5. Next, 1-chlorobutane was added, and the samples were centrifuged to collect the organic phase. 4-phenylphenol was used as internal standard. The organic phase was concentrated for 1 hour at 37 °C and incubated with 35 µL methoxyamine in pyridine for 2hrs at 37 °C. A 50 µL N-Methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) was added, which was then incubated at 37 °C for half an hour while being vortexed every 5 minutes. After 15 minutes of centrifugation at 15,000 rpm, the samples were loaded into the GC-MS.

GCMS analysis was done using Agilent GC 7890 coupled to a 5977B mass-detector (Agilent Technologies, USA). The separation of chemicals with helium as a carrier gas was accomplished using an HP-5 MS column (21). With splitless mode, the injection volume was 1 µL. The temperature of the injector was set to 280 °C. The oven temperature was set at 80 °C for 1.5 minutes, then ramped to 220 °C at a rate of 10 °C/min without holding, then increased to 310 °C at a rate of 20 °C/min held for 10 minutes and with a 5-minute solvent delay. The flow rate across the column was 1 mL/min.

The conditions for the operation of the mass spectrometer were set as follows: ion source temperature 230 °C, MS Quad temperature 150 °C, electron energy (70eV), and scanning range of m/z, 40-1000 amu. The compounds were identified by comparing the mass spectra of the components to that of the mass spectral library from NIST 17 (National Institute of Standards and Technology) (21). The identification of metabolites was done by matching the mass spectra of each compound from the library (3:1, signal: noise) using the NIST-17 mass spectral library. For the mass spectra comparison, the matching value of the metabolite identity taken was more than 70. To check for co-elution, the mass spectra of all peaks were analysed at three different points, the beginning, middle, and end of each peak width. Furthermore, the compounds were identified based on matching of retention index and mass spectrum with authentic standard or with library search. Observers were blinded about participant details.

Statistical analysis

Multivariate statistical analysis using MetaboAnalyst 6.0 (http://www.metaboanalyst.ca) was done (22). A reference feature (using an internal standard), a log transformation, and a Pareto scaling were used to normalize the data. Based on the Pearson distance measure and the Ward clustering algorithm of MetaboAnalyst, a heatmap of metabolites was generated. The 'Biomarker Analysis' module of the MetaboAnalyst was used to identify potential biomarkers associated with chest injuries. To identify the promising biomarkers with high sensitivity and specificity, exploratory multivariate ROC curve analysis was used. SVM algorithms were used to calculate the importance of all variables and rank them. Based on ROC (receiver operating characteristic) analyses, changes in normalized concentrations of the top nine biomarkers were calculated. Repeated measures analysis of variance (ANOVA)/Friedman test was used when comparing >2 continuous variables for paired analysis. The $\chi 2$ test was used for group comparisons of categorical data. Linear correlation between two continuous variables was explored using Pearson's or Spearman's correlation coefficient.

RESULTS

A total of 31 isolated chest trauma patients and 31 healthy controls were enrolled. Out of 31 participants, 28 (90.3%) were males and three (9.7%) were females. The mean age of participants was 45·32 ± 13·81 years, ranging from 22 to 65 years (Table 1).

A total of 250 (mean values) metabolites were identified, of which 101 metabolites of significance were identified (Supplemental Digital Content: Table 2).

On multivariate ROC curve analyses using MetaboAnalyst 6.0, the top 15 potential biomarkers based on their frequency of selection during cross validation were azelaic acid, glycerol, anthranilic acid, 2-palmitoylglycerol, 2-hydroxyhippuric acid, 4-hydroxybenzeneacetic acid, arachidic acid, 3-hydroxyhippuric acid, adenine, sucrose, butanedioic acid, 4-hydroxy-

Table 1. Patient characteristics

Variable	Mean ± SD	Median (IQR)	Min-Max
Age (Years)	45.32±13.81	47.00 (34.50-57.00)	22.00-65.00
Gender (No; %)			
Male	28 (90.3)		
Female	3 (9.7)		
Mode of injury (No; %)			
RTI	16 (51.6)		
Fall from height	8 (25.8)		
Physical assault	4 (12.9)		
Fall on ground	2 (6.5)		
Fall of object	1 (3.2)		
Passenger details (No; %)			
Others	18 (58.1)		
Driver	7 (22.6)		
Pilon rider	5 (16.1)		
Co-driver	1 (3.2%)		
Injury to reporting time interval (hours)	14.94±14.39	7.00 (5.00-21.50)	1.00-48.00
Duration of hospital stay (days)	7.23±4.39	7.00 (4.50-8.00)	3.00-22.00
Duration of hospital stay (days) (No; %)			
<5	8 (25.8)		
5-10	20 (64.5)		
>10	3 (9.7)		
Duration of ICU stay (days)	1.26±3.94	0.00 (0.00-1.00)	0.00-22.00
Thoracic Trauma Severity Score	7.87±2.70	8.00 (6.00-9.50)	4.00-14.00
Thoracic Trauma Severity Score category (No; %)			
<5	7 (22.6)		
6-10	17 (54.8)		
>10	7 (22.6)		
Rib fractures (No; %)			
None	2 (6.5)		
1-3 Ribs Unilateral	8 (25.8)		
4-6 Ribs Unilateral	10 (32.3)		
>6 Ribs Unilateral	3 (9.7)		
<3 Ribs Bilateral	1 (3.2)		
>3 Ribs Bilateral	6 (19.4)		
Flail Chest	1 (3.2)		
Lung contusion (No; %)			
None	19 (61.3)		
Unilateral Unilobar	9 (29.0)		
Unilateral Bilobar or Bilateral Unilobar	3 (9.7)		
Outcome (No; %)			
Discharged	30 (96.8)		
Expired	1 (3.2)		
Haemoglobin (mg/dL).	11.99±1.56	12.60 (10.50-13.40)	9.30-14.20

SD (Standard Deviation); IQR (Interquartile range); ICU (Intensive Care Unit); RTI (Road Traffic Injury)

phenyllactic acid, butanoic acid, 4-hydroxybenzoic acid and 2-hydroxybenzeneacetic acid (Figure 1). Predictive accuracy of 66.5% of the most accurate 50-feature panel of model

Table 3. Metabolites with a statistically significant difference in the trend over time in relation to length of hospital stay and thoracic trauma score

Metabolite (p)	Metabolite (p)
Metabolites in relation to length of hospital stay	
Azelaic acid (p= 0.001)	Stearic acid (p<0.001)
L-5-Oxoproline (p<0.001)	Butanoic acid (p<0.001)
Palmitic Acid (p<0.001)	Sorbitol (p<0.001)
9H-Purine (p=0.011)	Hexanedioic acid (p=0.001)
5-Hydroxyindoleacetic acid (p<0.001)	1-Monomyristin (p=0.001).
p-Tolyl-β-D-glucuronide (p= 0.012)	3-Hydroxyisovaleric acid (p=0.024)
3-Hydroxyanthranilic Acid (p<0.001)	Myristic acid (p=0.006)
Glycerol monostearate (p<0.001)	3-Methyladipic acid (p<0.001)
2-Palmitoylglycerol (p=0.029)	Adenine (p=0.041)
Anthranilic acid (p<0.001)	
Metabolites in relation to thoracic trauma score	
Butanedioic acid (p=0.026)	Glycerol monostearate (p<0.001)
L-5-Oxoproline (p=0.032)	3-Hydroxyphenylacetic acid (p<0.001)
4-Hydroxybenzoic acid (p<0.001)	3-Hydroxyanthranilic Acid (p=0.047)
Azelaic acid (p=0.001)	Heptadecanoic acid (p=0.019)
3-Indoleacetic acid (p<0.001)	Adenine (p=0.042)
Arachidic acid (p=0.013)	2-Hydroxyhippuric acid (p=0.020)
Anthranilic acid (p<0.001)	2-Palmitoylglycerol (p=0.028)

5 (Figure 1B) with the AUC of 0.708 with 95% confidence interval was found (Figure 1C). There were fifteen potential biomarkers (Figure 1D), of which nine were selected for boxplot representation to show the differences in metabolite levels between control and others (Figure 2).

A total of 9 metabolites were selected based on their percent rank frequency in ROC analyses with metabolites having rank frequency greater than 0.6 being selected. Of these 9 biomarkers, adenine, glycerol, anthranilic acid and 4-hydroxybenzeneacetic acid showed significant increase in levels in chest trauma patients compared to healthy controls. As compared to healthy controls, 2-hydroxyhippuric acid, 3-hydroxyhippuric acid, arachidic acid, 2-palmitoylglycerol and azelaic acid were downregulated in patients (Figure 3).

Statistical analysis of the same data set (GCMS peak intensities converted numeral values) found seven metabolites showing a statistically significant difference in the trend over time between the three groups in relation to both length of hospital stay and thoracic trauma severity score: azelaic acid, anthranilic acid. glycerol monostearate, adenine, L-5-Oxoproline, 2-palmitoylglycerol and 3-hydroxyanthranilic acid. Twelve metabolites including palmitic acid, 9H-purine, 5-hydroxyindoleacetic acid, p-Tolyl-β-D-glucuronide, stearic acid, butanoic acid, sorbitol, hexanedioic acid, 1-monomyristin, 3-hydroxyisovaleric acid, myristic acid and 3-methyladipic acid showed a statistically significant difference in the trend over time between the three groups in relation to the length of hos-

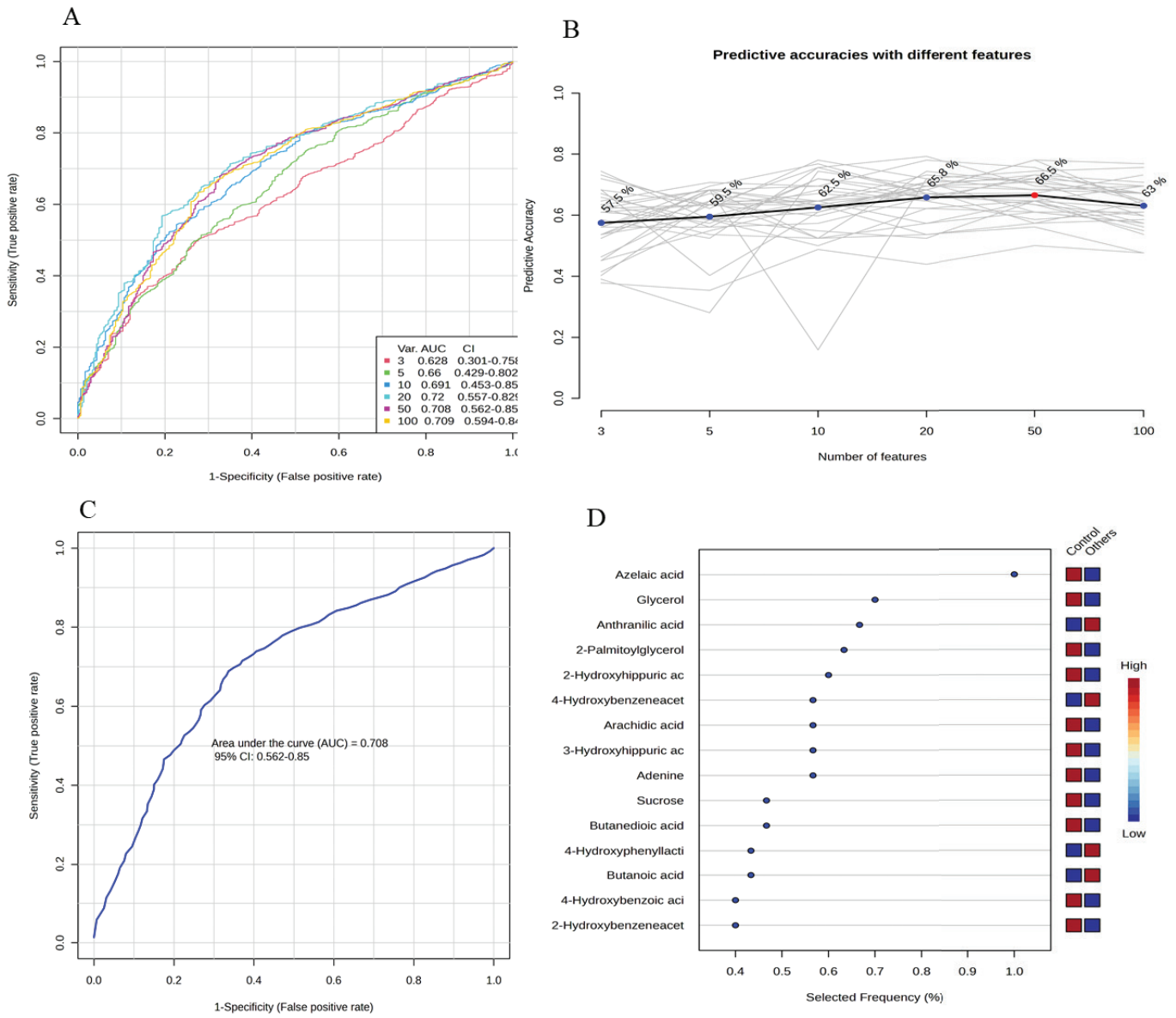

Figure 1. Biomarker identification from human urine samples of isolated chest trauma patients. Biomarkers were identified by Multivariate ROC curve analyses using MetaboAnalyst 6.0. The ROC curves were generated using Monte-Carlo cross validation (MCCV) algorithm linear SVM (Support vector machine) for the classification method and SVM built-in for the feature ranking method. A) ROC curves created by MetaboAnalyst using different numbers of features (5, 10, 15, 25, 50 and 100) with respective AUC values and confidence intervals; B) Graph showing the predictive accuracy of 6 different biomarker models. A red dot indicates the most accurate 50-feature panel of model 5; C) ROC curve for a selected biomarker model 5; D) The top 15 potential biomarkers predicted based on their frequency of selection during cross validation.

pital stay only, whereas seven metabolites including butanedioic acid, 4-hydroxybenzoic acid, 3-indoleacetic acid, arachidic acid, 3-hydroxyphenylacetic acid, heptadecanoic acid and 2-hydroxyhippuric acid showed a statistically significant difference in the trend over time between the three groups in relation to thoracic trauma severity score only (Table 3).

In patients with LOS (length of hospital stay) <5 days, L-5-Oxoproline, palmitic acid, 9H-purine, adenine, 3-hydroxyanthranilic acid, 3-hydroxyisovaleric acid, and 3-methyl adipic acid decreased between day 0 to 3, and then increased on day 7 with the tendency to return towards values in normal healthy controls. The opposite trend was seen in patients with LOS between 5 - 10 days with day 3 values returning towards control values. In patients with LOS >10 days, these metabolites showed either very low values or very high not returning towards control values. Glycerol monostearate and butanoic acid showed only minimal change between days 0 to day 7 in patients with LOS<5 days or 5-10 days, but significantly increased between day 3 to day 7 in the LOS >10 days group. The 1-monomyristin showed an increasing trend in the LOS <5 days group, an overall decreasing trend in the LOS 5-10 days group, and very high and increasing trend as compared to controls in LOS >10 days. Anthranilic acid, 2-palmitoylglycerol, and sorbitol showed mild change in patients with LOS <5 days and 5-10 days, but showed significantly higher values on day 3 in patients with LOS >10 days. There was a moderate association (Point-Biserial Correlation = 0·31; p=0·045) between the glycerol level and LOS with highest values in patients with LOS >10 days. Myristic acid showed an increasing trend between day 0 and day 3 in LOS <5 days and LOS 5-10 days and a decreasing trend in LOS >10 days group. Azelaic acid and 5-hydroxyindoleacetic acid increased between day 3, and day

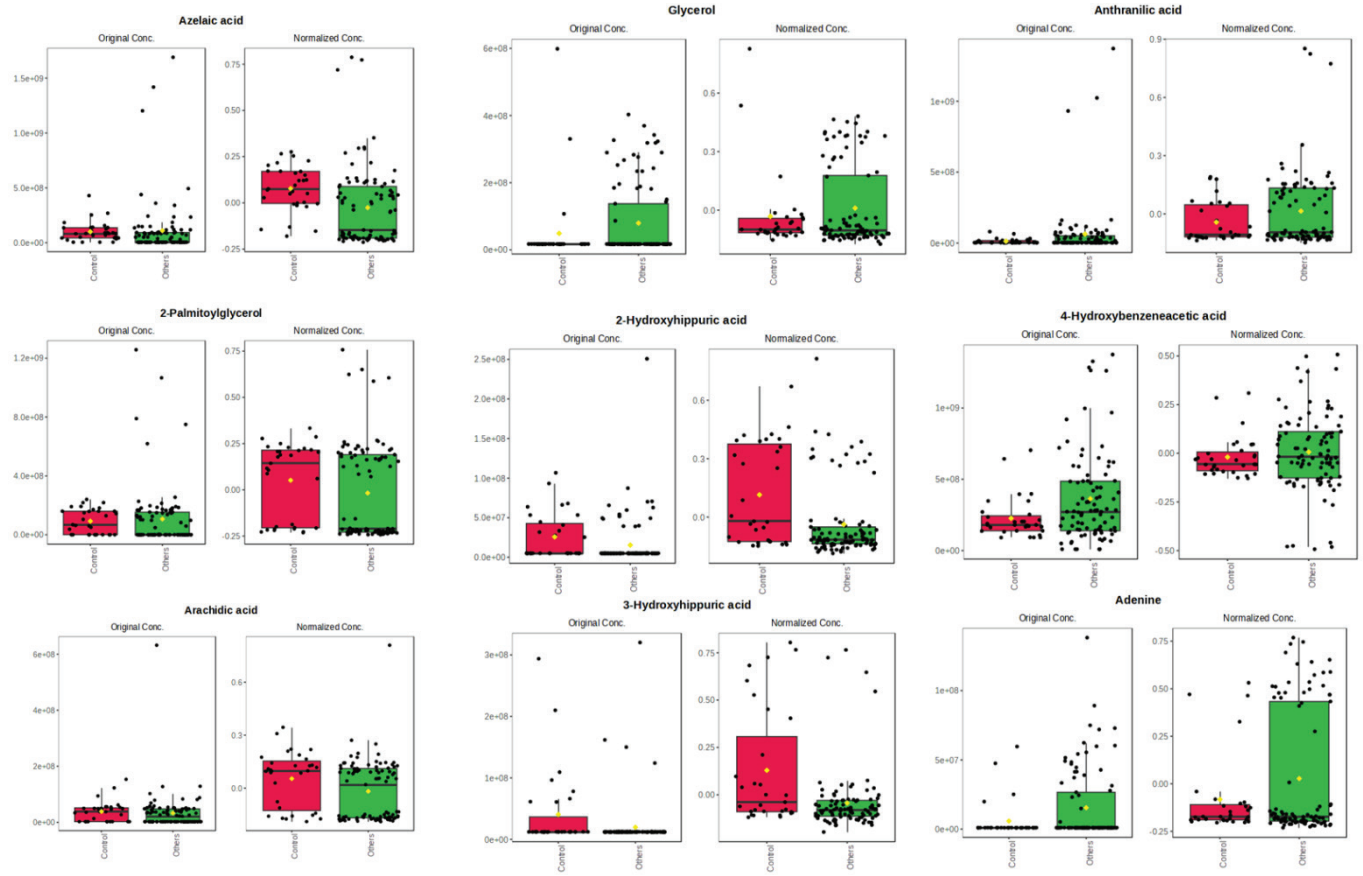

Figure 2. Based on ROC analyses between healthy and chest trauma samples, the original and normalized abundance of top 9 urinary metabolites (potential biomarkers). Each dot represents an individual value. Boxplot was created using ROC analyses module of MetaboAnalyst.

7 in LOS <5 days and LOS 5-10 days groups whereas it was either absent or remained low throughout in LOS >10 days group. The strength of association between LOS and metabolite levels was strong in myristic acid, moderate in arachidonic acid and 3-hydroxyanthranilic acid (Supplemental Digital Content: Figure 4A). A weak strength of association was seen in butanoic acid in relation to LOS, whereas a high strength of association was observed between the death versus discharge group with urinary butanoic acid levels being highest in the mortality group.

Butanedioic acid and L-5-oxoproline acid showed an overall increasing trend between days 0 and 7 in patients with TTSS <6, minimal increase in patients with TTSS 6-10 and a decreasing trend in patients with TTSS >10. Arachidic acid showed an increasing trend in patients with TTSS <6, a decreasing trend in patients with TTSS 6-10. 3-hydroxyphenylacetic acid showed a minimal decreasing trend between day 0 to day 7 in patients with TTSS <6 whereas it increased in 6-10 and >10 groups. 3-hydroxyanthranilic acid showed a gross increasing trend between days 0 and 7 in patients with TTSS <6, a decreasing trend in the TTSS 6-10 and > 10 groups with gross decrease in the latter. Moderate positive correlation with TTSS was seen in 3-hydroxyanthranilic acid (Supplemental Digital Content: Figure 4B).

DISCUSSION

Metabolic and endocrine pathways play a major role in the body's response to trauma by mobilizing energy sources, minimizing blood loss, and helping in recovery. While investigating mineral metabolism of patients with bony fractures, Cuthbertson in 1932 noticed metabolic changes in response to injury (23). A significant number of severely injured patients have associated chest injuries that may affect the outcomes. Computed tomography is an important diagnostic modality used in the management of trauma patients (24) and whole-body CT in polytrauma patients has been reported to identify clinically significant injuries (25,26). The radiographically detected lung parenchymal injuries have a closer association with the outcome but are often not diagnosed until 24 hours after injury (27). Metabolomics can provide a comprehensive understanding of metabolism in injured patients, potentially aiding in personalized treatment plans.

Nitrogenous bases have essential functions in human metabolism. Ischemia or systemic hypoxia leads to an increase in purines in systemic circulation (20,28,29). Increased adenosine (nucleoside containing adenine) levels in the brain have been reported after trauma (30). We found a statistically significant difference in adenine levels in different LOS groups with the highest values in patients with LOS >10 days. In our study, the levels of glycerol monostearate and 2-palmitoylglycerol were highest on day 7 and day 3 respectively in patients with LOS >10 days. During the initial post injury phase of metabolic response, glycerol levels increase by lipolysis. Suppression of α/β-hydrolase domain 6 (ABHD6) leads to an elevation in 1- and 2-MAG (monoacylglycerols) in different tissues, which leads to anti-inflammatory effects, and neuroprotective effects (31). Glycine and Glutathione have multiple functions in humans (32-36). Glutamine (precursor of glutathione) has been reported to have dose-dependent effect on macrophage activation and TNF-α synthesis (37). Elevated L-5-oxoproline (pyroglutamic acid) levels have been associated with oxidative

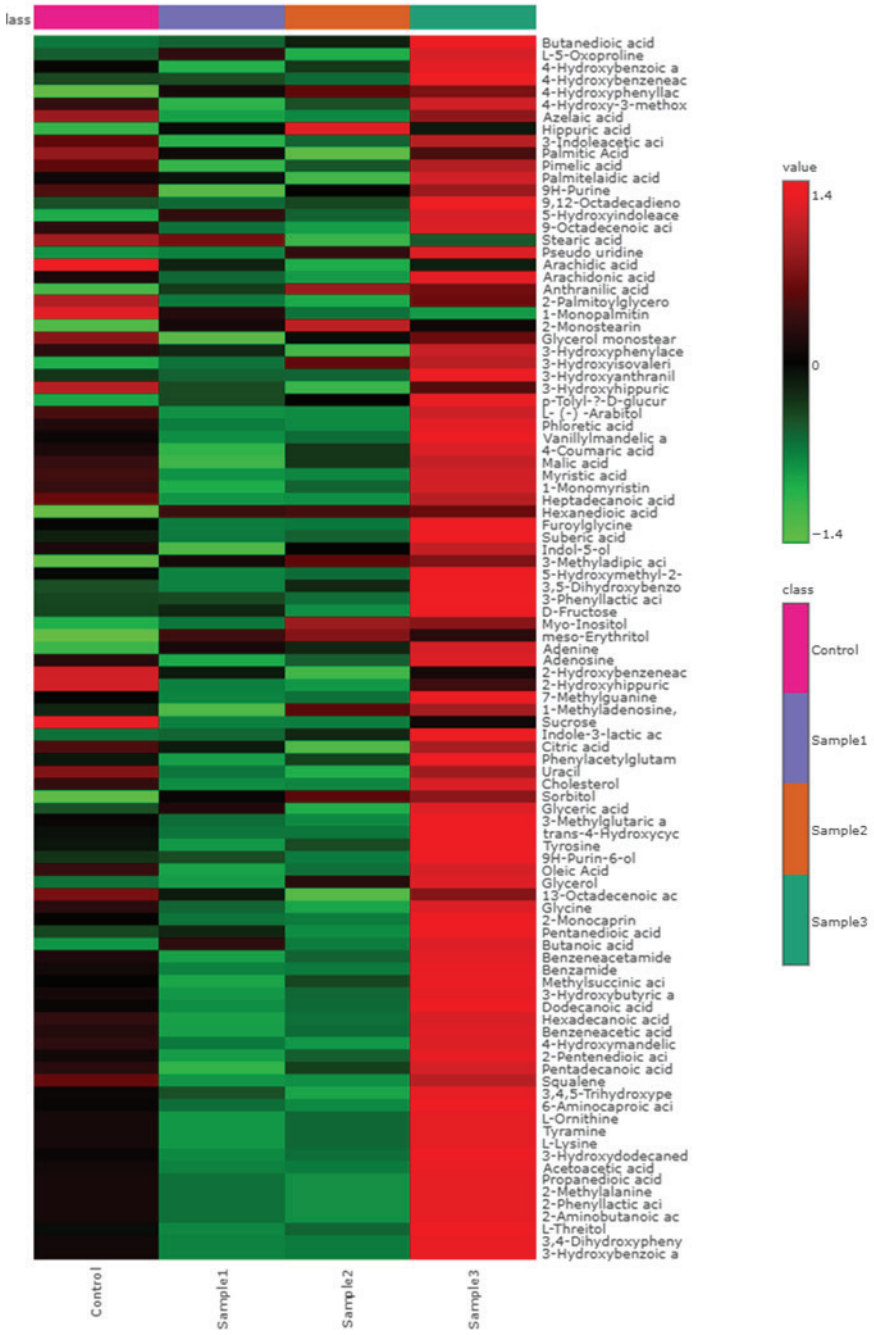

Figure 3. Heatmap of annotated metabolites from four different sampling groups. Healthy Control; Sample-1 (Day 0); Sample-2 (Day 3); Sample-3 (Day 7).

stress, glutathione (38) and glycine depletion in humans (39). Our results also showed a significant difference in L-5-oxoproline between LOS groups.

Biotin is involved in many metabolic pathways in humans (40) like cofactor for biotin-dependent carboxylases (41), role in cell physiology and gene regulation (42,43). Increased urinary excretion of 3-hydroxyisovaleric acid has been reported as a marker of biotin deficiency (44). We observed increased 3-hydroxyisovaleric acid levels in trauma patients in comparison to healthy controls, and the highest values in patients with LOS >10 days.

The primary source of energy after trauma are free fatty acids, and triglycerides provide nearly 50–80% of the total energy demand following trauma and critical illness (1). Insulin also stimulates the formation of triglycerides and inhibit their breakdown. Free fatty acids along with glucose and amino acids are released into the bloodstream after stress response including trauma (1). Our study demonstrated overexpression of arachidonic acid and butanoic acid in patients with LOS >10 days. There was a moderate positive association between LOS and arachidonic acid levels, LOS and butanoic acid levels. It was reported that arachidonic acid and butanoic acid levels were raised immediately after birth in the asphyxiated infants of *Macaca Nemestrina* (45). The finding of an increase in the levels of arachidonic acid and butanoic acid levels after asphyxia and in patients with longer length of stay suggest that raised arachidonic acid and butanoic acid levels might be associated with poor outcomes after trauma. Similarly, arachidic acid and butanedioic acid were differently expressed between control and trauma groups in our study.

The maintenance of tissue concentration of palmitic acid is essential for physiological roles like surfactant activity. Excess

fatty acids are exported to plasma via the liver (46). Unlike other fatty acids, the level of palmitic acid on day 0 was highest in patients with LOS <5 days, and lowest in patients with LOS >10 days. 3-hydroxyanthranilic acid (an intermediate in tryptophan metabolism) has immunomodulatory effects and anti-inflammatory activity on various immune cells (47). The high level of kynurenine metabolites enhances the generation of reactive oxygen species (48). Potential neurological actions of anthranilic acid *in vivo* are conflicting with neuroprotective effects in some cases and neurotoxic effects in others (49,50). In our study, 3-hydroxyanthranilic acid was very low in patients with LOS <5 days with a moderate positive correlation between 3-hydroxyanthranilic acid levels, LOS, and TTSS, respectively. The 3-hydroxyanthranilic acid concentrations in serum are associated with endothelial dysfunction and cardiac disease (51), which further validates its association with deranged healing and metabolism.

By recruiting only chest trauma patients, we tried to remove multiple confounding factors due to other injuries. We used age matched healthy relatives of injured patients for comparison to minimize the effect of genetic variation and age on metabolomic profile. We performed untargeted metabolomic analysis to obtain the greatest number of possible biomarkers. For the analysis, we used the patient's urine, which is a non-invasive process with no potential side effects. The limitations of our study are small sample size, using urine samples (which may have different metabolites and/or levels as compared to blood samples).

Based on the findings of our study using ROC analyses, top nine metabolites (azelaic acid, glycerol, anthranilic acid, 2-palmitoylglycerol, 2-hydroxyhippuric acid, 4-hydroxybenzeneacetic acid, arachidic acid, 3-hydroxyhippuric acid and adenine) based on their selected frequencies of more than 0.6% can be considered as biomarkers in isolated chest trauma patients.

Credit Statement:

Conceptualization: M.U.
Methodology, Visualization, Writing Original Draft, Review & Editing: I.A.
Software and Formal Analysis: D.S.
Resources: I.A., D.S. and N.J.
Data Curation: M.M. and I.A.
Supervision: M.Q.A.
Project Administration: M.U., A.K. and B.S.

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TRANSPARENCY DECLARATION:

Conflict of interests: None to declare.

SUPPLEMENTAL DIGITAL CONTENT (SDC)
(Supplemental Digital Content 1)

Table 2. Mean values ± SD of the Area Under Curve (AUC) of the peak intensities on Gas Chromatography-Mass Spectrometry (GCMS) analysis converted to numerical values of metabolites in different samples.

Figure 4. Correlation/association between 3-Hydroxyanthranilic acid level and duration of hospital stay (LOS) /thoracic trauma severity score (TTSS). A) Scatterplot depicting the correlation between 3-hydroxyanthranilic acid (Sample 1) and LOS (p = 0.023); B) Scatterplot depicting the correlation between 3-hydroxyanthranilic acid (Sample 1) and TTSS (rho=0.39).

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