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ORIGINAL RESEARCH

Metabolomic Profiling of Drug-Induced Liver Injury: A Biochemical Approach to Predicting Hepatotoxicity in Commonly Prescribed Pharmaceuticals

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ABSTRACT

Background: Drug-induced liver injury (DILI) is a significant clinical challenge, often leading to acute liver failure and the withdrawal of pharmaceutical agents from the market. Metabolomic profiling has emerged as a promising tool to identify biomarkers for early detection of hepatotoxicity. This study aims to investigate the biochemical alterations associated with DILI in commonly prescribed pharmaceuticals using metabolomic analysis.**Materials and Methods:** A total of 60 adult patients, categorized into drug-exposed (n=40) and control (n=20) groups, were enrolled. Blood and urine samples were collected at baseline, 4 weeks, and 12 weeks following drug administration. High-performance liquid chromatography-mass spectrometry (HPLC-MS) and nuclear magnetic resonance (NMR) spectroscopy were employed for metabolomic profiling. Key biochemical markers, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, and metabolic signatures of hepatotoxicity, were analyzed using multivariate statistical techniques.**Results:** Significant alterations in metabolite profiles were observed in the drug-exposed group compared to controls. Elevated levels of ALT (72 ± 15 U/L), AST (89 ± 18 U/L), and total bilirubin (2.4 ± 0.6 mg/dL) were detected in patients exhibiting hepatotoxicity. Metabolomic analysis revealed an upregulation of lipid peroxidation markers and a reduction in glutathione levels (1.8 ± 0.4 µmol/L). Principal component analysis (PCA) demonstrated distinct clustering between hepatotoxic and non-hepatotoxic patients, suggesting a predictive role of metabolic perturbations in DILI.**Conclusion:** Metabolomic profiling provides a biochemical approach to predicting DILI, offering potential biomarkers for early detection and risk assessment. The findings highlight the need for metabolomics-based screening in drug safety evaluation to minimize hepatotoxic risks in clinical practice.

Keywords: Drug-induced liver injury, metabolomics, hepatotoxicity, biomarker discovery, mass spectrometry, liver function markers.

INTRODUCTION

Drug-induced liver injury (DILI) is a major cause of acute liver failure and accounts for a significant proportion of drug withdrawals from the market (1). The liver, being the primary site for drug metabolism, is highly susceptible to toxic insults caused by pharmaceuticals, leading to hepatocellular damage, cholestasis, or mixed injury patterns (2). The complexity of DILI arises from inter-individual variability, drug metabolism, and immune-mediated responses, making its early prediction challenging in clinical settings (3).

Traditional liver function tests, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bilirubin, are widely used for DILI detection; however, they lack specificity and often fail to identify early-stage hepatotoxicity (4). Advanced biochemical and omics-based approaches, such as metabolomics, offer a more comprehensive insight into the molecular perturbations associated with liver injury, providing a promising tool for early diagnosis and risk assessment (5). Metabolomics is a high-throughput analytical technique that enables the identification and quantification of small-molecule

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metabolites, reflecting the biochemical changes occurring in response to drug exposure (6). This approach has been successfully utilized to detect metabolic fingerprints of hepatotoxicity in preclinical and clinical studies (7).

Recent advancements in mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy have facilitated the identification of metabolite alterations linked to oxidative stress, mitochondrial dysfunction, and bile acid dysregulation in DILI (8). Several studies have reported distinct metabolic signatures associated with hepatotoxic drugs, including acetaminophen, antibiotics, and anti-tubercular agents, highlighting the potential of metabolomic biomarkers in predicting drug-induced liver damage (9,10). Despite these advancements, further research is needed to validate metabolomic markers and integrate them into clinical practice for improved DILI surveillance.

This study aims to explore metabolomic alterations associated with commonly prescribed pharmaceuticals and their role in predicting hepatotoxicity. By utilizing high-performance liquid chromatography-mass spectrometry (HPLC-MS) and nuclear magnetic resonance (NMR) spectroscopy, we seek to identify specific metabolic signatures that can serve as early indicators of DILI.

MATERIALS AND METHODS

Study Design and Participants

This study was designed as a prospective observational study to evaluate metabolomic alterations in patients exposed to commonly prescribed pharmaceuticals associated with drug-induced liver injury (DILI). A total of 60 adult participants were recruited, comprising 40 patients receiving potentially hepatotoxic medications and 20 healthy controls. Patients were included based on prior prescription of drugs known to cause liver injury, such as antibiotics, analgesics, and antiepileptic drugs. Exclusion criteria included individuals with pre-existing liver disease, alcohol abuse, or metabolic disorders that could influence liver function.

Sample Collection and Processing

Blood and urine samples were collected at baseline, four weeks, and twelve weeks following drug administration. Blood samples were centrifuged at 3,000 rpm for 10 minutes to separate plasma, which was then stored at -80°C until further analysis. Urine samples were collected in sterile containers and stored under similar conditions to prevent metabolic degradation.

Biochemical Analysis

Liver function tests, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, and alkaline phosphatase (ALP), were measured using an automated biochemical analyzer. Glutathione levels and oxidative stress markers were assessed using commercially available assay kits.

Metabolomic Profiling

Metabolomic analysis was conducted using high-performance liquid chromatography-mass spectrometry (HPLC-MS) and nuclear magnetic resonance (NMR) spectroscopy. Plasma and urine metabolites were extracted using methanol precipitation, followed by centrifugation at 12,000 rpm for 15 minutes. The supernatant was filtered and injected into the HPLC-MS system for metabolic profiling. NMR spectroscopy was performed using a 600 MHz instrument to detect low-molecular-weight metabolites associated with hepatotoxicity.

Statistical Analysis

Data were analyzed using SPSS version 26.0 and MetaboAnalyst 5.0. A principal component analysis (PCA) was conducted to identify clustering patterns between hepatotoxic and non-hepatotoxic groups. Metabolite variations were assessed using Student's t-test and ANOVA, with a p-value <0.05 considered statistically significant. Correlations between metabolite levels and liver function parameters were evaluated using Pearson's correlation coefficient.

RESULTS

Baseline Characteristics

The demographic and baseline biochemical characteristics of the study participants are presented in **Table 1**. The drug-exposed group (n=40) had a mean age of 45.2 years, while the control group (n=20) had a mean age of 44.1 years (p=0.312). The percentage of male participants was comparable between the two groups (60% vs. 55%, p=0.432). Baseline liver function tests, including ALT and AST levels, were higher in the drug-exposed group compared to controls, with ALT at 35.4 U/L and AST at 40.2 U/L in the exposed group, while in the control group, the values were 28.2 U/L and 32.8 U/L, respectively (p=0.045 and p=0.039).

Biochemical Alterations Over Time

Liver function markers demonstrated significant alterations in the drug-exposed group over the study period (**Table 2**). ALT levels increased from a baseline of 35.4 U/L to 58.7 U/L at 4 weeks and 72.1 U/L at 12 weeks (p=0.002). Similarly, AST levels rose from 40.2 U/L to 74.3 U/L at 4 weeks and 89.2 U/L at 12 weeks (p=0.001). Total bilirubin levels followed an increasing trend, reaching 2.4 mg/dL at 12 weeks (p=0.004).

Glutathione levels, a marker of oxidative stress, decreased significantly from 2.1 µmol/L at baseline to 1.5 µmol/L at 4 weeks and further to 1.1 µmol/L at 12 weeks (p=0.018). Conversely, oxidative stress markers increased progressively from 1.8 at baseline

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to 2.4 at 4 weeks and peaked at 3.1 at 12 weeks (p=0.006).

Metabolomic Profiling

Principal component analysis (PCA) revealed distinct clustering between the drug-exposed and control groups, suggesting significant metabolic perturbations in response to drug administration. Elevated lipid peroxidation markers and bile acid metabolites were identified in hepatotoxic patients, supporting the role of metabolomics in detecting early-stage liver injury.

These findings indicate that metabolomic profiling can provide an advanced biochemical approach to predicting drug-induced hepatotoxicity, offering potential biomarkers for early risk assessment and intervention.

Table 1: Baseline Characteristics of Study Participants

Parameter	Drug-Exposed Group (n=40)	Control Group (n=20)	p-value
Age (years)	45.2	44.1	0.312
Male (%)	60%	55%	0.432
BMI (kg/m²)	24.8	25.1	0.621
ALT (U/L)	35.4	28.2	0.045
AST (U/L)	40.2	32.8	0.039
Total Bilirubin (mg/dL)	0.9	0.7	0.051

Table 2: Biochemical Parameters at Different Time Points

Parameter	Baseline	4 Weeks	12 Weeks
ALT (U/L)	35.4	58.7	72.1
AST (U/L)	40.2	74.3	89.2
Total Bilirubin (mg/dL)	0.9	1.6	2.4
Glutathione (µmol/L)	2.1	1.5	1.1
Oxidative Stress Markers	1.8	2.4	3.1

DISCUSSION

Drug-induced liver injury (DILI) remains a major challenge in clinical pharmacology, often leading to drug discontinuation and severe hepatic complications. The present study utilized metabolomic profiling to investigate biochemical alterations in patients receiving commonly prescribed pharmaceuticals associated with hepatotoxicity. The findings suggest that metabolic perturbations, particularly in liver function markers and oxidative stress indicators, can serve as early biomarkers for predicting DILI.

Elevated levels of ALT, AST, and bilirubin observed in the drug-exposed group are consistent with previous studies reporting hepatic enzyme abnormalities in patients with DILI (1,2). The progressive increase in ALT and AST levels over 12 weeks suggests ongoing hepatocellular damage, which could be attributed to mitochondrial dysfunction and oxidative stress induced by drug metabolism (3). These enzymes are well-established indicators of liver injury, yet their specificity in distinguishing different types of hepatotoxicity remains limited (4).

Glutathione depletion and increased oxidative stress markers observed in this study further confirm the role of oxidative damage in drug-induced hepatotoxicity. Glutathione acts as a crucial antioxidant, protecting hepatocytes from reactive oxygen species generated during drug metabolism (5,6). Reduced glutathione levels have been widely reported in cases of acetaminophen-induced liver toxicity and other hepatotoxic drugs (7). Oxidative stress markers, which showed a significant increase over time, highlight the involvement of lipid peroxidation and mitochondrial dysfunction in DILI (8).

Metabolomic analysis has emerged as a valuable tool for identifying novel biomarkers of liver injury. In the current study, principal component analysis (PCA) revealed distinct clustering patterns between hepatotoxic and non-hepatotoxic patients, indicating metabolic shifts due to drug exposure. Similar findings have been reported in studies using metabolomics to assess hepatotoxicity caused by antibiotics and chemotherapeutic agents (9,10). The detection of altered bile acid metabolites in the hepatotoxic group aligns with previous research indicating the role of bile acid dysregulation in drug-induced cholestasis (11).

Mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy were instrumental in detecting metabolic signatures associated with liver toxicity. MS-based metabolomics has shown high sensitivity in identifying low-molecular-weight biomarkers predictive of liver injury (12). In contrast, NMR spectroscopy provides comprehensive metabolic insights, enabling the detection of dynamic biochemical changes in response to hepatotoxic drugs (13). Combining these techniques enhances the ability to identify early biomarkers before clinical symptoms of hepatotoxicity manifest (14).

Despite its strengths, this study has certain limitations. The sample size was relatively small, and variations in drug dosages and patient compliance may have influenced the results. Future studies should include larger cohorts and a broader range of hepatotoxic drugs to validate these metabolomic biomarkers.

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Additionally, integrating multi-omics approaches, including proteomics and transcriptomics, could provide a more comprehensive understanding of DILI mechanisms (15).

CONCLUSION

In conclusion, this study demonstrates the potential of metabolomic profiling in predicting drug-induced liver injury. The observed biochemical alterations, including elevated liver enzymes, glutathione depletion, and oxidative stress, provide valuable insights into hepatotoxicity mechanisms. Further research is warranted to validate these findings and develop metabolomics-based screening strategies for early DILI detection in clinical settings.

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