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A Metabolomic Approach for Screening of Acute Liver Failure Patients in Hepatitis E Virus Infection

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Abstract

Background: Hepatitis E is mainly an acute and self-limiting disease which is endemic to resource poor regions of the world. Some patients have an increased susceptibility to develop fulminant hepatitis which is a rare disorder with high mortality and resource cost. In this study, a metabonomic approach was used to investigate the biochemical perturbation of the serum samples from acute liver failure patients induced by hepatitis E virus. **Materials and Methods:** Serum samples from hepatitis E virus-related acute liver failure patients (n = 20) and healthy controls (n = 20) were studied. Gas chromatography–mass spectrometry technique integrated with a commercial mass spectral library for the peak identification was used to detect the serum metabonome. **Results:** Out of the 24 metabolites detected, the serum levels of benzenepropanoic acid, lactic acid, hexadecanoic acid, L-proline, serine, and butanoic acid were significantly higher in the acute liver failure patients than those in the healthy control, whereas octadecanoic acid, N-formylglycine, and isoleucine were significantly higher in healthy controls compared to cases. These metabolites are suggested to be involved in various metabolic activities. **Conclusion:** These results may indicate that metabonomic analysis of the serum samples can provide integrative information to assess the severity of the liver failure, which is beneficial for predicting the pathogenetic condition and the course of liver disease.

Keywords: GC-MS, Metabonomic, HEV

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Introduction

Acute liver failure is the clinical manifestation of sudden and severe hepatic injury. The main causal agent that triggers the onset of liver failure shows wide geographical variation and depends on the prevalent hepatotropic virus infection.[1,2] Hepatitis E is an acute and largely self-limiting form of viral hepatitis that is caused by hepatitis E virus (HEV). The virus is transmitted through fecal–oral route and is endemic throughout tropical and subtropical countries, with periodic epidemics through contaminated drinking water. Sporadic cases of viral hepatitis E infection also arise at much higher rates in endemic than in non-endemic regions.[3,4] Hepatitis E is now the most common cause of acute liver failure in India and Pakistan, China, and Southeast Asia.[5] Viral infections follow a more severe course, although children are infected, the highest attack rates are observed in young adults, aged 15–40 years.[6] Especially pregnant women show the propensity to develop acute hepatic failure with high mortality.[7,8] Liver is the main metabolic, biosynthetic, storage, and detoxifying organ, its infection by HEV causes a broad range of clinical manifestations, most of which are related to hepatocellular damage.[6] Hepatitis E patients show the differential expression of various acute-phase proteins that are synthesized in the liver, in their plasma, and urine.[9] Liver being a major metabolic organ, its infection is also expected to result in measurable changes in metabolite levels.

The information obtained from metabonomic study is also complementary to that from proteomics and genomics. Recent advances in analytical techniques have enhanced the capability for the global assessment of entire classes of biomolecules such as genome, proteome, and metabonome. For understanding and elucidating the etiology and mechanisms of human diseases, molecular systems biology has been proposed as an alternative and promising resolution.[10] Metabonomics, defined as "the quantitative measurement of the multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification,"[11,12] is a novel methodology arising from the post-genomics era for the study of molecular systems biology. Nowadays, metabonomics has been applied to various biomedical and toxicological studies. Because the alteration of the physiological status can disrupt homeostasis, resulting in perturbations of the levels of endogenous biochemicals involved in different kinds of key metabolic processes, the metabonomic study may provide both invaluable information to understand the molecular mechanisms and provide novel insights into the status of dysfunction in biological system.

The "-omics" approaches, especially the proteomics with the global analysis of protein expression, have been employed to screen the molecular markers which can be used as serological biomarkers. Because liver failure will induce severe metabolic disturbance, it is generally believed that the comprehensive detection and characterization of the alternative endogenous metabolites may

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be useful to develop the metabolic phenotyping of liver diseases. In this study, we applied gas chromatography–mass spectrometry (GC–MS) technique to profile the metabolic composition of serum samples from both healthy subjects and HEV-induced liver failure patients. The detailed analysis of metabonomic data with pattern recognition techniques may reveal valuable information for the diagnosis and therapy of liver failure patients.

Materials and Methods

Patients and Controls

Twenty HEV-induced acute liver failure patients visiting the medical OPD of the Department of Medicine, Lok Nayak Hospital, New Delhi, between February 2012 and February 2015 were enrolled in this study with the patients' consent. All of the patients had severe liver malfunction with hepatic encephalopathy. The control serum samples from 20 healthy volunteers were collected. After the centrifugation (3000 rpm) for 10 min at 4°C, the serum samples were stored at –80°C until use.

Sample Preparation

The serum samples were thawed at room temperature. A 300 μL aliquot of acetonitrile was added into 200 μL of sera to precipitate the protein, and the mixture was shaken vigorously for 30 s. After it was kept on ice for 5 min, the mixture was ultrasonically extracted for 10 min and then followed by centrifugation (10,000 rpm) for 10 min at 4°C. Then, 300 μL supernatant was transferred to the GC vial and evaporated to dryness under a stream of nitrogen gas. The chemical derivatization of the serum metabonome was carried out using the combination of methoxymation and silylation. A 50 μL aliquot of methoxyamine pyridine solution (15 μg/μL) was added into the vial. The methoxymation was performed at room temperature for 16 h. Then, 90 μL of MSTFA with 1% TMCS as catalyst was added to the vial. After the silylation for 1 h at room temperature, 150 μL of *n*-heptane was added into each GC vial to dilute the solution. The sample solution was transferred to the GC microvial for GC–MS analysis after filtration.

GC–MS Analysis

A 2.0 μL aliquot of the derivatized sample was injected with splitless mode by an Agilent 7683 Series autosampler (Agilent Technologies) into an Agilent 6980 GC system equipped with a fused-silica capillary column chemically (30 m × 0.25 mm i.d.) bonded with 0.25 μm ZB-5MS stationary phase (Phenomenex). The injector temperature was set at 270°C. Helium (He) was used as the carrier gas with a constant flow rate set at 0.8 mL/min through the column. To acquire a well separation, the column temperature was initially maintained at 70°C for 5 min and then increased from 70 to 100°C at a rate of 10°C/min for 5 min. Then, the column temperature was increased to 200°C at 10°C/min for another 5 min. After that, the temperature was increased to 280°C at 10°C/min and held for 10 min. The column effluent was introduced into the ion source of an Agilent 5973 mass selective detector (Agilent Technologies). The temperature of the ion source was set at 230°C, and for the quadrupole, it was set at 150°C. MS detection was implemented with electron impact mode and full scan monitoring mode (m/z 60–600). AMDIS software (National Institute of Standards and Technology, Gaithersburg, MD) was used for the peak deconvolution. Identification of the interested peaks was supported by NIST v1.0.0.12 mass spectra library.

Data Processing and Pattern Recognition

The serum samples from the patient group and control group were blindly analyzed in a random order. Each sample was represented with a GC–MS total ion current (TIC) chromatogram, and the peaks with the intensity higher than 3-fold of the ratio of signal-to-noise were detected.

Statistical Analysis

Statistical analyses were performed using online GraphPad software. Values are represented as mean± SD; significant difference between the HEV patients and healthy group was assessed using the two-tailed Student's *t*-test; $P = -0.05$ was considered statistically significant.

Results and Discussion

GC–MS Analysis of Serum Samples

GC–MS TIC chromatograms of serum samples from the HEV-related acute liver failure patient group and healthy controls are shown in Figures 1 and 2. In these chromatographic profiles, endogenous metabolites such as amino acids, fatty acids, organic acids, and carbohydrates were identified based on the NIST mass spectra library. These components are involved in multiple biochemical processes, especially the energy metabolism, protein metabolism, amino acid metabolism, and lipid metabolism.

Approximately 80% of the amino acids were absorbed form digestive tract used in the process of protein synthesis, deamination, and transamination in liver.[13] As shown in Table 1, in the liver failure patients, the concentration of multiple amino acids was increased in the serum samples. Mainly when the liver is injured, the metabolic enzymes of amino acids may be damaged and the normal metabolic pathway of the amino acids is deteriorated results in increased amino acids in serum. Moreover, when the process of protein synthesis in the liver was disturbed, the absorption of the amino acids into the liver from the blood was decreased. Furthermore, the increase of amino acids in the blood was enhanced by plasmatorrhexis and necrobiosis from hepatic cells. In addition, glucose, proteins, and lipids were absorbed less in liver failure patients, amino acids will be generated by the decomposition of endogenous proteins for energy metabolism further leads to increase amino acids in the blood.

Serum levels of benzenepropanoic acid, lactic acid, hexadecanoic acid, l-proline, serine, and butanoic acid were significantly higher in the acute liver failure patients than those in the healthy control [Table 1], whereas octadecanoic acid, N-formylglycine, and isoleucine were significantly higher in healthy controls compared to cases [Table 1]. These metabolites are suggested to be involved in energy metabolism, lipid metabolism, protein metabolism, and amino acid metabolism.

In clinical diagnosis of liver failure cases, the liver function examination which is used is limited by small number of parameter and is insufficient to give comprehensive information about the physiopathological state. There is a lack of global index for evaluation of the severity of liver disease and prognosis. A rapid diagnosis in clinical practice is beneficial for improving the

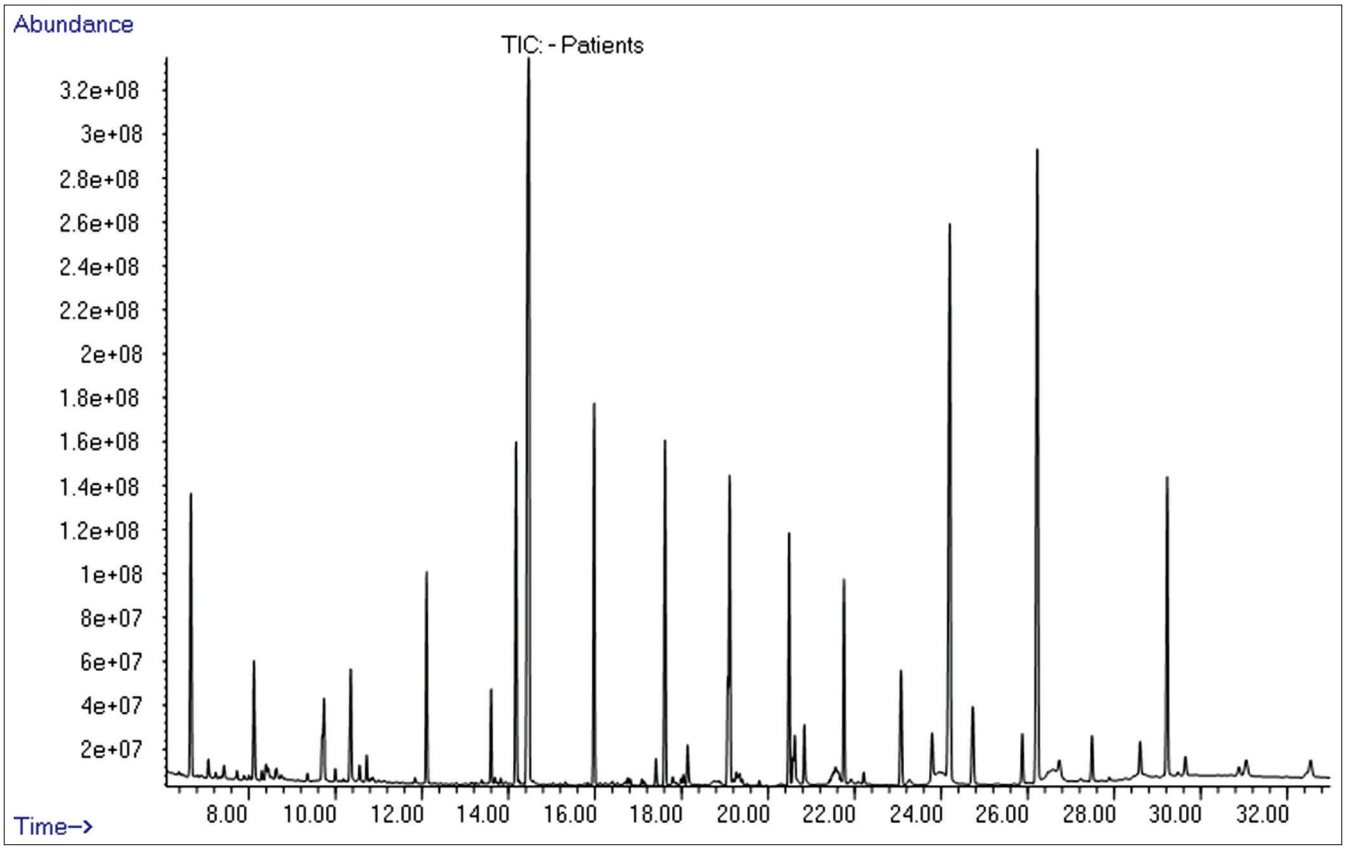

Figure 1: Gas chromatography–mass spectrometry total ion current profile of serum metabolites of hepatitis E virus patient group. The identified peaks are listed in Table 1

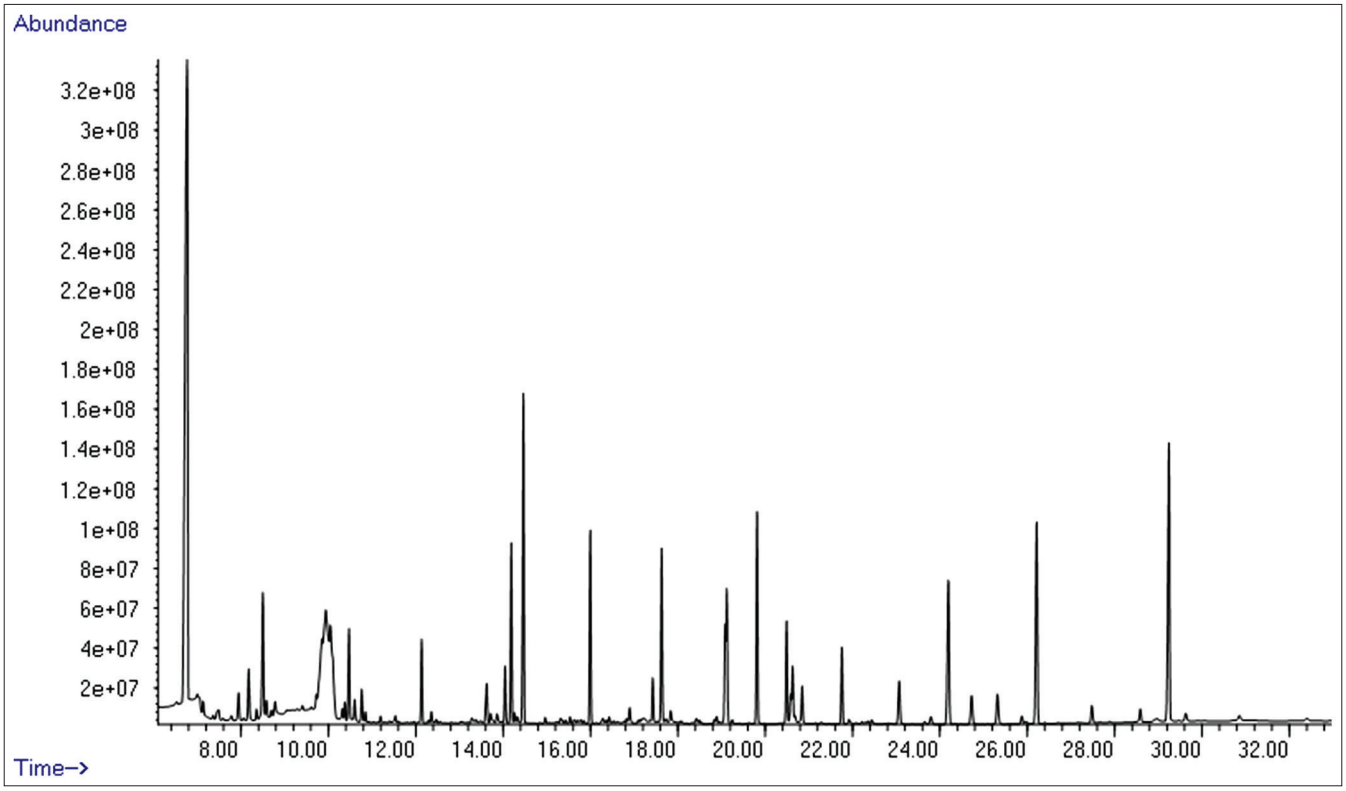

Figure 2: Gas chromatography–mass spectrometry total ion current profile of serum metabolites of healthy group. The identified peaks are listed in Table 1

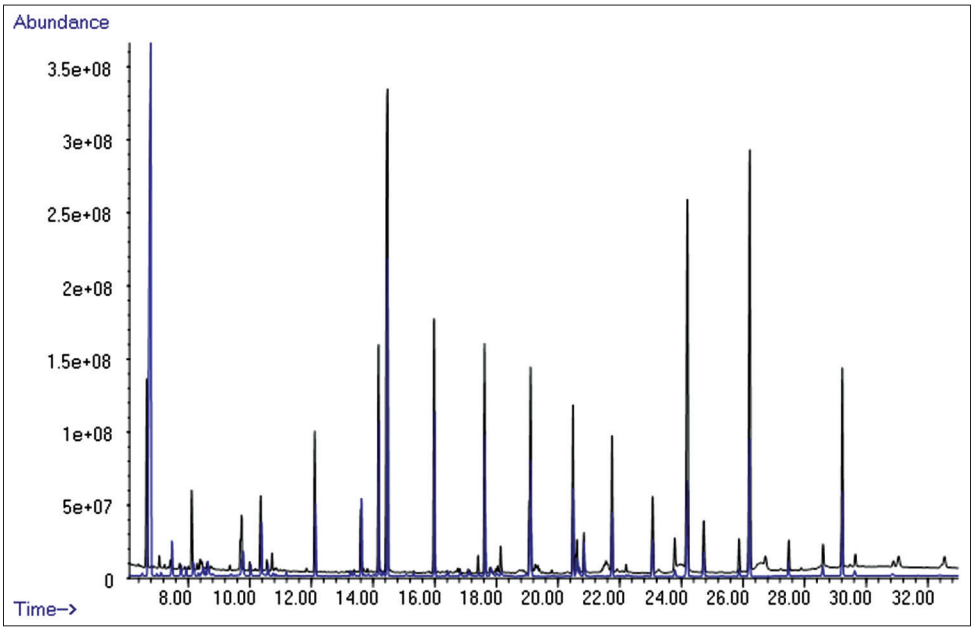

Figure 3: Over laid gas chromatography–mass spectrometry total ion current profile of serum metabolites of hepatitis E virus patients and healthy group

Table 1: Serum metabolites of the hepatitis E virus-related acute liver failure patients and healthy group measured by gas chromatography–mass spectrometry

Peak no.	*Retention time*	*Metabolites*	*Patient group (n=20)*	*Healthy group (n=20)*
1	25.72	Octadecanoic acid	1.8e7±2.7e7	4.9e6±4.7e6*
2	20.59	Octadecadienoic acid	4.3e7±2.9e7	4.6e7±4.2e7
3	17.83	Benzenepropanoic acid	5.0e6±5.1e6	1.0e8±2.2e8**
4	10.87	Butanedioic acid	8.9e6±4.3e6	1.1e7±5.7e6**
5	26.98	Cholesterol	6.2e7±1.3e8	2.9e7±7.6e7
6	7.18	Lactic acid	7.7e7±1.7e8	2.5e7±6.3e7**
7	22.51	Ethanedioic acid	1.4e6±2.9e6	1.2e6±1.1e6
8	7.87	Glycine	9.8e7±5.1e7	3.0e6±6.8e6**
9	19.09	Hexadecanoic acid	6.4e8±3.1e8	2.2e8±1.6e8**
10	8.71	Isoleucine	5.8e6±2.8e6	9.8e6±3.5e6**
11	7.52	l-Alanine	1.5e7±9.8e7	2.6e7±1.5e7
12	8.37	l-Leucine	1.2e7±7.3e7	1.8e7±1.0e7
13	13.62	L-Proline	5.5e7±2.6e7	2.6e7±2.1e7**
14	14.26	L-Threonic acid	6.6e6±4.1e6	8.1e6±3.3e6
15	7.11	l-Valine	1.5e7±1.2e7	1.6e7±1.5e7
16	19.81	Myo-inositol	1.4e7±1.1e7	2.2e7±1.9e7
17	19.25	N-Formylglycine	4.3e6±1.4e7	7.8e5±5.0e5**
18	14.32	Pentanedioic acid	1.3e7±5.3e7	2.3e7±2.6e7
19	16.52	Phosphoric acid	1.0e7±1.1e7	1.4e7±1.4e7
20	6.50	Propanoic acid	1.8e8±3.5e8	1.9e7±2.6e7
21	10.04	Serine	6.7e7±4.9e7	2.4e7±5.7e7*
22	20.31	2-Octenoic acid	8.4e5±1.1e5	6.7e5±6.8e5
23	17.43	Sorbitol	5.0e7±5.7e7	4.6e7±2.9e7
24	16.599	Butanoic acid	3.1e7±1.3e7	2.0e7±1.2e7**

The relative intensity of serum metabolites in the healthy and patients group is expressed with their peak area. Values are represented as mean±SD; significant difference between the hepatitis E virus patients (20) and healthy group (20) is based on the two-tailed Student's *t*-test (*$P<0.05$, **$P\leq0.01$). Serum metabolites were identified based on NIST mass spectra database

patient care quality and reducing the health-care costs. In this study, we attempted to use metabonomic approach based on the GC–MS technique to evaluate the physiopathologic state of acute liver failure patients for clinical diagnosis and prognosis. The result of multivariate analysis of the metabonomic data showed that the metabolism pattern of the acute liver failure patients was distinctive from the healthy control group [Figures 3 and 4]. These results may indicate that metabonomic analysis of the serum samples can provide integrative information to assess the severity of the liver failure, which is beneficial for predicting the pathogenetic condition and the course of liver disease.

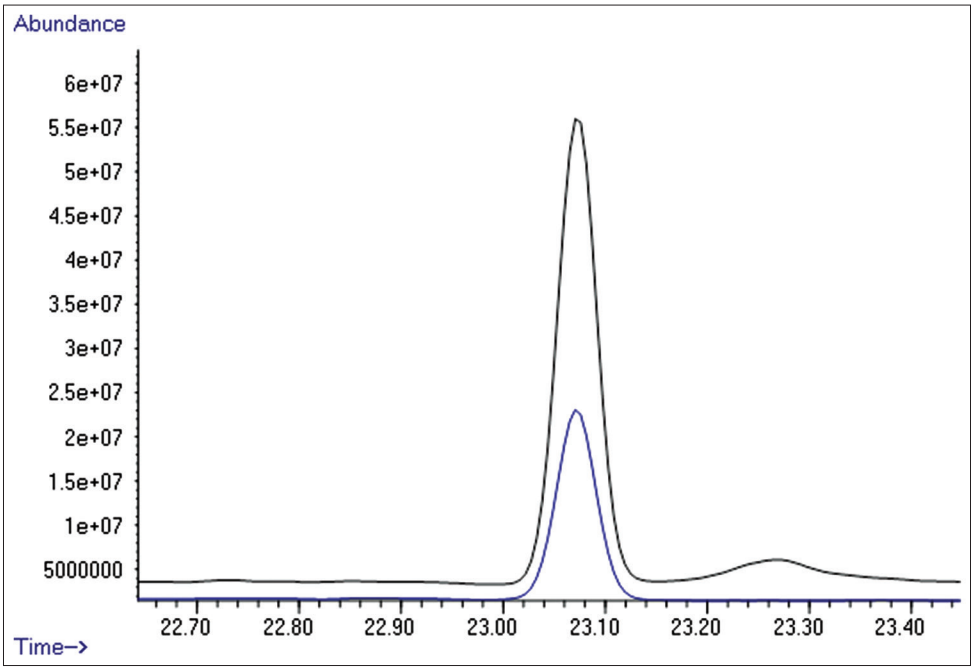

Figure 4: Over laid gas chromatography–mass spectrometry total ion current profile of serum metabolites of hepatitis E virus patients and healthy group, a peak with significant difference zoomed in

Conclusion

In the current study, the result of multivariate analysis of the metabonomic data may indicate that the serum samples can provide integrative information to assess the severity of the liver failure, which is beneficial for predicting the pathogenesis and the course of liver disease.

References

1. Bhatia V, Singhal A, Panda SK, Acharya SK. A 20-year single-center experience with acute liver failure during pregnancy: Is the prognosis really worse? Hepatology 2008;48:1577-85.
2. Acharya SK, Batra Y, Hazari S, Choudhury V, Panda SK, Dattagupta S. Etiopathogenesis of acute hepatic failure: Eastern versus Western countries. J Gastroenterol Hepatol 2002;17 Suppl 3:S268-73.
3. Chadha MS, Walimbe AM, Chobe LP, Arankalle VA. Comparison of etiology of sporadic acute and fulminant viral hepatitis in hospitalized patients in Pune, India during 1978-81 and 1994-97. Indian J Gastroenterol 2003;22:11-5.
4. Dalton HR, Stableforth W, Thurairajah P, Hazeldine S, Remnarace R, Usama W, *et al*. Autochthonous hepatitis E in Southwest England: Natural history, complications and seasonal variation, and hepatitis E virus IgG seroprevalence in blood donors, the elderly and patients with chronic liver disease. Eur J Gastroenterol Hepatol 2008;20:784-90.
5. Dalton HR, Bendall R, Ijaz S, Banks M. Hepatitis E: An emerging infection in developed countries. Lancet Infect Dis 2008;8:698-709.
6. Aggarwal R, Naik S. Epidemiology of hepatitis E: Current status. J Gastroenterol Hepatol 2009;24:1484-93.
7. Khuroo MS, Kamili S. Aetiology, clinical course and outcome of sporadic acute viral hepatitis in pregnancy. J Viral Hepat 2003;10:61-9.
8. Hamid SS, Atiq M, Shehzad F, Yasmeen A, Nissa T, Salam A, *et al*. Hepatitis E virus superinfection in patients with chronic liver disease. Hepatology 2002;36:474-8.
9. Taneja S, Sen S, Gupta VK, Aggarwal R, Jameel S. Plasma and urine biomarkers in acute viral hepatitis E. Proteome Sci 2009;7:39.
10. Craig A, Sidaway J, Holmes E, Orton T, Jackson D, Rowlinson R, *et al*. Systems toxicology: Integrated genomic, proteomic and metabonomic analysis of methapyrilene induced hepatotoxicity in the rat. J Proteome Res 2006;5:1586-601.
11. Nicholson JK, Lindon JC, Holmes E. "Metabonomics": Understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. Xenobiotica 1999;29:1181-9.
12. Nicholson JK, Connelly J, Lindon JC, Holmes E. Metabonomics: A platform for studying drug toxicity and gene function. Nat Rev Drug Discov 2002;1:153-61.
13. Tsukada K, Moriyama T, Lieberman I. Liver amino acid pool may be homogeneous with respect to protein synthesis. J Biochem 1971;70:173-4.