1H Nuclear Magnetic Resonance spectroscopy for the prediction of therapeutic outcome in patients with Fulminant Hepatic Failure

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SYNOPSIS: The use of [1]H-NMR spectroscopy for the assessment of therapeutic outcome in patients (n=14) with Fulminant-Hepatic-Failure (FHF) is presented. Serum- and urine- glutamine and urine-urea were found to change over an order of magnitude in two groups (survivors (n=9) and non-survivors (n=5)). No major difference in the conventionally employed clinical data of the two groups was found. The results authentically predict the probability of survival of patients on standard treatment alone. The studies promise the potential of NMR in quickly deciding on the need for advanced therapeutic intervention such as artificial liver support or emergency liver transplantation in FHF.

INTRODUCTION: In patients with FHF, hyperammonemia leads to less urea and more glutamine production. Diagnosis of FHF is conventionally made on the basis of a certain clinical picture and associated prolonged prothrombin time, low serum glucose levels, hyperbilirubinemia and grossly elevated transaminase values with AST values sometimes exceeding 1000 IU/L. However, all these estimations are time consuming and labor intensive, with a small but significant probability of error. Additionally, none of these parameters, used singly, can predict the outcome in a given patient of FHF. Urinary and serum estimation of urea and glutamine by NMR spectroscopy demonstrates the utility of these markers in distinguishing between those patients likely to recover and those likely to succumb to FHF on standard treatment protocol.

MATERIALS AND METHODS: This study has been conducted on 14 patients, aged 4-55 years, of acute viral hepatitis,. All the patients were admitted with grade III-IV encephalopathy. The patients underwent serum transaminases, serum bilirubin, and prothrombin time estimation, besides other parameters and imaging.

Blood and urine samples were collected for NMR spectroscopic studies. 500 □l of serum and urine samples were then taken in 5mm NMR tube with a sealed reusable capillary containing 25□l of 0.375% sodium salt of tri-methyl silyl propionic acid (TSP) in D_2O. All experiments were performed on a Bruker Avance 400 MHz spectrometer. Serum spectra were obtained using Carr-Purcell-Mieboom-Gill (CPMG) sequence with water suppression by presaturation and urine spectra were obtained using one pulse sequence with water suppression using WATERGATE sequence. The parameters used were: spectral width: 8000 Hz; data points: 32 K; recycle delay: 18s (CPMG), 3s (Watergate); spectrum size: 32K; line broadening: 0.3Hz; Length of CPMG pulse train: 269 ms.

Concentrations of the metabolites were determined from the integral areas of the respective signals with reference to that of TSP and the quantities of the metabolites calculated using a computer program.

RESULTS: All patients were treated according to a standard uniform protocol for management of FHF - nine recovered and five expired following the treatment. Clinical and NMR parameters of patients with FHF (survivors) and FHF (non-survivors) are expressed in median and range.

Conventional clinical laboratory parameters					**Metabolites from NMR**			
Parameter	FHF (Survivors)*	FHF (non-survivors)*	p-value[+]		Parameter	FHF (Survivors)*	FHF (non-survivors)*	p-value[+]
AST (IU/L)	334 (28-1116)	197 (150-1052)	0.84		Serum glutamine(mg/dl)	5 (4-12)	74 (35-84)	0.0001
ALT (IU/L)	696 (14-3652)	1180 (201-1720)	0.87		Urinary glutamine(mg/dl)	ND	460 (24-972)	0
PT (seconds)	18.7 (13.7-48.4)	49 (29-92)	0.05		Urinary urea(mg/dl)	665 (342-2628)	133 (80-215)	0.02
Bilirubin[&](mg/dl)	5.01 (1.9-8.3)	8.9 (5.2-19)	0.09					
Bilirubin[$](mg/dl)	1.79 (0.5-2.9)	3.2 (1.9-8.1)	0.10					

*values are in median and range, [+] two-tailed p- test, [&] Total, [$]direct, ND=not detected

Clinical parameters show higher values (than expected for a normal subject) for both survivors and non-surviving patients with prothrombin time as the only parameter approaching difference with significance, whereas the data from [1]H-NMR show distinctly different values for both categories thus correctly predicting the outcome of patients on standard treatment. Results of NMR experiments indicate that the patients with non-recoverable FHF have highly elevated levels of glutamine in serum (74 mg/dl, median) and urine (460 mg/dl, median) while the serum-glutamine in surviving cases was 5 mg/dl (median) and glutamine could not be detected in urine.

DISCUSSION: In functioning human liver, the toxic ammonia generated by amino acid metabolism in different parts of the body gets converted to urea through urea cycle. In case of hepatic failure, the resultant impairment in urea cycle leads to elevated levels of toxic ammonia, thereby triggering the formation of glutamine from glutamate. As a consequence, there is elevation in serum-glutamine and depletion in urinary urea. A comparison of clinical parameters and NMR results demonstrates that while no single clinical parameter predicts the outcome of patients, the finding of serum-glutamine levels in excess of 30 mg/dl in patients with FHF predicts un unfavorable outcome, and should warrant an aggressive resort to more advanced therapy for FHF in the form of artificial liver support or liver transplantation. Further studies are in progress to define the precise serum- glutamine levels in patients with FHF which could definitely predict an unfavorable therapeutic outcome.