Assessment of Fulminant Hepatic Failure and Liver Grafts by 1H NMR Spectroscopy of Serum

P. Tripathi[1], A. Gupta[1], R. Roy[1], S. K. Mandal[1], R. Saxena[2], S. K. Yachha[3], and C. L. Khetrapal[1]

[1]Centre of Biomedical Magnetic Resonance, Centre of Biomedical Magnetic Resonance, Lucknow, Uttarpradesh, India, [2]Department of Surgical Gastroenterology, Sanjay Ghandhi Post-graduate Institute of Medical Sciences, Lucknow, Uttarpradesh, India, [3]Department of Gastroenterology, Sanjay Ghandhi Post-graduate Institute of Medical Sciences, Lucknow, Uttarpradesh, India

INTRODUCTION:

The mortality of Fulminant Hepatic Failure (FHF) in patients is very high and liver transplant is the most effective treatment for FHF. In routine clinical practice, the functional status of liver in FHF and transplanted liver grafts are being assessed by a battery of biochemical parameters such as prolonged prothrombin time, low serum glucose levels, hyperbilirubinemia, grossly elevated values of alanylaminotransferase and aspartylaminotransferase, blood urea, international normalized ratio (INR) and colour doppler imaging[1]. In patients of FHF, periodic assessment of therapeutic outcome is very essential in deciding whether a particular patient requires liver transplantation at an initial stage or not, followed by monitoring the success of the liver grafts. Dysfunction of the liver and its failure results in multiple metabolic abnormalities with special reference to amino acid metabolism and protein synthesis[2]. Along with other clinical parameters ^{1}H NMR spectroscopy can also be successfully used to demonstrate the early detection of end stage liver disease (FHF) and graft dysfunction[3, 4]. Our present study focuses on the ^{1}H NMR investigation of serum samples in FHF and liver graft patients in order to reveal important amino acids and other metabolites, if any, as clinical biomarkers, which may accomplish snap shots of hepatocyte functions in FHF and liver graft dysfunction aimed at deciding appropriate therapeutic intervention in patient management.

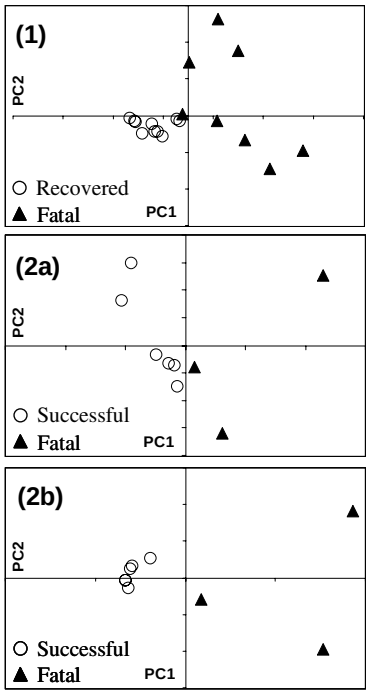
MATERIALS AND METHODS:

This study includes eighteen FHF [fatal (n=8), recovered (n=10)] and nine liver transplant [fatal (n=3), successful (n=6)] cases. In FHF patients, blood samples were collected at the time of admission and in liver grafts before transplant followed by post transplantation every 24 hours till the patients were discharged from the hospital or till patient's death. ^{1}H NMR experiments on the serum samples were performed on a Bruker Avance 400 MHz Spectrometer at 25ºC. Serum (0.5 ml) was taken in 5-mm NMR tubes and a sealed co-axial capillary tube containing 35 µl of 0.375% sodium salt of trimethylsilylpropionicacid-d_4 (TSP) in deuterium oxide (D_2O) was inserted into the NMR tube containing the serum samples for recording the spectra. TSP served as chemical shift reference as well as the standard signal for quantitative estimation of the metabolites. The spectra were recorded using one pulse sequence with suppression of water signal by presaturation and Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence. The semi-quantitative measurements of the metabolites were performed using the CPMG experiment. The statistical significance between the groups was determined by Mann-Whitney U-test. The data were further subjected to principal component analysis (PCA).

RESULTS:

Concentrations of alanine, lysine, glutamine, tyrosine, histidine and phenylalanine in fatal FHF cases were significantly higher (p=0.04, 0.04, 0.001, 0.001, 0.004 and 0.02 respectively) compared to the recovered cases. However in multivariate PCA performed with all the metabolites and clinical variables; the best possible group separations were observed with combination of lysine, glutamine, tyrosine and histidine [Fig (1)].

On the final day of fatal liver grafts (before the patient died) lactate, alanine, lysine, glutamine, methionine, citrate, tyrosine, histidine and phenylalanine were found to be significantly higher (p=0.02, 0.02, 0.01, 0.02, 0.01, 0.05, 0.02, 0.02 and 0.02 respectively) when compared to the successful cases (on the day patient discharged from the hospital). The PCA were performed on the metabolites second day after transplant for early assessment of the graft considering all the metabolites and clinical variables but the best possible group separations were observed with combination of metabolites viz. lysine, glutamine, tyrosine and histidine between fatal and successful cases [Fig (2a)], whereas the clinical variables didn't provide appropriate separation in PCA on second day. In the final day the combination of similar metabolites provided correct classification [Fig (2b)]. On the final day correct classification of groups were also obtained using clinical variables. It was observed that lysine, glutamine, tyrosine and histidine amino acids are common in both FHF and liver grafts to correctly classify the patient groups.



DISCUSSION:

It is a well-known fact that during hepatic failure in plasma, level of branched chain amino acids (BCAA) decrease and the aromatic amino acids (AAA) increase. These changes in plasma were thought to be caused by increased BCAA catabolism in muscle and decreased AAA breakdown in the failing liver. The increase in plasma AAA in combination with increased blood brain permeability for neutral amino acids has been suggested to contribute to an increased influx of AAA in the brain, as they compete for the same transporter (large neutral amino acid transporter). This leads to imbalances in neurotransmitter synthesis and accumulation of false neurotransmitters, such as octapamine in the brain, which may contribute to liver failure[2]. An increase in circulating amino acids is reflective of acute hepatocyte dysfunction[3]. The rise in glutamine takes place in hepatic failure due to the impairment in urea cycle as reported earlier[1,3,4]. Even though the quantitation of BCAA has not been carried out due to overlapping of the signals, but the results obtained using univariate analysis, showed increase in concentration of several amino-acids both in non-survived FHF and liver grafts. The results are clearly suggestive of complex physiological abnormalities in hepatocytes. The ^{1}H NMR analysis wih PCA and other clinical parameters may be very helpful in deciphering the status of FHF patients and liver grafts at an early stage.

REFERENCES:

1. Singh HK etal. A new dimension of ^{1}H-NMR spectroscopy in assessment of liver graft dysfunction. NMR Biomed. 2003; **16**: 185-188.
2. Dejong CH etal. Aromatic amino acid metabolism during liver failure. J. Nutr. 2007; **137**: 1579S-1585S.
3. Serkova NJ etal. Early detection of graft failure using the blood metabolic profile of a liver recipient. Transplantation 2007; **83**: 517-52.
4. Saxena V etal. ^{1}H NMR spectroscopy for the prediction of therapeutic outcome in patients with fulminant hepatic failure. NMR Biomed. 2006; **19**: 521-526.