Potential of in-vitro Proton MRS of Seminal Plasma in the Diagnosis of Male Infertility Diseases

P. K. Chaturvedi[1], U. Sharma[2], A. Kumar[1], N. R. Jagannathan[2]

[1]Reproductive Biology, All India Institute of Medical Sciences, New Delhi, Delhi, India, [2]Dept. of NMR, All India Institute of Medical Sciences, New Delhi, Delhi, India

INTRODUCTION

Male infertility disease, oligoasthenoteratospermia is characterized by low sperm count, morphology and motility defect. Astheno-teratozoospermia sperm have both motility and morphology defect while in teratozoospermia only morphological defect was observed. Hamamah et al. reported that peak area ratios for Cho/Cit as well as Cho/Lac can be used to differentiate patients of spermatogenic failure and obstructive azoospermia using ^{1}H MRS (1). Recently, we reported that the absolute concentration of metabolites of seminal plasma could be used to differentiate between the subjects having partial obstructive azoospermia due to injection of contraceptive, Reversible Inhibition of Sperm Under Guidance (RISUG) in the lumen of vas deferens and normal controls (2). The objective of the present study is to determine the concentration of biochemicals of human seminal plasma using in-vitro ^{1}H MRS and to find a suitable biochemical marker for diagnosis of various male infertility diseases.

PATIENTS AND METHODS

Semen samples were collected by masturbation from healthy volunteers and subjects presenting with OAT, AT and T at the infertility clinic of our Institute. Liquefaction of each sample was carried out at 37^0C for 20 min to reduce viscosity. An aliquot of 0.5 ml was removed to determine semen parameters using standard WHO procedures. The remaining semen sample was centrifuged at 1000 g for 15 min for removal of cells and spermatozoa. The supernatant was separated, 45 µl of supernatant was diluted in order to further reduce the viscosity of the sample and for field/frequency lock with 555 µl of D_2O. Sodium trimethylsilyl-[2,2,3,3-$^2H_4$] -1- propionate (TSP) was added as a chemical shift reference (δ 0.0) and internal quantitation standard. Proton NMR spectra were acquired at a frequency of 400.13 MHz using Bruker DRX-400 FT-NMR spectrometer. Typical parameters for one-dimensional proton NMR experiments were: pulse width 90^0, number of data points 32 K, spectral width 5000 Hz, number of scans 64-128 and relaxation delay of 14 sec. Two dimensional (2D) total correlation spectroscopy (TOCSY) and double quantum filtered correlation spectroscopy (DQF COSY) of the samples were carried out using standard software package. Concentration of the various metabolites in the seminal plasma ejaculates of patients with OAT, AT, T and controls subjects were expressed as mean values ± SD. Data obtained from patients was compared with that of controls as well as among different patient groups using the Student's t-test with the level of significance set at $P<0.05$.

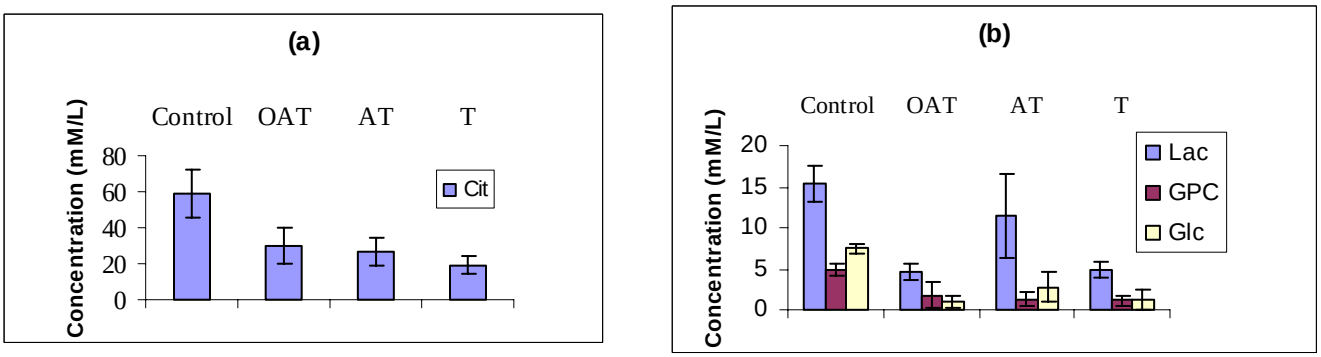

Fig. 1 (a&b) Comparison of the concentration of metabolites in seminal plasma of control OAT, AT and T group of patients.

RESULTS AND DISCUSSION

In this study we have evaluated the potential of proton NMR spectroscopy in estimating the concentration of four prominent metabolites of human seminal plasma and their potential as markers in distinguishing OAT, AT and T group of patients. Assignment of the various metabolites of the seminal plasma was carried out using 2D NMR methods (2). The concentration of metabolites like Lac, Glc, glycerophosphocholine (GPC) and Cit were observed to be statistically significantly lower in the entire patient groups compared to the control group implying that these biochemicals may be used as markers of infertility. Within the patient groups, significantly higher concentrations of Lac and Glc was observed for the AT group compared to both the OAT and T groups. However, there is no significant difference in the concentration of Cit between the patients of OAT and AT groups (Fig. 1a). The concentration of Cit is significantly reduced in T group compared to both OAT and AT group of patients, which may be used to discriminate between different groups of patients. GPC is the marker for epididymal function and Cit is considered as marker of prostatic function. Variation in the levels of these metabolites in these groups of patients may also be useful in assessing the functioning of accessory glands. Hamamah et al. documented that peak area ratios for Cho/Cit as well as Cho/Lac can be used to differentiate patients of spermatogenic failure and obstructive azoospermia using ^{1}H MRS (1). Results of the present study demonstrate that analysis of seminal plasma using simple 1D proton MRS may be used as a technically simple and economical tool for providing additional diagnostic information in distinguishing male infertility diseases at the biochemical level.

REFERENCES

1. Hamamah S, Seguin F, Barthelemy C et al. J Reproduction & Fertility 1993; 97: 51-55.

2. Sharma U, Chaudhury K, Jagannathan N R et al. Reproduction 2001; 122: 431-436.