(1)H-HRMAS for quantitative measurement of choline concentration in amniotic fluid as a marker of fetal lung maturity: inter- and intra-observer reproducibility study

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Introduction: Respiratory distress syndrome is a significant cause of neonatal morbidity and mortality related to insufficient surfactant production at birth. Diagnosis currently requires amniocentesis, an invasive procedure, to measure surfactant:albumin ratio. Choline (surfactant component) and other metabolites may be potential noninvasive markers of fetal lung maturity detectable by MR spectroscopy. To determine the degree of change in concentration measurements that can be measured by high resolution magic angle spinning (HR-MAS) spectroscopy, we studied the intra- and inter-observer reproducibility of metabolite concentration measurements related to a semi-automated peak fitting process

Methods: Ex vivo high resolution spectroscopy was performed at 11.7T (500 MHz for ^{1}H) using a Varian INOVA spectrometer equipped with a 4 mm gHX nanoprobe with HR-MAS capabilities. Twenty-three amniotic fluid samples obtained for evaluation of fetal lung maturity were analyzed quantitatively using trimethylsilylpropionic acid (TSP) as a reference compound. Four blinded readers independently processed the 23 randomized spectra offline using ACD/Labs 1D NMR processor (ACD/Labs, Toronto). Following phasing and frequency shifting, peaks were quantified using a semi-automated Lorentzian-Gaussian peak fitting algorithm and peak areas exported to an Excel spreadsheet. Each reader tabulated 8 metabolite peak areas (TSP, lactate doublet, alanine, citrate, creatinine, choline, lactate quartet, glucose) twice after a wash-out period. Concentrations were calculated based on the mass of TSP added to the sample. Intra- and inter-rater reproducibility was determined by Pearson correlation coefficients and intra-class correlation (ICC) coefficients for each metabolite concentration.

Results: A sample spectrum is shown in Fig 1. There was excellent inter and intra-observer reproducibility for measurements. Results of inter-observer variability based on intra-class correlation (ICC) coefficients are given in Table 1. ICC ranged from 0.654 to 0.995. A high correlation of 0.973 was seen for choline in particular, a major component of surfactant. Excellent intra-reader reproducibility was also seen. Fig 2 shows the results for choline with intra-observer correlation coefficients of 0.95 to 0.99 for Readers 1 to 4. In general, Pearson correlation coefficients were all higher than 0.65 except for alanine for one reader who fit different numbers of peaks (1 versus 3) between the first and second analyses, thus explaining the increased variability. Results are summarized in Table 2 below.

Conclusion: Quantification of choline and other metabolite concentrations in amniotic fluid by high resolution MR spectroscopy can be performed with high inter- and intra-observer reproducibility. Reproducible choline concentration measurements are a critical first step for establishing choline as a potential biomarker of fetal lung maturity.

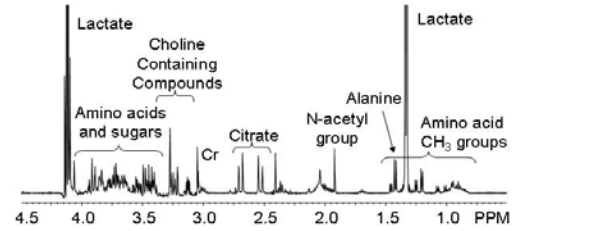

Fig. 1 Sample high resolution spectrum from 3rd trimester amniotic fluid sample.

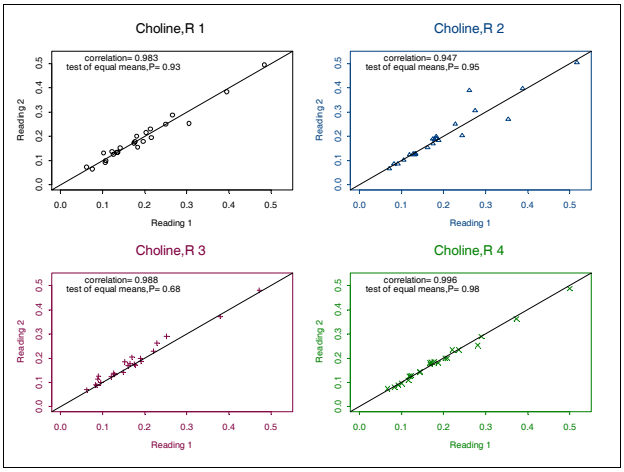

Fig 2. Graphs show excellent intra-observer correlation for choline concentration measurements using ex vivo high resolution MR spectroscopy at 11.7T. R1 to R4 refers to readers 1 to 4 who independently analyzed 23 spectra in a blinded fashion. Reading 1 and Reading 2 were performed at separate sittings after a washout period to avoid recall bias

TABLE 1: Inter-observer reproducibility based on *intra-class correlation (ICC) coefficients among 4 blinded readers

Metabolite	ICC*
Lactate Doublet	0.995
Alanine	0.654
Citrate	0.923
Creatinine	0.665
Choline	**0.973**
Lactate Quartet	0.969
Glucose	0.875

TABLE 2: Correlation coefficients showing high intra-observer reproducibility for measurement of choline and other metabolite concentrations.

Metabolite	Reader 1	Reader 2	Reader 3	Reader 4
Lactate Doublet	0.991	0.999	0.996	0.997
Alanine	0.478*	0.912	0.716	0.949
Citrate	0.930	0.920	0.910	0.991
Creatinine	0.679	0.757	0.738	0.931
Choline	**0.983**	**0.947**	**0.988**	**0.996**
Lactate Quartet	0.990	0.925	0.991	0.980
Glucose	0.987	0.738	0.972	0.992

*Reader 1 fit one peak in the alanine range at first sitting and 3 peaks at second sitting accounting for the increased intra-rater variability for alanine.