Statistical Analysis of metabolites of histologically confirmed preinvasive and invasive cervical cancer tissue using 1H HR MAS

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Introduction: The development of invasive cervical cancer is preceded by a well-defined pre-invasive stage called cervical intraepithelial neoplasia (CIN). Elevated levels of choline have previously been identified in a number of tumours including cervical cancer [1] but whether these metabolites are elevated in CIN tissue is still unclear. The aim of this study is to determine whether significant increases in concentration of choline-containing metabolites such as phosphocholine (PC) and choline (Cho), Taurine (Tau), Alanine (Aln) and Creatine (Cr) are observed in histologically confirmed normal cervical tissue, CIN tissue and cancer tissue using ^{1}H MAS NMR.

Methods: ***Tissue Collection:*** All women were studied with their written informed consent and with the approval of the local ethics committee. Criteria for inclusion- Abnormal smears (all grades CIN or Cancer). Regions were biopsied at colposcopic examination or on visual inspection of the cervix by the gynecologist when invasive disease was clinically evident. Average tumour biopsy sample size ~30 mg, Average CIN tissue biopsy sample size ~10 mg.

MAS: Tissue samples were thawed, and washed with phosphate buffered saline solution (PBS) to remove excess blood, loaded into 40µl sample inserts, topped up with D2O and then placed inside 4mm ZrO rotors. Nuclear Magnetic Resonance (NMR) measurements were performed on a Bruker Avance 11.74T spectrometer. All spectra were acquired with spin rate of 3kHz, number of scans 512 and temp 4°C. 1H MAS data were acquired using a CPMG sequence (TE=134 ms, TR=4.8s), expt time 41 mins, internal chemical shift ref-creatine 3.03 ppm. Peak assignments were based on chemical shift. Peak areas were measured using the AMARES algorithm included in the jMRUI software package [2]. Concentrations from ^{1}H spectra were obtained using the peak area of a reference compound in a separate measurement (50 µ l of 9.64 mM 3-(Trimethylsilyl)- Propionic acid-D4, sodium salt (TSP)), and corrected for the weight of each sample. All calculations are based on the assumption that the total reference volume is detected by the coil and gives rise to the area under the peak. Metabolite concentrations of biopsy tissue were differentiated and grouped with the aid of histology. Statistical analysis was performed using parametric statistical test ANOVA data analysis with SPSS for Windows and *p*- value of less than 0.05 was chosen as the criterion for statistical significance. The concentrations of Alanine (Aln), Choline (Cho), Creatine (Cr), Phosphocholine (PCh) and Taurine (Tau) were compared between preinvasive and invasive carcinoma NMR data from CPMG experiments.

Results:

Patient category on diagnostic biopsy	group	Histology of biopsy studied by MRS	Aln	Cr	Cho	PCh	Tau
CIN	1	normal N=13	0.58 ± 0.28	1.02 ± 0.90	0.48 ± 0.26	1.19 ± 0.44	2.11 ± 2.74
	2	CIN N=15	0.41 ± 0.24	0.64 ± 050	0.54 ± 0.39	1.31 ± 1.77	1.46 ± 1.00
Cancer	3	normal N=5	0.50 ± 0.32	0.68 ± 0.53	0.39 ± 0.25	0.95 ± 0.59	1.33 ± 0.89
	4	CIN N=3	0.66 ± 0.09	1.27 ± 0.70	1.72 ± 1.49	1.90 ± 1.68	1.86 ± 1.53
	5	cancer N=6	1.12 ± 0.60	0.84 ± 0.63	1.41 ± 1.12	2.11± 1.14	3.38 ± 3.79

Table 1 Metabolite concentrations (µmol/g wet wt) (mean ± sd) obtained from of patients with diagnostic biopsies positive for CIN or cancer ^{1}H MAS spectra that have been histologically analysed

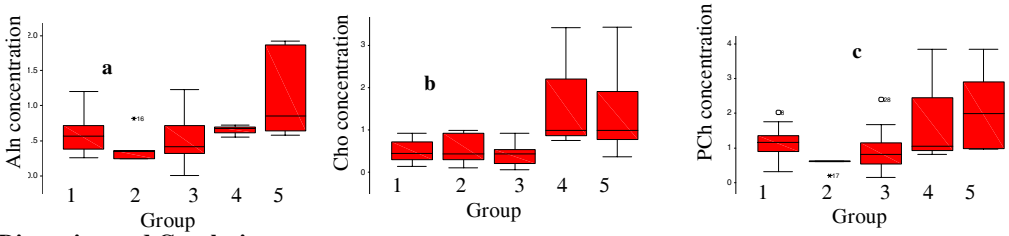

Figure 1 Concentration (µmol/g wet wt) of (a) Aln (b) Cho and (c) PCh in all five groups. The mean value of Cho appears to be significantly different in all groups..

Discussion and Conclusion

One way ANOVA showed significant differences in Aln (p=0.008) and Cho (p=0.001) between the CIN and tumour patient categories but not for any other metabolites. It was surprising to find that there were no differences in metabolite concentrations between CIN tissue and cancer tissue from cancer patients but that there was a difference between CIN tissue from CIN patients and cancer from cancer patients: Aln (t-test p=0.007), Cho (t-test p=0.003), PCh (t-test p=0.006) were all increased in the cancer tissues while increases in Tau (t-test p= 0.056) also approached significance. These results suggest that there may be a difference between the CIN depending on whether it came from the CIN or tumour patients. In fact an increased concentration of Cho (t-test p=0.002) was observed in the CIN tissue from the cancer patients. There were no differences in measured metabolite concentrations between any other groups.

In conclusion, these results suggest that CIN tissue show a difference in metabolite concentrations depending on whether the biopsy came from CIN or tumour patient.

References [1] deSouza, NM *et al*. NMR in Biomedicine, 2004;17:144-153. [2] L Vanhamme JMR **129**:35(1997)

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