HR-MAS Spectroscopic Analysis of Biopsies of Thyroid Tumors; a Pilot Study

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INTRODUCTION:

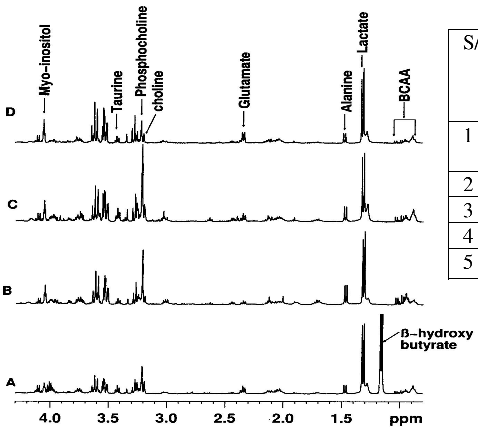
The thyroid nodules are most common thyroid disorders and are frequently benign in nature and their incidence increases with advancing age. Thyroid cancer is comparatively rare with an incidence of approximately four per 100 thousands individuals per year. In India the incidence of thyroid cancer in endemic goiter area is about 1.6 per lakh per year. The differential diagnosis of benign and malignant tumors is not straightforward even by the use of battery of techniques like thyroid tumor scanning, ultrasonography, fluorescent, therefore histopathological examination is a gold standard technique for differential diagnosis in biopsied tissues or after the excision of tumor. Few studies in magnetic resonance e.g. measurement of relaxation times for nodular and extra nodular tissues,[(1)] evaluation of Magnetization transfer rate constant for thyroid tissues which provided *in vivo* discrimination between follicular cancer and follicular adenoma.[(2)] Proton MRS has also been utilized for the evaluation of malignant thyroid tumors larger than 1cm^3, the spectra of malignant tumors differ from that of normal thyroid tissues[(3)]. With these objectives ^{1}H-HRMAS NMR studies have been performed on thyroid tissues of tumor patients in order to differentiate tumor type and to correlate with subsequent histopathological examination.

MATERIALS AND METHODS:

Tissues sampling (n=48) were obtained from the patients (n=16) undergoing surgery for thyroid disorders (nodules or cancers). All the samples were immediately snap frozen in liquid nitrogen (at -80^{0}C) stored in liquid nitrogen until NMR experiments were performed. Prior to NMR analysis the tissues were thawed, and 30-35 mg of wet weight thyroid tissues were dissected from the sample for HR-MAS analysis. The HR-MAS experiments were performed on a Bruker Avance 400 MHz FT NMR spectrometer equipped with ^{1}H-^{13}C MAS Probe with gradient aligned with the magic angle axis. All samples were then spun at 4 KHz in order to keep the rotation side bands out of the acquired window and all the experiments were performed at 25^{0}C. One Dimensional ^{1}H NMR experiments were performed using NOESY Pulse sequence with water presaturation during relaxation delay 2 sec. One dimensional CPMG experiments were also recorded for all the samples. The tissue samples after the NMR measurement over after 29 minutes, the samples were immediately fixed in formalin for histopathological studies. In few samples 2-D COSY and 2-D ^{1}H-^{13}C HSQC experiments were acquired exclusively for resonant assignment purposes. The statistical analysis was done by the SPSS version 11.5.

RESULTS:

The typical ^{1}H NMR spectra of the thyroid tissues sampled from the core of the tumors from 4 different diseases (Hashimoto's thyroidites.,Follicular carcinoma, Follicular adenoma, Colloid goiter) histologically proven malignant and non malignant tissues are shown in Fig. 1. The spectra comprises of large number of resonances associated with lipid signals overlapped with small molecular weight metabolites in the high field region. The metabolites identified in the NMR spectra of the samples consisted of lipids, branched Chain amino acids (leucine, isoleucine, valine), lactate, threonine, alanine, lysine, glutamine, glutamate, myo-inositol, α-glucose, β-glucose, choline, phosphocholine, GP-choline, phenylalanine, asparagine, tyrosine,taurine and fatty acids. The confirmations of resonances of all these metabolites were further carried out by the combined use of 2-D COSY and HSQC Spectra. In malignant case Glutamate/alanine, Choline/alanine, Lactate/alanine ratios were found to be significantly reduced which is shown in table 1.The tissue specimen (n=6) from Hashimoto's thyroidites showed the presence of additional metabolite,viz. β-hydroxybutyrate which was not observed in other thyroid tumor tissue type .



S/No.	Metabolites	Malignant (n=4)	Non-Malignant (n=12)	P-values
1	Glutamate/alanine	1.19± 0.62	1.96± 0.68	0.01*
2	Choline/alanine	0.19± 0.14	0.34 ± 0.18	0.02*
3	Lactate/alanine	0.63 ± 0.36	1.29 ± 0.83	0.007*
4	Phosphocholine/alanine	1.20 ± 0.61	0.88 ± 0.39	0.20
5	Myo-inositol/alanine	7.52 ± 6.8	5.55 ± 2.93	0.45

Table.1

Figure 1. Typical CPMG ^{1}H NMR spectrum of Thyroid Tissue. (A) Hashimoto's thyroidites, (B) Malignant(follicular carcinoma), (C) Non-malignant (follicular adenoma), (D) Non malignant (Colloid goiter), BCAA- Branched chain amino acid.

DISCUSSION:

The NMR spectral patterns in malignant tissues were quite different from the non-malignant ones. Statistically it was found that glumate/alanine ,choline/alanine ratios were significantly reduced in the case of malignant due to higher rate of anabolic reaction in malignant cases. Lactate/alanine ratio was found to be significantly reduced in the studied malignant cases, indicating non –hypoxic state of the tumor tissue and may probably have a low incidence of metastasis in later stage of the disease. Spectra of Hashimoto' s thyroidites is different from the other spectra of thyroid disorder due to presence of strong signal of β-hydroxybutyrate ,which can be used as fingerprint marker in this particular tumor type. We believe that the data present here has demonstrated a potential utility of HR-MAS tissue NMR analysis as a diagnostic tool in synergism with histopathological examination. Further studies are under progress involving a larger sample size in order to validate our findings.

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