의학박사 학위논문

Metabolomic profiles of thyroid nodules using nuclear magnetic resonance analysis of percutaneous fine needle aspiration specimens: Potential application for the diagnosis of papillary thyroid carcinoma

갑상선 결절의 경피적 세침 흡인물에 대한 핵자기 공명 대사체학적 성분 분석: 갑상선 유두암 진단 방법으로서의 가능성 평가

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갑상선 결절의 경피적 세침 흡인물에 대한 핵 자기 공명 대사체학적 성분 분석: 갑상선 유두암 진단 방법으로서의 가능성 평가

Metabolomic profiles of thyroid nodules using nuclear magnetic resonance analysis of percutaneous fine needle aspiration specimens: Potential application for the diagnosis of papillary thyroid carcinoma

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ABSTRACT

Introduction: Cytopathologic analysis of fine needle aspiration biopsy (FNAB) specimens is the standard diagnostic test for thyroid nodules. However, its accuracy and adequacy has been reported to be limited for the diagnosis of thyroid nodules. The purpose of this study is to present the differences in the metabolomic profiles of malignant (papillary thyroid carcinoma [PTC]) and benign thyroid nodules using metabolomic analysis (nuclear magnetic resonance [NMR]) of FNAB specimens of thyroid nodules and to evaluate the potential of the metabolomic approach (NMR) as an ancillary method for diagnosing PTC.

Methods: In total, 230 samples from patients with thyroid nodules were collected by ultrasonography-guided percutaneous FNAB; among them, 35 samples diagnosed as malignant (PTC) and 69 samples diagnosed as benign follicular nodules on conventional cytopathologic analysis were spectroscopically analyzed using 1.7 mm tube NMR. Metabolomic profiles were statistically generated based on the NMR results, and their correlation with conventional

cytopathologic diagnoses from the same samples were assessed to determine the feasibility of using these profiles as a diagnostic tool for thyroid nodules. Furthermore, we analyzed the correlation between the metabolomic profiles of surgically confirmed 25 PTC samples and clinicopathologic factors such as tumor multiplicity, T stage, N stage, and BRAF mutation status.

Results: Benign nodules and PTCs could be distinguished according to the different relative concentrations of several metabolites. The citrate (2.6 ppm), glutamate (2.0 ppm), and glutamine (2.1 ppm) levels in benign samples were greater than those in PTC samples and the lactate (1.3 ppm) and choline (3.2 ppm) levels in PTC samples were greater than those in benign samples. Receiver operating characteristic (ROC) curve analysis indicated that seven metabolites could serve as discriminators (area under the ROC curve value, 0.64–0.85), of which citrate was the most significant discriminator.

However, in PTC subgroup analysis, we did not observe a significant correlation between clinicopathologic factors and metabolomic profiles.

Conclusions: This study results indicate the potential of the metabolomic approach (NMR) as an ancillary method for diagnosing PTC using FNAB specimens of thyroid nodules.

Keywords: thyroid cancer, metabolomics, nuclear magentic resonance, fine needle aspiration biopsy

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LIST OF ABBREVIATIONS

AUC = area under the receiver operating characteristic curve

AUS = atypia of undetermined significance

FLUS = follicular lesion of undetermined significance

FNAB = fine needle aspiration biopsy

HRMAS = high-resolution magic-angle spinning

MRS = magnetic resonance spectroscopy

NMR = nuclear magnetic resonance

OPLS-DA = orthogonal projections to latent structure discriminant analysis

PCA = principal component analysis

PTC = papillary thyroid carcinoma

ROC = receiver operating characteristic

TSP = trimethylsilane propionic acid

USG = ultrasonography

INTRODUCTION

Thyroid nodules are very common, and are detected in 19–67% of the general population with ultrasonography (USG) evaluations (1–4).

Cytopathologic analysis of fine needle aspiration biopsy (FNAB) specimens is current gold standard in diagnosing thyroid nodules before surgery. Moreover, nine to 15% of all FNAB specimens from thyroid nodules are identified as malignant thyroid nodules (1–5). Although the mortality rate is low among such cases–particularly in well differentiated thyroid cancers that encompass the majority of thyroid cancers including papillary thyroid carcinoma–the recurrence rate is 20–30% (6, 7). These high recurrence rates correlate with patients' morbidity.

However, cytopathologic analysis of FNAB specimens of thyroid nodules shows high rates of inconclusive results such as nondiagnostic or unsatisfactory (Bethesda category I), atypia of undetermined significance (AUS), or follicular lesion of undetermined significance (FLUS; Bethesda category III) (8–12). Up to 40% of cytopathologic examinations of FNAB specimens from thyroid nodules yield inconclusive results (9–

12). Currently, the Bethesda system recommends repeat FNAB for the diagnosis of initial inconclusive nodules. Nevertheless, up to 50% of these repeated examinations yield indeterminate or insufficient results (11-14). Moreover, repeated examinations are not only associated with psychological and physical stress, but also associated with increased medical expenses and wasting time to the patients and physicians .

There have been several studies which evaluated various methods other than conventional cytopathologic examinations, for the diagnosis of thyroid cancer. These studies included immunohistochemical, immunocytochemical, or genetic analysis, however all these results are still unsatisfactory (15-19).

Many recent studies have analyzed the metabolomic profiles of thyroid nodules to assess the feasibility of the metabolomic approach in the diagnosis of thyroid cancer (20-25). However, all these studies used postoperative surgical specimens and none of them showed a correlation between metabolomic profiles and clinicopathologic factors of thyroid cancer. In the present study, we analyzed metabolomic spectra of thyroid nodules obtained through percutaneous FNAB preoperatively using nuclear magnetic resonance (NMR) to demonstrate the

potential of the metabolomic approach in the diagnosis of thyroid cancer (papillary thyroid carcinoma [PTC]). In addition, we assessed the correlation between the metabolomic profiles of PTC and the prognostic factors such as tumor multiplicity, T stage, N stage, and BRAF mutation status.

MATERIALS AND METHODS

This study was approved by the institutional review board of Seoul National University Hospital. Informed consent was obtained from all patients.

1. Sample collection

From November 2012 to June 2013, 230 samples were collected from thyroid nodules via USG-guided FNAB of patients who visited Seoul National University Hospital for the diagnosis of thyroid nodules. During FNAB, a major proportion of the aspiration specimens was smeared on a slide for conventional cytologic analysis, whereas the remainder (20-40 μL) was collected in Eppendorf tubes. Immediately after collection, the tubes were kept in a dry-ice box and stored in a liquid nitrogen tank until metabolomic analysis was performed.

2. Nuclear magnetic resonance (NMR) spectroscopy data acquisition

Samples (20-40 μL) from thyroid nodules were thawed slowly in an ice box after which they were centrifuged at 13000 rpm. The supernatant was collected with a pipette and placed

into 1.7 mm SJ tubes with 0.25% trimethylsilane propionic acid (TSP) buffer in D_2O to make a final volume of 35 ul. The one-dimensional spectra of the thyroid samples were measured using an NMR spectrometer (BrukerBiospin, AVIII700, Billerica, MA, U.S.A.) equipped with a 1.7 mm PATXI probe, operating at a proton NMR frequency of 700.193 MHz.

The acquisition parameters were: pulse, CPMG; time domain size, 32,768; relaxation delay, 2 s; number of scans, 128; spectral width, 14,097 Hz; mixing time, 76 ms; and temperature, 25°C. The lactate signal ((δ = 1.342 ppm) was used as a reference value.

3. Data processing

All the time-domain NMR data underwent Fourier transformation, phase correction, and manual baseline correction. The resulting frequency-domain data were binned at a 0.0031 ppm interval to reduce the complexity of the NMR data for pattern recognition. The signals were normalized (area normalization) against the total integration values and 0.025% TSP buffer to exclude the effects of different volumes and NMR measurement variations, and then converted into an ASCII text

file. The regions corresponding to water (4.71–5.1 ppm) were removed from all the spectra. The binning, normalization, and conversion were performed using a Perl program written in-house.

4. Statistical analysis

The signals in specific bins which showed significant difference (P value < 0.05) in terms of the area normalization values between the benign and malignant (PTC) groups were determined using the unpaired Student t test. Thereafter, the metabolites were identified using Chenomx (Spectral database, Edmonton, Alberta, Canada) by fitting the experimental spectra (significant signals) to those in the database.

The resultant spectral data sets were then imported into the SIMCA-P version 11.0 program (Umetrics, Umeå, Sweden), and mean-centering with Pareto scaling for multivariate statistical analysis was performed. Furthermore, orthogonal projections to latent structures discriminant analysis (OPLS-DA) were performed with one predictive component and two orthogonal components.

Class discrimination models were created while ensuring that

the cross-validated predictability value did not significantly increase, in order to avoid over-fitting of the statistical model. Diagnostic performance was obtained by prediction of leave-*n* (one-third of the samples)-out samples on the basis of the distinction model constructed using the rest of the samples. An *a priori* cut-off value of 0.5 was used to evaluate the prediction results(26). The signals specific for each group were identified by performing the Wilcoxon rank-sum test on all the ppm variables using an in-house written R script (Q2 and R2). Eventually, the specific signals were compared to the metabolites identified using the Chenomx data base.

The performance of the prediction derived from OPLS modeling of the NMR spectra was evaluated by computing the area under the receiver operating characteristic (ROC) curve (AUC), using an open source ROC curve analysis tool for metabolomics data (ROC Curve Explorer & Tester, www.roccet.ca).

RESULTS

Cytopathologic analysis of the samples

Of the 230 collected samples, 53 were malignant thyroid nodules (consistent with PTC), 85 were benign nodules (consistent with benign follicular nodules), 22 were suspicious for malignancy, five were follicular neoplasm or suspicious for a follicular neoplasm, 39 were AUS, and 23 were nondiagnostic or unsatisfactory on conventional cytologic results. Three samples were discarded due to improper storage.

NMR metabolomic discrimination between benign and malignant (PTC) thyroid nodule aspiration specimens

Of the 53 malignant samples, 18 were excluded due to an insufficient sample amount (< 20 μL); thus, only 35 malignant (PTC) samples were used for metabolomic analysis. Of the 85 benign samples, 16 samples were excluded due to an insufficient sample amount (< 20 μL); thus, 69 benign samples were included for metabolomic analysis (Figure 1).

To test the feasibility of using NMR-based metabolomics for discriminating between benign and malignant (PTC) thyroid nodules, we eventually analyzed 104 samples. Principal

component analysis (PCA) was unable to yield a clear discrimination between the two sample types, and thus, supervised statistics were employed. Specifically OPLS-DA was performed to differentiate between benign nodules and malignant (PTC) nodules.

As indicated in Figure 2, the OPLS-DA score plot (created using one predictive component and two orthogonal components) shows a statistically significant discrimination between the benign nodules and malignant (PTC) nodules with a Q^2 value of 0.33 (R^2Y = 0.59). In the prediction validation study with leave-*n* (one-third of the samples)-out cross validation, the accuracy, sensitivity, and specificity for diagnosing PTC were 88.6%, 75%, and 95.6%, respectively.

According to the OPLS-DA loadings of the predictive latent variable, the relative amounts of citrate (2.6 ppm), glutamine (2.1 ppm), and glutamate (2.0 ppm) were greater in benign samples than in PTC samples whereas the relative amounts of lactate (1.3 ppm) and choline (3.2 ppm) were greater in PTC samples than in benign samples. Among the other metabolites, O-phosphocholine (3.2 ppm) and glycine (3.6 ppm) showed slightly higher relative amount in the PTC group as compared to

the benign group (Figure 3). These findings corresponded with the significant metabolites identified in the unpaired Student t test (Table 1).

In the ROC curve analysis, the malignancy grade (0 for benign sample and 1 for PTC samples) and the grades predicted by the OPLS-DA model were used. In the group discrimination analysis, the AUCs ranged from 0.64 (glycine) to 0.85 (citrate). Moreover, in multiple ROC analysis, we noted that all seven metabolites were useful for group discrimination, of which citrate was the most significant discriminator (Figure 4).

Correlation between metabolomic profiles of PTC and clinicopathologic factors

Of the 35 analyzable malignant samples, 25 nodules were surgically confirmed to be PTC; the pathologic staging was performed using surgical specimens. Of the 25 patients, 17 were female and eight were male, with a mean age of 51.4 ± 12.1 years. Fifteen tumors had more than one tumor in the ipsilateral or contralateral lobe of the thyroid gland. In T stage analysis, four patients had T1a and 21 patients had T3 stage

tumors. In N stage analysis, 13 patients had N0, 10 patients had N1a, and two patients had N1b stage tumors.

BRAF mutation analysis was performed in only 22 patients among the 25 surgically confirmed PTC patients. We noted that 18 patients had BRAF mutation-positive tumors and the remaining four patients had BRAF mutation-negative tumors.

In the correlation study with metabolomic profiles, no significant correlation between the clinicopathologic factors and metabolomic profiles was noted.

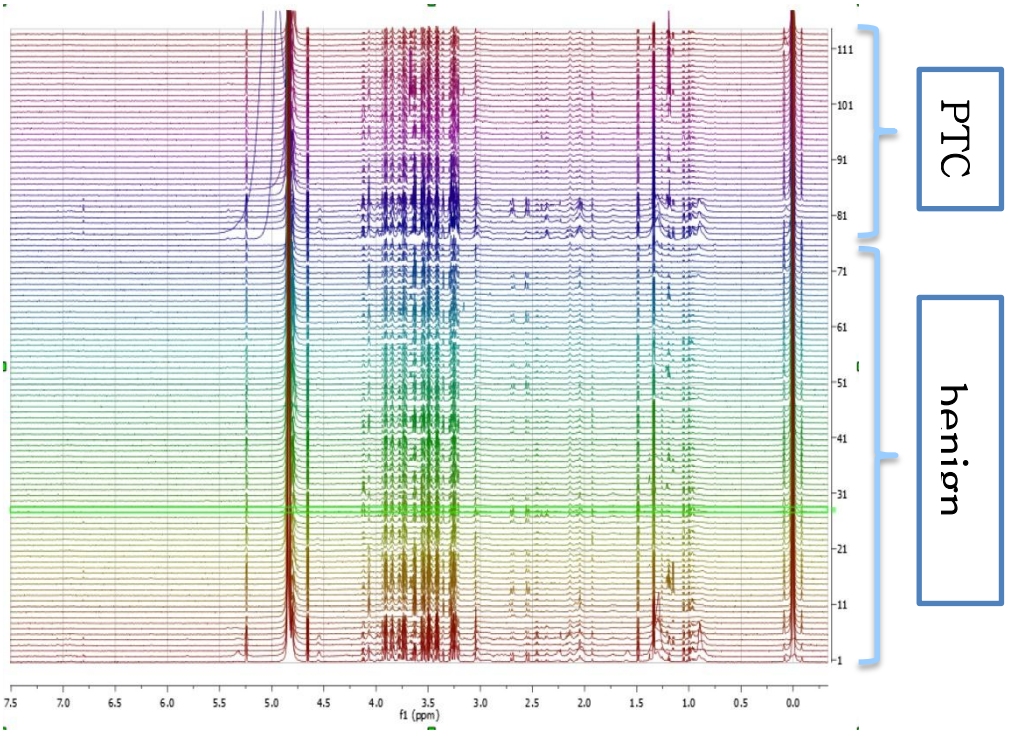


Figure 1. Metabolomic spectra of fine needle aspiration biopsy specimens of malignant (papillary thyroid carcinoma) and benign thyroid nodules

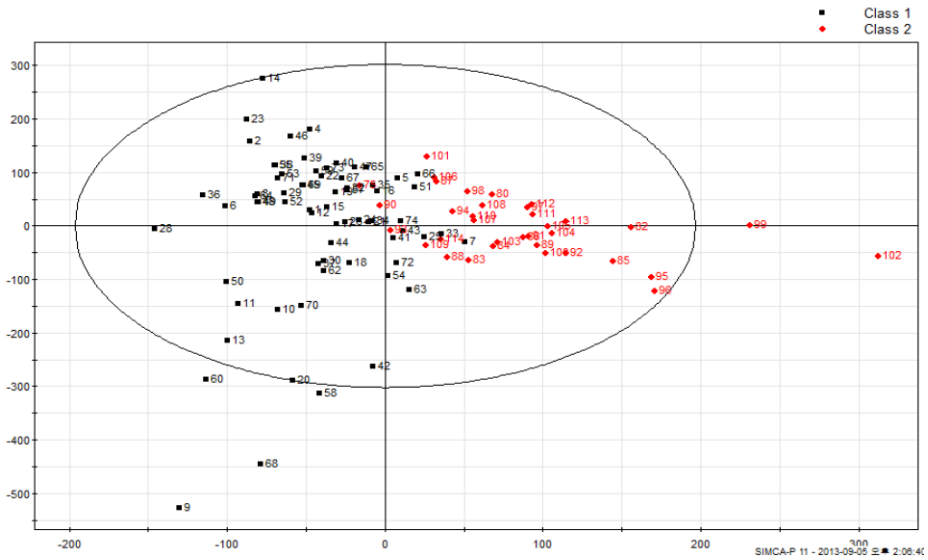
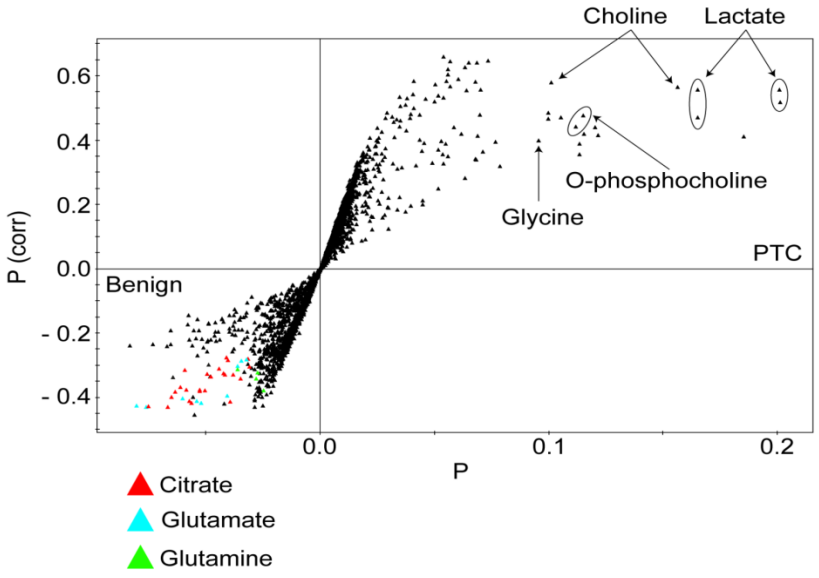
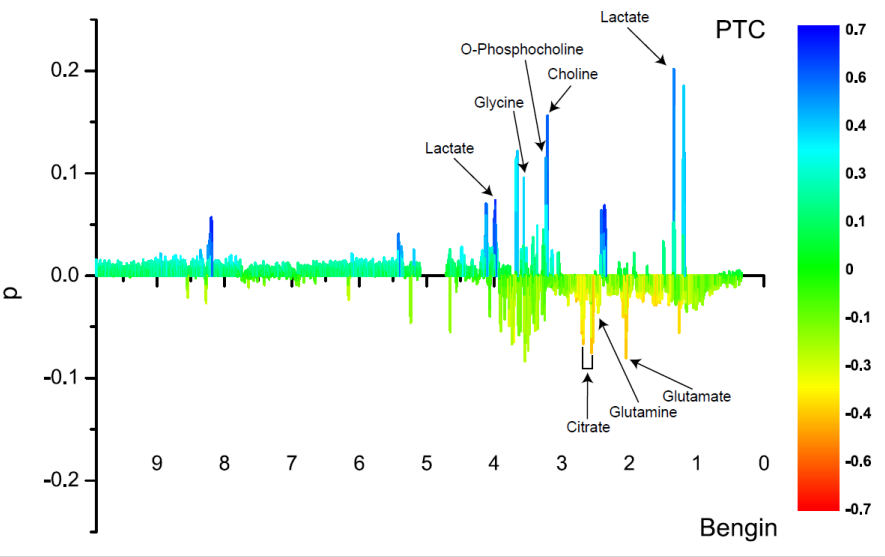


Figure 2. OPLS-DA score plot showing the discrimination between malignant (papillary thyroid carcinoma) and benign thyroid nodules. Benign group: class 1 (black dots), malignant group: class 2 (red dots)

(A)


Choline
Lactate
O-phosphocholine
Glycine
PTC
Benign
P (corr)
P
0.6
0.4
0.2
0.0
- 0.2
- 0.4
0.0
0.1
0.2
Citrate
Glutamate
Glutamine

(B)


Lactate
PTC
O-Phosphocholine
Choline
Glycine
Lactate
p
0.2
0.1
0.0
-0.1
-0.2
9
8
7
6
5
4
3
2
1
0
Glutamate
Glutamine
Citrate
Bengin
0.7
0.6
0.4
0.3
0.1
0
-0.1
-0.3
-0.4
-0.6
-0.7

(C)

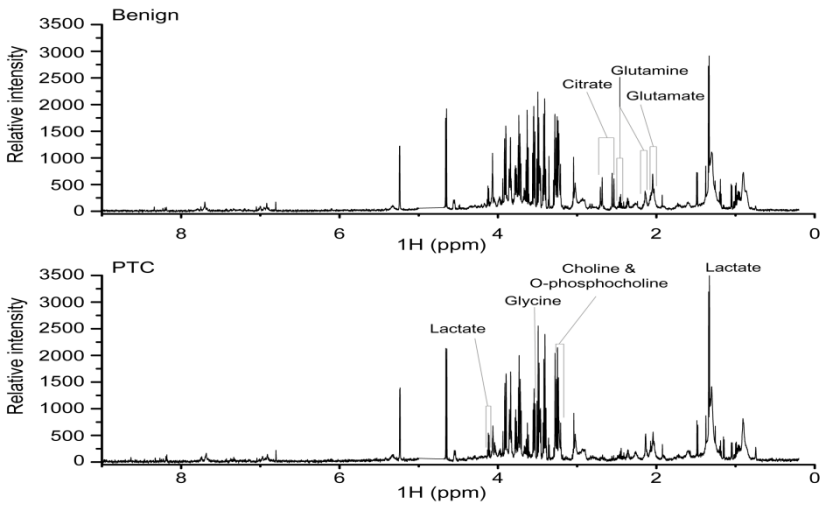


Figure 3. (A) OPLS-DA score plot showing the metabolites of papillary thyroid carcinoma (PTC) and benign groups for marker identification. (B) OPLS loadings plot (S-TOCSY) showing the model coefficients for each NMR variable. The signals are color coded according to their weights as a discriminator between benign and PTC groups. Metabolites that significantly discriminate the two groups were annotated on the model coefficient plot. (C) Representative spectra from the fine needle aspiration specimens of benign thyroid nodule and PTC. Metabolites that significantly discriminate the two groups were annotated on the spectra.

Table 1. Relative concentrations of metabolites in benign and papillary thyroid carcinoma samples.

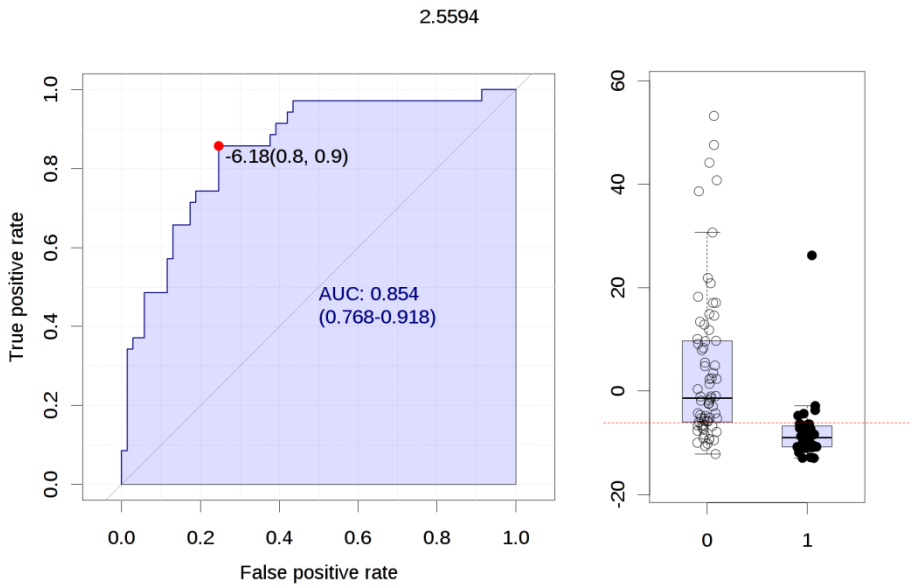
Metabolite	Area normalization value		
	Benign	PTC*	*P* value†
Citrate	2.45±1.37	1.33±0.49	0.004
Glutamate	6.45±1.58	5.50±0.81	0.003
Glutamine	1.82±0.52	1.59±0.43	0.01
Lactate	6.89±2.65	9.54±3.15	0.003
Choline	1.94±0.52	2.67±0.92	0.0008
O-phosphocholine	2.72±0.96	3.50±1.12	0.002
Glycine	0.63±0.31	0.83±0.41	0.005

Note – Unless otherwise specified, the data are the means ± standard deviations.

*PTC, papillary thyroid carcinoma

†*P* value for the comparison of means was calculated using the unpaired Student t test.

(A)



(B)

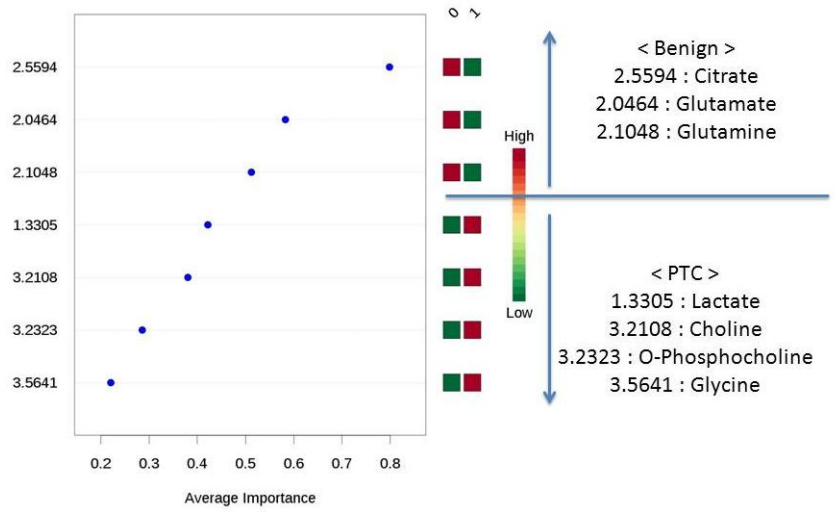


Figure 4. (A) The ROC curve of citrate showing the ability of citrate as a discriminator of a thyroid nodule. (B) Multiple ROC curve analysis showing that all the seven metabolites had

additive values in discriminating between benign thyroid nodules and papillary thyroid carcinomas (PTC). The single most important discriminator was citrate which was more abundant in benign thyroid nodules than in PTC.

DISCUSSION

In the present stud, we noted that the metabolomic profiles of FNAB specimens from thyroid nodules differed between malignant (PTC) and benign thyroid nodules. Compared to benign thyroid nodules, PTCs showed a higher relative concentration of lactate and choline and a lower relative concentration of citrate, glutamine, and glutamate. These results are generally consistent with the results of previous studies (22, 24, 25). The concentrations of lactate and choline have been frequently reported to be increased in malignant tumors (20−24, 27−29). Increased lactate levels could indicate an increase in the glycolytic flux due to hypoxia and ischemia in tumor tissues or could be a result of the so called Warburg effect (24, 25, 27−30). In addition, accelerated cancer cell metabolism has also been indicated to produce more waste products, such as lactate, for extrusion and neutralization (24, 30). Furthermore, since choline usually forms the phospholipids of cell membranes, malignant tissue cells that have increased multiplication and proliferation can also exhibit increased choline contents(20−23).

However, the presence of lower relative concentrations of citrate, glutamine, and glutamate in PTC samples (or higher relative concentrations in benign samples) was the unique finding of the present study. Since proliferating cells exhibit aerobic glycolysis and convert glucose to lactate (lactate fermentation, or the Warburg effect) at high levels, pyruvate-derived citrate synthesis in mitochondria may be reduced in these cells (31, 32). Moreover, several recent studies have reported that ATP citrate lyase, which uses citrate to synthesize acetyl CoA in a lipogenesis pathway for cell proliferation, is upregulated in some human cancers such as lung, colorectal, and ovarian cancers, and also that its inhibition suppresses the proliferation of certain types of tumor cells (33, 34). As noted in these various cancers, the proliferating thyroid cancer cells may also use citrate for lipogenesis and may have a lower concentration of citrate compared to that in benign cells. Recent publications on cell metabolism emphasize that the proliferating cells exposed to hypoxic conditions rely almost exclusively on the reductive carboxylation of glutamine-glutamate-derived α-ketoglutarate for *de novo* lipogenesis (31, 32). Furthermore, renal cell lines deficient in the von

Hippel-Lindau tumor suppressor protein preferentially utilize this type of reductive glutamine metabolism even in the normoxic state (32). Therefore, several research groups are currently studying the possibility of developing a glutaminase inhibitor as an anti-cancer drug. These observations from earlier studies could possibly explain why proliferating cells or cancer cells might have lower glutamine or glutamate levels as compared to normal cells.

However, in the subgroup analysis with surgically confirmed PTC, we did not observe any significant correlation between metabolomic profiles and clinicopathologic factors such as tumor multiplicity, T stage, N stage, and presence of BRAF mutation.

Due to the limitations of the conventional cytopathologic analysis of FNAB specimens from thyroid nodules, several attempts have been made to find methods other than cytopathologic analysis to diagnose thyroid cancer (15-19). During the last decade, several researches also showed that the metabolic analysis of thyroid nodules could be useful in the diagnosis of thyroid cancer. Some studies using magnetic resonance spectroscopy (MRS) showed that thyroid cancer had

an increased choline peak or choline/creatinine ratio (20, 21, 23). However, the relatively small size of the thyroid gland and its anatomical adjacency to the airway as well as the long scanning time and expensive costs associated with MRS limit its utilization in the diagnosis of thyroid nodules.

Recently several studies using metabolomic analysis of thyroid nodule FNAB specimens with high-resolution magic-angle spinning (HRMAS) NMR have shown that malignant thyroid nodules had an increased amount of lactate and taurine and decreased amount of phosphocholine, myo-inositol, and scyllo-inositol (22, 24, 25); the results of the present study are consistent with these findings. However, all the previous studies used surgical specimens rather than the percutaneous aspiration technique. Moreover, no study has attempted to assess the correlation between metabolomic profiles and various prognostic factors of thyroid cancer. In the clinical setting, since the diagnosis of thyroid cancer should be performed prior to surgery using percutaneous FNAB of thyroid nodules to avoid an unnecessary operation, our study using thyroid nodule specimens obtained by percutaneous FNAB could have important meaning in terms of its clinical

applicability. Furthermore, considering that our study using percutaneous FNAB specimens showed similar results with previous studies using surgical specimens, the preoperative application of metabolomic analysis could be a reliable test for the characterization of the metabolomic profiles of thyroid nodules.

In addition, metabolomic analysis is convenient to use in the clinical setting as it requires only a very small amount of samples (approximately 20 μL), and unlike BRAF mutation analysis, it does not require consent for genetic analysis.

There're several limitations in this study. First, since the reference used for comparison was the results of conventional cytologic analysis of FNAB specimens from thyroid nodules, the actual pathologic diagnosis of some thyroid nodules could be differ from the cytologic results. Although most of the nodules indicating PTC on FNAB cytologic analysis were confirmed to be true PTC on the final pathologic report of thyroidectomy specimens, most of the nodules indicating benign follicular nodules on cytologic analysis did not undergo surgery. Therefore, the final pathologic analysis of the benign nodules could not be confirmed.

Second, metabolomic analysis requires only a small amount of sample (20–40 μL); it is unclear whether the amount of tissue assessed could represent the whole nodular tissue. However Jordan *et al.* reported that the findings of metabolic spectral analysis of tissue (10 mg) and FNAB sample (10 μL) did not significantly differ(22).

Third, many samples (34/138) in the present study were insufficient to analyze metabolic profiles. However, the amount of FNAB samples remaining after the collection for conventional cytologic analysis was used for metabolomic analysis, and as the sample quantity required for metabolomic analysis is minimal (more than 20 μL), it would not be a huge matter in clinical application.

Last, all the malignant samples were PTCs. Although the majority of thyroid cancers in general are PTCs, there are several other types of thyroid cancers as well, such as follicular thyroid carcinoma. However, as we could only collect PTCs as the malignant samples, we could not evaluate other types of malignant nodules.

Based on the results of the present study, we can believe that the metabolomic approach can be applied for diagnosing thyroid

nodules with indeterminate or insufficient cytologic results that have been very problematic in thyroid nodule management over the last several decades. However, further studies would be warranted to facilitate the clinical application of this metabolomic approach in diagnosing thyroid nodules.

In summary, the present study indicated the differences in the metabolomic profiles between benign thyroid nodules and PTC FNAB specimens using NMR. For thyroid samples obtained through USG-guided percutaneous FNAB, we noted that the metabolomic approach has potential for clinical applications in diagnosing PTC before surgical treatment.

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국문 초록

서론: 현재 갑상선 결절의 수술 전 진단법은 세침 흡인물에 대한 세포병리학적 분석이다. 그러나 이 방법은 현재까지 갑상선 결절의 진단에 있어 정확도와 시료의 적절성 (adequacy) 측면에서 제한점을 갖고 있다. 본 연구의 목적은 갑상선 암 (갑상선 유두암)과 양성 결절의 세침 흡인물에 대한 핵 자기 공명 (NMR) 대사체학적 분석을 통해 대사체 성분이 갑상선 유두암과 양성 결절 사이에 서로 다름을 보임으로서 갑상선 유두암의 수술 전 진단에 대사체학적 접근의 적용 가능성을 알아보고자 한다.

방법: 갑상선 결절을 가진 환자로부터 초음파 유도하 경피적 세침 흡인술로 총 230 개의 시료를 확보하였다. 그 중 세포병리학적 검사상 악성(갑상선 유두암)으로 진단된 35 개의 시료와 양성 여포상 결절로 진단된 69 개의 시료를 이용하여 1.7 mm tube NMR 분석을 시행하였다. 대사체 성분은 NMR 분석 결과를 바탕으로 통계적으로 분석하여 세포병리학적 검사 결과와 비교함으로써 갑상선 결절의 진단 방법으로서의 가능성을 평가하였다. 35 개의 갑상선 유두암 시료 중 수술을 통해 병리학적 병기가 확정된 25 개의 시료를 이용하여 종양의 다발성, T 병기(stage), N 병기, BRAF 유전자 변이 유

무와 같은 임상병리학적 요인들과 대사체학적 성분과의 상관 관계 역시 분석하였다.

결과: 갑상선 양성 결절과 갑상선 유두암은 몇 개 대사체군의 상대적인 농도차로 구분되었다. 구연산 (citrate, 2.6 ppm), 글루타메이트 (glutamate, 2.0 ppm), 글루타민 (glutamine, 2.1 ppm)은 갑상선 유두암보다 양성 결절에서 상대적으로 높은 농도를 보였고 젖산 (lactate, 1.3 ppm)과 콜린 (choline, 3.2 ppm)은 양성 결절보다 갑상선 유두암에서 상대적으로 높은 농도를 보였다. 수신자 조작 특성 (receiver operating characteristic) 곡선 분석에서 총 7 가지의 대사체가 통계적으로 유의하게 두 군을 구분하는 요소로 평가되었으며 구연산이 가장 중요한 요소로 평가되었다.

그러나 수술로 병리학적 병기가 확정된 갑상선 유두암 군을 이용한 임상병리학적 요인과 대사체학적 성분과의 상관 관계 분석에서는 유의한 상관 관계를 도출할 수 없었다.

결론: 본 실험 결과는 갑상선 유두암의 보조적 진단법으로서 갑상선 결절의 세침 흡인물을 이용한 NMR 대사체학적 분석 방법의 적용 가능성을 보여주었다.

주요어 : 갑상선 암, 대사체학, 핵 자기 공명, 세침 흡인
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