Serum-plasma matched metabolomics for comprehensive characterization of benign thyroid nodule and papillary thyroid carcinoma

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Metabolomics offers a noninvasive methodology to identify metabolic markers for pathogenesis and diagnosis of diseases. This work aimed to characterize circulating metabolic signatures of benign thyroid nodule (BTN) and papillary thyroid carcinoma (PTC) via serum-plasma matched metabolomics. A cohort of 1,540 serum-plasma matched samples and 114 tissues were obtained from healthy volunteers, BTN and PTC patients enrolled from 6 independent centers. Untargeted metabolomics was determined by liquid chromatography-quadrupole time-of-flight mass spectrometry and multivariate statistical analyses. The use of serum-plasma matched samples afforded a broad-scope detection of 1,570 metabolic features. Metabolic phenotypes revealed significant pattern differences for healthy versus BTN and healthy versus PTC. Perturbed metabolic pathways related mainly to amino acid and lipid metabolism. It is worth noting that, BTN and PTC showed no significant differences but rather overlap in circulating metabolic signatures, and this observation was replicated in all study centers. For differential diagnosis of healthy versus thyroid nodules (BTN + PTC), a panel of 6 metabolic markers, namely myo-inositol,
**Introduction**

Thyroid nodules are a common clinical manifestation of the head and neck, affecting about 50 to 60% of patients without obvious indication of disease.\(^1,2\) Most thyroid nodules are benign with only 5–10% being malignant.\(^3\) In the past decades, the incidence of thyroid nodules has had a dramatic global increase and epidemiological surveys indicate that thyroid cancer would surpass colorectal cancer as the fourth leading cancer by 2030 in the United States of America,\(^4\) owing to the introduction of new diagnostic techniques.\(^5\)

The gold standard for the diagnosis of thyroid nodules is ultrasound-guided fine-needle aspiration and cytological evaluation. This method is however unable to accurately differentiate between benign follicular adenomas and malignant follicular carcinomas or a variant of PTC, resulting in the diagnosis of these cases as “indeterminate”. Due to this challenge, complementary immunological and genetic tests are usually performed to aid in the diagnosis. Among the panel of biomarkers analyzed, determinations of BRAF or RAS mutations, RET/PTC or PAX8/PPAR translocations hold promise in the diagnosis of the “indeterminate” subtypes of thyroid cancers.\(^6\)

Basically, thyroid cancers can histologically be classified into papillary, follicular, poorly differentiated and anaplastic cancers. PTC alone accounts for about 80% of all thyroid cancers with a 10-year survival rate as high as 90% in the early stage.\(^7\) They are usually associated with indolent disease cause and mostly curable with a generally low mortality rate.\(^6\)

Metabolomics is simply defined as the comprehensive analysis of the metabolome of a biological system under defined conditions.\(^8,9\) Metabolome is the final downstream product of gene expression and changes in the proteome and transcriptome.\(^10,11\) It is thus, a snapshot of any biological system representing the comprehensive profile of biochemical pathways and functions.\(^8\) The use of metabolomics for the diagnosis and prognosis of diseases is currently receiving worldwide attention by the scientific community. With respect to thyroid nodules, several studies have reported the use of metabolomics to differentiate between benign and malignant tumors particularly PTC. However, these studies had the after limitations: small sample size, no multicenter validation, and the use of either plasma, serum or tissue samples.\(^12-17\)

In our study we used serum-plasma matched samples of a large cohort of patients with BTN and PTC as well as healthy persons with the after underlying aims: (1) to differentiate with the aid of metabolomics among the healthy individuals, benign thyroid nodule (BTN), and PTC and (2) to provide evidence from a metabolomic point of view for the diagnosis and management of persons with PTC.

**Material and Methods**

**Participants and study design**

The discovery phase of the study consisted of healthy volunteers and patients with BTN and PTC recruited from Center 1 (Jiangsu Province People’s Hospital, Nanjing, China). Patients enrolled from the other independent centers formed the external validation phase: Center 2 (Shanghai General Hospital, Shanghai, China), Center 3 (Yixing People’s Hospital, Yixing, China), Center 4 (Jiangsu Provincial Cancer Hospital, Nanjing, China) and Center 5 (Northern Jiangsu People’s Hospital, Yangzhou, China). Additional experiments were performed using tissue samples obtained from Center 6 (Nanjing Drum Tower Hospital, Jiangsu, China). All participants were enrolled from February 2014 to June 2017 and were between the ages of 11 and 83 years. Ultrasound-guided fine-needle aspiration was used for the determination of the tumor sizes. A biopsy of the fine-needle aspiration aspirate was used for the confirmation of either benign or malignant tumors by microscopic examination of the morphology of the cells upon staining.

The exclusion criterion included patients with comorbid conditions such as other forms of cancer, thyroid dysfunction (hyperthyroidism or hypothyroidism), immunodeficiency

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**What’s new?**

When thyroid nodules are classified “indeterminate,” is it better to wait and see, or take out the thyroid? Usually, doctors remove the thyroid, resulting in a lifetime of levothyroxine replacement, yet most often the nodules are not cancerous. Here, the authors investigated whether metabolic profile could give a more accurate prediction of whether a thyroid nodule is cancerous. They tested healthy patients, those with benign nodules, and those with papillary thyroid carcinoma. Healthy patients showed distinct differences from those with benign nodules and those with carcinomas, while significant overlap was observed between circulating metabolites from BTN and PTC patients.

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\(\alpha\)-N-phenylacetyl-L-glutamine, proline betaine, L-glutamic acid, LysoPC(18:0) and LysoPC(18:1) provided area under the curve of 97.68% in the discovery phase and predictive accuracies of 84.78–98.18% in the 4 validation centers. Taken together, serum-plasma matched metabolomics showed significant differences in circulating metabolites for healthy versus nodules but not for BTN versus PTC. Our results highlight the true metabolic nature of thyroid nodules, and potentially decrease overtreatment that exposes patients to unnecessary risks.
diseases and other diseases of the nervous system that potentially affect metabolism. Patients with histories of long-term drug use and those who had undergone any form of cancer therapy such as radiation, chemotherapy, and surgical operation three months prior to the enrollment were also excluded. Informed consent was obtained from all participants. The stages of PTC were determined according to the Tumor Node Metastasis Classification of Malignant Tumors, Union for International Cancer Control (6th edition). The study complied with the guidelines of the Helsinki Declaration and the Conference on Harmonization-Good Clinical Practices (ICH-GCP) and approved by all centers. Serum, plasma and tissue samples were immediately stored at −80 °C until metabolomics analysis. Detailed sample preparation methods are available in the Supporting Information material.

Metabolomics study
Chromatographic separations of processed serum and plasma samples were achieved on an ACQUITY UPLC HSST3 column (2.1 × 100 mm, 1.8 μm) using an Agilent 1,290 liquid chromatography (LC) infinity set-up (Agilent technologies, USA). The separated components were detected in an Agilent 6,545 quadrupole time-of-flight spectrometric (Q/TOF-MS) system in both positive and negative ion modes. Quality control samples (QC samples) were analyzed five times at the beginning of the run and injected once after every 16 injections of the random sequenced samples. The raw data obtained from the LC–MS run were transformed to the .mzdata format using MassHunter Workstation Software (Version B.06.00, Agilent Technologies). Data pretreatment including nonlinear retention time alignment, peak discrimination, filtering, alignment, matching, and identification were done using the XCMS package (http://metlin.scripps.edu/download/) in R-3.3.3. Differential metabolites were tentatively identified by database matching, i.e., Human Metabolome Database, MassHunter METLIN Metabolite PCDL (Agilent technologies, USA) and METLIN (http://metlin.scripps.edu). Some were unambiguously confirmed with available reference compounds. Genomes (KEGG) database and MetaboAnalyst (http://www.metaboanalyst.ca) were used to elucidate their related metabolic pathways.

Data analysis
All the pretreated data were normalized by LOESS before multivariate analyses. The Mann–Whitney–Wilcoxon test with false discovery rate correction was used to measure the significance of each metabolite. Orthogonal partial least-squared discriminant analysis (OPLS-DA) was conducted to identify the discrimination of variables. The models of OPLS-DA were sevenfold cross-validated and the quality was evaluated by the R²Y and Q² values. R²Y indicates the interpretation rate of model, while Q² indicates the prediction rate. Higher values of R²Y and Q² usually indicate that the model was reliable and highly predictive. Benjamini-Hochberg false discovery rate (FDR) procedure was employed for the multiple test adjustments. Adjusted p-values less than 0.05 were considered statistically significant. Differential metabolites were defined those with variable importance in the projection (VIP) >1.0 obtained from OPLS-DA and adjusted p-values less than 0.05. VIP indicates the contribution of each variable to group differences. Heatmaps were obtained based on spearman correlation and cluster analyses. The dissimilarity test among groups (ANOSIM) were conducted by the “vegan” package in R-3.4.3. Principal component analysis (PCA), orthogonal partial least-squared discriminant analysis (OPLS-DA), and other statistical analysis were performed using R-3.3.3.

Results
Participants’ characteristics and study design
A total of 334 participants comprising 100 healthy individuals, 93 BTN patients and 141 patients diagnosed with PTC from Center 1 constituted the discovery phase. The validation set composed of 68 healthy volunteers (from Center 1) and 368 patients diagnosed with thyroid nodules from Centers 2–5. Tissue samples (from 57 participants) obtained from Center 6 were also analyzed. In total, 1,540 serum-plasma matched and 114 tissue samples were used. The baseline characteristics of these participants in the discovery phase are summarized in Table 1 while that for the external validation phase and Center 6 are shown in Supporting Information Tables S1 and S2. The entire study is outlined in Figure 1.

Metabolomic analysis of serum-plasma matched samples.
The extraction efficiencies of different solvents were optimized. Methanol/acetonitrile (3:1, v/v) was chosen as the optimal extraction solvent over methanol/methyl tert-butyl ether (1:1, v/v), methanol/acetonitrile (1:1, v/v), methanol (100%),

<table>
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<th>Characteristic</th>
<th>Healthy (n = 100)</th>
<th>BTN (n = 93)</th>
<th>PTC (n = 141)</th>
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<tr>
<td>Female</td>
<td>75 (75)</td>
<td>70 (75)</td>
<td>110 (78)</td>
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<tr>
<td>Male</td>
<td>25 (25)</td>
<td>23 (25)</td>
<td>31 (22)</td>
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<tr>
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<td>93</td>
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Abbreviations: BTN, benign thyroid nodule; PTC, papillary thyroid carcinoma.
analysis (PCA) further confirmed the high reproducibility and instrumental stability throughout the run (Supporting Information Fig. S5). The distribution of metabolic ion features in serum and plasma is presented as venn diagram in Supporting Information Figure S6. Serum-plasma matched metabolomics afforded a broad-scope detection of 1,570 metabolic features. Among them, 884 ion features were present both in serum and plasma, while 378 ion features were detected only in serum, and 308 detected only in plasma.

**Comparisons of healthy group to BTN**
The PCA showed a clear separation in serum metabolic phenotypes between healthy and BTN (Fig. 2a). A significant discrimination was further observed by the orthogonal partial least-squared discriminant analysis (OPLS-DA) (Fig. 2b). The cumulative R2Y and Q2 were 0.949 and 0.920, respectively. For visualizing the relationship between the altered metabolites, hierarchical clustering was used to arrange the metabolites on the basis of their relative levels across samples (Fig. 2c). Plasma metabolomics also confirmed the significant differences (Supporting Information Fig. S7A-C). Using the selection criterion of variable importance in the projection (VIP) > 1.0 and p < 0.05 from Mann–Whitney-Wilcoxon test with false discovery rate correction, differential metabolic features were obtained. Totally, 300 and 352 differential metabolic features were detected in serum (Supporting Information Fig. S8A) and plasma (Supporting Information Fig. S8B), respectively. Among them, 18 differential metabolites were identified from the serum samples (Supporting Information Table S3), and 42 differential metabolites identified from the plasma samples (Supporting Information Table S4).

**Comparisons of healthy group to PTC**
The PCA showed obvious differences in serum metabolic phenotypes between healthy and PTC (Fig. 2d). The OPLS-DA demonstrated a significant distinction with R2Y of 0.945 and Q2 of 0.926 (Fig. 2e). The differential metabolic ion features across samples were displayed as a heatmap (Fig. 2f). Plasma metabolomics confirmed the phenotype differences in terms of PCA, score plots of OPLS-DA, and heatmap (Supporting Information Figs. S7D, E, and F). Totally, 259 and 334 differential metabolic features were detected in the serum (Supporting Information Fig. S8A) and plasma samples (Supporting Information Fig. S8B), respectively. Most differential metabolites showed strong overlap between healthy versus BTN and healthy versus PTC (Supporting Information Fig. S8). Among them, 17 differential metabolites were identified from the serum (Supporting Information Table S5), while 42 differential metabolites were identified from the plasma (Supporting Information Table S6).

**Comparison between BTN and PTC**
The serum metabolomics phenotypes by PCA showed complete overlap between BTN and PTC (Fig. 3a) in the discovery phase.
The OPLS-DA further showed no significant distinction between the groups with R2Y of 0.436 and Q2 of −0.174 (Supporting Information Fig. S9A). Additionally, the unsupervised clustering analysis cannot discriminate BTN individuals from PTC patients (Supporting Information Fig. S9B). The log2 fold change and p-values distribution of the metabolic features indicated that there is no difference between BTN and PTC (Supporting Information Fig. S9C). Only 2 metabolic features with fold change value >2.0 and p-value <0.05 were detected in serum samples. As expected, 1,261 out of 1,262 ion features were found to be non-statistically significant between BTN and PTC in serum (Fig. 3b). To further validate the similarities and differences in the metabolome of the two groups, the ANOSIM analysis based on 10,000 permutation tests of the Bray-curtis index matrix for all individuals was conducted. The results indicated there was no significant difference between BTN and PTC (R = −0.035, p-value = 0.977). To confirm this pattern of non-statistical significance between the two groups, multicenter samples were employed for validation. The PCA plots of serum metabolomics showed no obvious separations but rather overlap in validation Centers 2–5 (Fig. 3a). Correspondingly, over 99.50% ion features were not statistically different in the validation centers (Fig. 3b). A similar pattern of non-significance between the two groups was observed in the plasma samples in the discovery phase and multi-center validation sets (Supporting Information Fig. S9D, E, F and Figs. 3b and 3c).

Perturbed metabolic pathways of thyroid nodules

For comparison of healthy and thyroid nodules (BTN + PTC) in serum, the PCA score plots showed a significant separation (Supporting Information Fig. S10A). A total of 233 metabolic features were detected (Supporting Information Fig. S8A), and 16 differential metabolites were identified (Supporting Information Table S7). The concentration changes of the identified metabolites are summarized as a heatmap (Fig. 4a). Perturbed metabolic pathways of the identified differential metabolites from the serum samples include glycerophospholipid metabolism, arachidonic acid metabolism, linoleic acid metabolism, alanine, aspartate and glutamate metabolism, and D-glutamine and D-glutamate metabolism (Fig. 4b). Plasma metabolomics also confirmed the phenotype differences between healthy and thyroid nodules (BTN + PTC) in terms of PCA (Supporting Information Fig. S10B). A total of 328 metabolic features were detected in the plasma (Supporting Information Fig. S8B), and 41 differential metabolites were identified (Supporting Information Table S8). The average normalized concentration changes of the identified differential metabolites are presented as a heatmap (Fig. 4c). The perturbed metabolic pathways of the identified differential metabolites from the plasma samples include alanine, aspartate and glutamate metabolism, aminoacyl-tRNA biosynthesis, cysteine and methionine metabolism, taurine-hypotaurine metabolism, and selenoamino acid metabolism (Fig. 4d).

Differential diagnosis of thyroid nodules

Since metabolic profiles show complete overlap between BTN and PTC, metabolomics-based noninvasive blood detection is not appropriate for their differential diagnosis. These findings

Figure 2. Metabolomic comparison of healthy volunteers versus BTN (a–c) and healthy volunteers vs. PTC (d–f) using serum samples. (a) PCA score plots of H vs. BTN. (b) Discriminative OPLS-DA score plots of H vs. BTN. (c) Heatmap of the differential metabolites from H vs. BTN. (d) PCA score plots of H vs. PTC. (e) Discriminative OPLS-DA score plots of H vs. PTC. (f) Heatmap of the differential metabolites from H vs. PTC. The colors from green to red in the heatmap indicate elevation in the normalized abundance of the metabolites. PCA: principal component analysis, OPLS-DA: orthogonal projection to latent structure-discriminant analysis, H: healthy, BTN: benign thyroid nodule, PTC: papillary thyroid carcinoma. [Color figure can be viewed at wileyonlinelibrary.com]
led us to screen metabolic markers for diagnosis of healthy group against thyroid nodules. A panel of 6 metabolites were screened out as the metabolic markers in terms of VIP value and peak abundance, namely, myo-inositol, alpha-N-phenylacetyl-L-glutamine, proline betaine, L-glutamic acid, LysoPC (18:0) and LysoPC (18:1). The receiver-operating characteristic presentations, on the basis of the logistic regression of the biomarker panel from the discovery phase, appear in Figure 4e. The average areas under the curve is 0.977 with the sensitivity at 91.5% and specificity at 95.0% (n = 334). The optimal cut-off value of 0.317 was used to predict the healthy individuals and thyroid nodule patients in the multicenter sets. Predictive value was 89.71% for healthy group (n = 68), 91.45% for Center 2 (n = 152), 93.91% for Center 3 (n = 115), 98.18% for Center 4 (n = 55), and 84.78% for Center 5 (n = 46) in Figure 4f.

Comparison using tissue samples
Using tissue samples, the BTN samples were discriminated from the PTC samples using the OPLS model with appreciably good interpretability (i.e., $R^2_Y = 0.774$) as shown in

Figure 3. Comparison of benign thyroid nodule (BTN) versus papillary thyroid carcinoma (PTC). (a). Principal component analysis (PCA) score plots of the comparison at the five study centers using serum samples. (b). Differential metabolic features detected in the serum-plasma matched samples at the various study centers. The number and percentage of non-statistically significant ions from the comparison between BTN and PTC are illustrated in the pie chart. (c). PCA score plots of the comparison at the five study centers using plasma samples. The inclusion criteria of the differential metabolic features are variable importance in the projection (VIP) > 1.0 and false discovery rate adjusted p-values < 0.05. [Color figure can be viewed at wileyonlinelibrary.com]
Figure 4. Differential metabolites, diagnostic biomarkers and their predictive outcomes in the study centers from the comparison of H vs. TN. (a). Heatmap of the 16 identified metabolites from the comparison of H vs. TN in serum. (b). Disturbed metabolic pathways identified from the comparison of H vs. TN using serum samples. (c). Heatmap of the 41 identified differential metabolites from the comparison of H vs. TN in plasma. (d). Disturbed metabolic pathways identified from the comparison of H vs. TN using plasma samples. (e). ROC curve showing the average AUC of the 6 diagnostic biomarkers using serum samples from H vs. TN. (f). The average predictive values of the 6 diagnostic biomarkers in 5 study centers. The blue color in heatmap represents a relatively low abundance while the red color represents a relatively elevated level of the various metabolites. Asterisk (*) means that the metabolites were confirmed with reference compounds. The diagnostic biomarkers are highlighted red. H: healthy, TN: thyroid nodules, vs: versus, ROC: receiver operating characteristic, AUC: area under the curve. [Color figure can be viewed at wileyonlinelibrary.com]
Supporting Information Figure S11A. This difference was also observed in the comparisons of either BTN against adjacent non-tumor tissues or PTC tissues versus adjacent non-tumor tissues (Supporting Information Fig. S11B, C).

Discussion

This work describes a comprehensive metabolomics profiling of thyroid nodules for 1,540 serum-plasma matched and 114 tissues samples from 6 independent centers. The serum-plasma matched metabolomics offers a broader scope to better capture the metabolizing molecular features.

The functions of the thyroid gland, i.e., to increase basal energy expenditure by modulating carbohydrate, lipid and protein metabolism, are affected to varying degrees in diseased states particularly cancer. The metabolome of the thyroid nodule patients in comparison with the healthy persons is characteristic of tumor cells, reflecting in the pathways involved in energy production. The Warburg effect which is central in most cancer cells leads to altered metabolism so as to produce the required energy and biosynthetic needs of the uncontrolled proliferating cells. The perturbed metabolic pathways from both the serum and plasma samples can be broadly categorized into those involved in: (1) Amino acids metabolism, i.e., D-glutamine and D-glutamate metabolism; alanine, aspartate and glutamate metabolism; cysteine and methionine metabolism; aminoacyl-tRNA biosynthesis and selenoamino acid metabolism and (2) Lipid metabolism, thus, arachidonic acid metabolism, glycerophospholipid metabolism, linoleic acid metabolism and taurine-hypotaurine metabolism.

The levels of the various amino acids differed according to their respective metabolic pathways. For instance, L-alanine was found to be down-regulated in the serum. This could be due to increased catabolism since it is pivotal in most of the perturbed pathways. However, the levels of tryptophan, L-proline and glutamic acid were elevated in the sera of the thyroid nodule patients. This phenomenon could be attributed to increased productions of these amino acids since they are substrates for nucleotide biosynthesis and their increased amounts could be to replenish the levels of the metabolites of the tricarboxylic acid cycle as a result of the aerobic glycolysis (Warburg effect). A panel of 15 phosphatidylcholine (PC) and lysophosphatidylcholines (lysoPCs) identified from the serum-plasma matched samples were found to be associated with glycerophospholipid metabolism. LysoPC is produced from PC through sequential mediated actions of phospholipase A1 or phospholipase A2 and lysophospholipase D under varying pathological or physiological conditions. Under normal conditions LysoPC is present at high concentrations, existing mainly as lipoprotein- or albumin-bound forms. They (i.e., LysoPCs) are bioactive proinflammatory and related compounds known as prostanoids or eicosanoids are related to homeostasis and inflammation. They are down-regulated probably due to higher rates of utilization as a result of increased demand for the membrane biosynthesis of tumor cells.

The previous studies broadly investigated the metabolic perturbations between healthy and thyroid nodules using serum, tissue and urine samples with advanced detection techniques such as gas chromatography–mass spectrometry (GC–MS), LC–MS, proton nuclear magnetic resonance (1H-NMR). The capacity of detecting differential metabolites depends on the method employed. The metabolic perturbations observed in this work mainly related to amino acids and lipids metabolisms. There were some common metabolites shared between our work and previous reports. The differential metabolites tryptophan, proline, and the LysoPCs (18:1, 18:0, 16:0, 20:2, 22:4, 22:5, 22:6) were detected both in a previous serum metabolomics by LC–MS and in our work. The amino acid alanine was a differential metabolite identified in serum and urine 1H NMR-based metabolomics, in tissue 1H NMR-based metabolomics and in this work.

The choice between watchful waiting and thyroidectomy remains a difficult one using the current diagnostic techniques where 15 to 30% cases are indeterminate. Most patients with these indeterminate nodules often undergo surgery though a majority turn out to have BTNs. Patients that undergo surgery usually require a life-long levothyroxine replacement therapy, suffering from its attendant side effects as a result. The combination of microscopy and genetic markers has yielded positive outcomes, albeit with limited sensitivity and predictive value. As part of our aims, we sought by our study to provide a potential guideline for the management of thyroid nodules that would minimize the potential risk associated with overtreatment especially in patients at low risk of disease-specific mortality. For the comparison between healthy versus BTN and healthy versus PTC, most differential metabolites showed strong overlap. For instance, 40 of 42 differential metabolites identified in plasma between the healthy and BTN were overlapping in the comparisons of healthy against PTC. Only 2 metabolites from each comparison were found to be different. A similar trend was observed using the serum of the participants. From the comparison between patients with BTN and PTC, we found no significant differences in terms of their metabolic phenotypic states in all study centers. This similitude in the global and holistic outlook of the metabolic phenotypes between BTN patients and those with PTC could be the underlying reason behind the high 10-year survival rates of the latter (i.e., they are at low risk of disease-specific mortality). This is therefore a clarion call to clinicians to watchfully manage patients diagnosed with BTN and even PTC instead of thyroidectomy. Our findings though novel, are contrary to earlier reports. These studies reported metabolic differences between PTC and BTN cases, but none employed a comprehensive protocol as ours. The shortfalls of these studies were addressed in this present study. The superiority and reliability of our study protocol lies in the fact that our findings were validated in multiple centers with high predictive accuracies using relatively larger
sample sizes. Thus, the observed phenomenon was repeated in all five study centers using serum and plasma samples.

Our results indicate that metabolomic profiles are sensitive enough to delineate differences in the comparisons of either BTN against adjacent non-tumor tissues or PTC tissues versus adjacent non-tumor tissues. These results were consistent with the differences observed from the comparisons of BTN versus healthy or PTC versus healthy using blood samples. Tissues from benign tumor and PTC exhibit different cell behaviors because of their fundamentally distinct gene expression patterns. In addition to histologic and cytologic changes, metabolic processes at the cellular level also display alterations during the development and progression of malignancies. The development of cancer requires the existence of particular conditions under which cell metabolism is reprogrammed to satisfy its bioenergetic and biosynthetic requirements. As a result, PTC tissue exhibits distinctly different profiles of low molecular weight metabolites against BTN. So, data obtained from BTN versus PTC using tissue samples though opposite to that for the sera and plasmas, point to the localized nature of the disease. This observation points to the fact that, difference in the morphology or even biochemistry of tissues from persons with BTN or PTC, does not necessarily reflect in their global metabolic states, which also explains at least in part the underlying reason for the low mortality rates of patients with PTC. This finding further buttresses our point and lends support to the option of watchful waiting for PTC.

The main limitation of our study lies in the composition of the study group. One limitation is the unavailability of follicular, poorly differentiated and anaplastic cancer samples, because of their low morbidities. It will be interesting to see whether or not significant metabolic differences exist between these thyroid carcinomas and healthy individuals. A second limitation is that most of the PTC patients (from the discovery phase) were assessed to be in stage I of the disease (129 out of a total of 141 patients). For future studies, it is recommended that patients with other stages of PTC be recruited to ascertain our findings or otherwise. A third limitation of our work stems from the determination and ascertainment of the metabolites. The metabolites were identified from relevant databases but confirmation of their identities remained a challenge due to the unavailability of reference compounds.

References

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