Original Article

Salivary biomarkers-assisted ultrasound-based differentiation of malignant and benign thyroid nodules

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Background: The incidence of papillary thyroid cancer (PTC) is increasing annually. ultrasonography (US) is the current primary method for evaluating thyroid nodules; however, there have been persisting challenges in diagnosing borderline malignancies. This paper aimed to establish the differential diagnostic value of salivary biomarkers for thyroid nodules geared towards improving the efficacy of US.

Methods: We recruited a total of 44 PTC patients and 42 benign thyroid tumor (BTT) patients to this study. The distribution of tumor markers and thyroid hormones in saliva and serum were compared between groups; then, uni-/multi-variate logistic analyses were used to determine the risk factors of PTC. Further, we estimated the differential diagnostic value of biomarkers in thyroid nodules, especially in borderline scenarios. Finally, a multi-index diagnostic model was constructed constituting biomarkers and US.

Results: The distributions of serum thyroglobulin (TG), salivary triiodothyronine (T3), free-triiodothyronine (FT3), and free-thyroxine (FT4) were significantly different in BTT and PTC ($P<0.05$); salivary FT3 was identified as an independent risk factor for PTC. By analyzing the diagnostic accuracy of various Thyroid Imaging Reporting and Data System (TI-RADS) categories, category 4A was shown to have the lowest diagnostic accuracy (48.39%) with the largest proportion (31 people, 36.05%). In 4A patients, the K-nearest neighbor (KNN) algorithm attained the highest sensitivity of 87.50% and specificity of 100.00% among the machine learning-based multi-biomarkers models. Eventually, by combing the US with the KNN-based biomarkers model, the sensitivity and specificity reached 90.91% and 83.33%, respectively.

Conclusions: Salivary biomarkers exhibit good potential in the differential diagnosis of borderline thyroid nodules and they significantly improve the prediction accuracy of the US. Additionally, we found that salivary FT3 is an independent risk factor for PTC and may be used as a key marker for PTC diagnosis.

Keywords: Saliva; free-triiodothyronine (FT3); ultrasound; papillary thyroid cancer (PTC)

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Introduction

The incidence of papillary thyroid cancer (PTC) has gradually been increasing over the past decades and it is currently the 6th leading cancer among the female population (1). In China, PTC is the most prevalent type of cancer among females under the age of 30 (2). The majority of thyroid nodules are benign, while only 5% are cancerous (2). Although PTC is less aggressive compared to other cancers, some patients are still at risk of metastasis and dissemination (3). In clinical practice, total thyroidectomy is the standard treatment method; however, PTC with metastases often requires an extended surgical resection. Therefore, early identification, diagnosis, and treatment are critical.

Ultrasonography (US) is currently the most common method of examining the properties of thyroid nodules (4). In 2009, Park *et al.* (5) established a thyroid imaging reporting and data system (TI-RADS) criteria, and based on ultrasound features, they developed 6 classifications to distinguish between thyroid benign and malignant nodules. Despite TI-RADS achieving significant clinical value, it has inherent shortcomings including experience dependence, poor reproducibility, and high heterogeneity among sonographers (6), specifically in the differentiation of borderline tumors. Based on previous reports, the TI-RADS 4B nodule has a 10–50% probability of malignancy (7). These patients often require invasive fine-needle aspiration biopsy, which markedly increases their burden and suffering. Besides, biopsy might also yield non-diagnostic or false-negative results (8). Therefore, more convenient tests are necessary to help in diagnosis and differentiation.

Among the current techniques, tumor markers are frequently used as adjuncts in the screening and diagnosis of malignant tumors including pancreatic cancer, lung cancer, liver cancer, and so on (9,10). Nonetheless, due to the low malignancy, serum biomarkers of thyroid cancers and their diagnostic value are usually restricted. Increasing recent studies have shown that salivary biomarkers are substantially increased in many cancers including breast cancer, pancreatic cancer, and parotid tumors, indicating their significant diagnostic value (11). Among these, certain biomarkers even exhibit higher expression levels in saliva, thereby making them more advantageous in examining the severity and outcomes of tumors (12). This research sought to evaluate the predictive value of salivary biomarkers and develop a diagnostic model combining with US to distinguish benign from malignant thyroid tumors. We present the following article in accordance with the STARD reporting checklist (available at https://gs.amegroups.com/article/view/10.21037/gs-21-864/rc).

Methods

Patients

Between July and December 2020, we enrolled 86 patients with thyroid nodules from Ruijin Rehabilitation Hospital. The experimental protocol was approved by the Shanghai Ruijin Rehabilitation Hospital Ethics Committee. Informed consent was waived for this non-interventional, observational study by the Ethics Committees, in which the patient data used were kept strictly confidential. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Among them, 42 individuals had benign thyroid tumors (BTT), whereas 44 patients had PTC. After hospitalization, the patients underwent thyroidectomy and were diagnosed through postoperative pathology. Either BTT or PTC was confirmed through post-operative pathological diagnosis by a team of specialists. All participants had salivary and serological assays comprising tumor markers and thyroid hormones. The inclusion criteria were as follows: (I) patients underwent thyroidectomy and were confirmed through postoperative pathological diagnosis; (II) complete clinical information; (III) the diagnosis of malignant cases restricted to PTC and benign tumors included thyroid adenoma and thyroid cyst. The exclusion criteria were as follows: (I) co-morbidity with other malignancy; (II) pregnant women or minors under 18 years old; (III) diagnosed with chronic thyroid inflammation or other thyroid disorder.

Data collection

The clinical characteristics of the participants included age, gender, body mass index (BMI), serum and salivary tumor markers, serum and salivary thyroid hormones, US reports, and so on. Tumor markers included carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), neuron-specific enolase (NSE), carbohydrate antigen 125 (CA125), carbohydrate antigen 153 (CA153), carbohydrate antigen 19-9 (CA199), carbohydrate antigen 242 (CA242), carbohydrate antigen 724 (CA724), squamous cell carcinoma antigen (SCC), and calcitonin (CT). Thyroid hormones included triiodothyronine (T3), thyroxine (T4),

free-triiodothyronine (FT3), free-thyroxine (FT4), thyroid-stimulating hormone (TSH), thyroglobulin (TG), anti-thyroglobulin antibody (A-TG), and thyroid peroxidase antibody (A-TPO). None of those salivary biomarkers were secreted by salivary glands, but derived from serum after absorption and concentration.

The patient diagnosis was performed through surgical resection and postoperative pathological analysis. Thyroid US was executed by 2 experienced sonographers and was scored using TI-RADS criteria. After admission, venous blood samples were drawn from the antecubital vein. The passive drool technique was used to collect saliva samples after cleaning of the oral cavity in the morning after fasting (13); notably, approximately 5 mL of non-stimulated saliva was collected during this step. For the detection of biomarkers, the specimens were examined using the chemiluminescence method (i2000 chemiluminescence immunoassay analyzer; Abbott, Chicago, IL, USA). Also, salivary biomarkers were determined using serum marker reagents (14).

Research process

First, serum and salivary tumor markers, as well as thyroid hormone levels were measured and compared between the BTT and PTC groups. Subsequently, the risk factors for thyroid malignant tumors were determined using logistic regression analysis. Then, the receiver operating characteristic (ROC) curve was used to establish the cut-off values for these risk factors to differentiate between benign and malignant thyroid tumors. Also, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), area under the curve (AUC), and Youden index were determined. Thereafter, the diagnostic value of US was evaluated for each TI-RADS grade. The PTC grade with the lowest prediction accuracy was selected to develop a salivary biomarker diagnostic model using K-nearest neighbor (KNN) and support vector machine (SVM) machine learning algorithms. In the ultimate diagnostic models, patients were classified first by the US, then the borderline cases between benign and malignant were further confirmed using a salivary biomarker diagnostic model.

Statistical analysis

The R language (4.0.2; https://cran.r-project.org/bin/windows/base/old/4.0.2/) was employed for data analysis and graphing. Data were presented by mean ± standard deviation (SD). The Mann-Whitney U test was performed for continuous variables. The chi-square test was used to establish the differences between subgroups. $P<0.05$ indicated a statistically significant difference. Further, the ROC curve, AUC, and Youden index were used to determine the predictive value of biomarkers. Machine learning algorithms were conducted to construct the multi-biomarkers-based diagnostic model, including KNN and SVM.

Results

Comparison of biomarker distributions in serum and saliva between PTC and BTT

A total of 86 patients were enrolled, among them, 44 had PTC, while 42 had BTT (*Figure 1*). Initially, the gender and age distributions of the 2 participant groups were evaluated, as a result, no statistically significant difference was found. Subsequently, the serum and saliva biomarkers were analyzed in the PTC and BTT groups, where tumor markers including CEA, AFP, NSE, CA125, CA153, CA199, CA242, CA724, SCC, and CT, and thyroid hormones including T3, T4, FT3, FT4, TSH, TG, A-TG, and A-TPO were identified. Among tumor indicators, serum AFP distribution was significantly different between benign and malignant thyroid tumors ($P<0.05$), whereas the distribution of other tumor markers was not statistically different both in saliva and serum. As for thyroid hormones, salivary T3, FT3, and FT4 had a considerably larger distribution in BTT than that in PTC ($P<0.05$). Additionally, BTT exhibited a higher level of T4 than PTC and the difference was statistically insignificant ($P=0.053$). In serum thyroid hormone, only TG revealed a considerably higher expression in the BTT group than that in the PTC group ($P=0.008$). The results are shown in *Table 1*.

Regression analysis of risk factors for benign and malignant thyroid tumors

The risk factors for distinguishing benign and malignant thyroid nodules were examined in this section. First, univariate regression analysis was performed including age, gender, US TI-RADS classification, and several biomarkers with significant differences including salivary T3, salivary FT3, salivary FT4, and serum TG. Consequently, data indicated that lower levels of salivary FT3, salivary FT4,

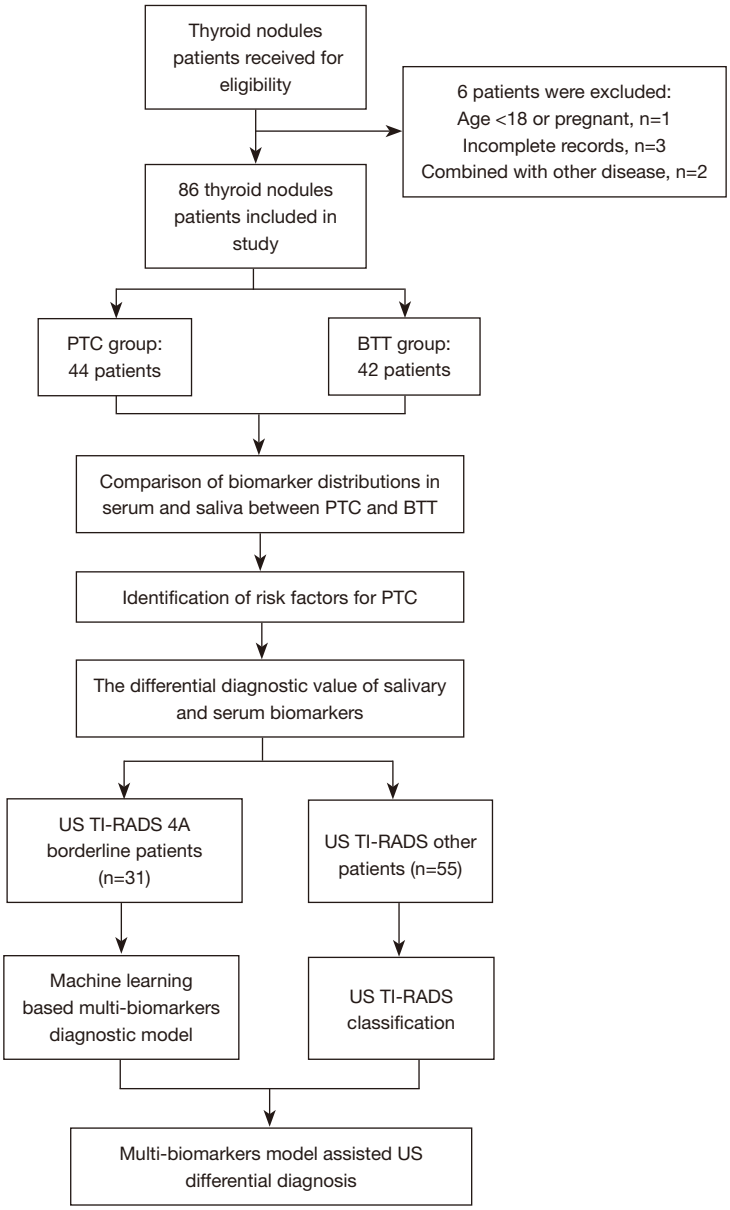

Figure 1 Flowchart of cohort integration. PTC, papillary thyroid carcinoma; BTT, benign thyroid tumor; US, ultrasound; TI-RADS, thyroid imaging reporting and data system.

serum TG, and higher US grades were associated with an increased risk of malignancy (P<0.05) (*Table 2*). In the multivariate logistic regression analysis, salivary FT3 and the US grade were independent risk factors of PTC (P<0.05). The salivary FT3 was negatively associated with the increased risk of PTC, whereas US grades were positively associated, which was broadly consistent with the trends presented above (*Table 3*).

The differential diagnostic value of salivary and serum biomarkers

The ROC curves were plotted for candidate biomarkers and their combination for differential diagnosis of benign and malignant nodules (*Figure 2*). The candidate diagnostic indicators included salivary T3, salivary FT3, salivary FT4, serum TG, and US. The diagnostic values of each

Table 1 Comparison of serum and salivary biomarkers between PTC and BTT patients

Variables	Serum biomarkers			Salivary biomarkers		
	PTC (n=44)	BTT (n=42)	P value	PTC (n=44)	BTT (n=42)	P value
Gender			0.307			–
Male	8	12		–	–	
Female	35	31		–	–	
Age, years	43.19±13.73	44.30±14.75	0.717	–	–	–
CEA	24.13±98.89	7.55±18.09	0.499	62.44±117.74	54.23±76.91	0.840
AFP	0.85±0.51	1.05±0.70	0.075	0.85±0.51	1.05±0.70	0.214
NSE	3.89±0.64	4.58±0.51	0.106	0.58±0.30	0.54±0.15	1.000
CA125	14.2±5.97	16.09±8.32	1.000	128.89±218.99	162.18±265.91	0.171
CA153	9.12±7.54	6.78±5.19	0.439	4.88±5.87	9.67±17.55	0.430
CA199	10.89±7.93	8.96±4.96	0.535	389.69±380.96	455.32±379.99	0.227
CA242	14.39±10.84	11.98±15.83	0.148	152.61±69.67	181.63±46.72	0.025
CA724	2.04±1.78	2.05±2.01	0.950	18.02±35.91	21.74±31.7	0.081
SCC	0.84±0.71	2.18±2.16	0.503	69.48±36.45	72.35±37.21	0.719
CT	4.32±2.1	4.60±2.59	0.828	40.51±62.21	40.3±53.84	0.843
T3	1.84±0.46	1.80±0.35	0.937	1.68±0.31	1.91±0.38	0.045*
T4	108.47±25.26	110.30±21.49	0.519	6.62±0.91	7.41±1.59	0.089
FT3	4.93±1.24	4.87±0.70	0.623	1.98±0.32	2.29±0.51	0.027*
FT4	17.57±3.46	18.00±2.69	0.464	0.82±0.41	1.12±0.49	0.016*
TSH	1.92±1.15	1.64±1.16	0.168	0.01±0.01	0.01±0.01	0.111
TG	81.45±149.50	189.31±210.37	0.008*	0.05±0.02	0.15±0.35	0.809
A-TG	252.09±725.63	88.24±322.94	0.923	10.50±1.67	11.03±2.45	0.420
A-TPO	35.52±59.77	34.56±100.08	0.687	7.68±6.82	13.75±18.54	0.260

*, P<0.05. PTC, papillary thyroid carcinoma; BTT, benign thyroid tumor; CEA, carcinoembryonic antigen; AFP, alpha fetoprotein; NSE, neuron-specific enolase; CA125, carbohydrate antigen 125; CA153, carbohydrate antigen 153; CA199, carbohydrate antigen 19-9; CA242, carbohydrate antigen 242; CA724, carbohydrate antigen 724; SCC, squamous cell carcinoma antigen; CT, calcitonin; T3, triiodothyronine; T4, thyroxine; FT3, free-triiodothyronine; FT4, free-thyroxine; TSH, thyroid-stimulating hormone; TG, thyroglobulin; A-TG, anti-thyroglobulin antibody; A-TPO, thyroid peroxidase antibody.

index were subsequently determined. As a consequence, the sensitivity, specificity, AUC, and cut-off of salivary T3 were 68.18%, 66.67%, 0.679, and 1.710, respectively. The diagnostic values of salivary FT3 were 72.73%, 59.09%, 0.695, and 1.995, respectively; those of salivary FT4 were 66.67%, 77.27%, 0.714, and 0.955, respectively, while those of serum TG were 67.5%, 64.29%, 0.671, and 32.25, respectively. In contrast, the US had a sensitivity of 59.09% and a specificity of 83.33%, respectively (*Table 4*).

Machine learning-based multi-biomarkers diagnostic model in TI-RADS borderline patients

First, the distributions of US TI-RADS grades and their respective diagnostic accuracy rates were analyzed. The US grades higher than 4A were classified as malignancy, while grade 4A and below was classed as benign. Based on *Table 4*, the sensitivity and specificity of US was 59.09% and 83.33% in differentiation of benign and malignant tumor, respectively. Among the TI-RADS US grades, grade 4A had

Table 2 Univariate logistic analysis of risk factors for benign and malignant thyroid tumors

Factors	Regression coefficient	OR	95% CI	P value
Age	–0.012	0.989	0.945–1.034	0.616
Gender	–1.478	0.228	0.052–1.007	0.051
Salivary T3	–1.949	0.142	0.020–1.013	0.052
Salivary FT3	–2.140	0.118	0.017–0.824	0.031
Salivary FT4	–1.588	0.204	0.043–0.981	0.047
Serum TG	–0.006	0.994	0.989–0.999	0.027
US	2.266	9.643	2.362–39.365	0.002

OR, odds ratio; CI, confidence interval; US, ultrasound; T3, triiodothyronine; FT3, free-triiodothyronine; FT4, free-thyroxine; TG, thyroglobulin.

Table 3 Multivariate logistic analysis of risk factors for benign and malignant thyroid tumors

Factors	Regression coefficient	OR	95% CI	P value
Salivary FT3	–2.372	0.093	0.011–0.828	0.033
US	3.036	20.815	3.369–128.620	0.001

OR, odds ratio; CI, confidence interval; US, ultrasound; FT3, free-triiodothyronine.

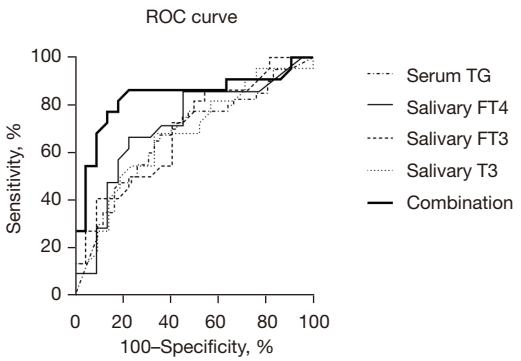

Figure 2 ROC curve of salivary T3, FT3, FT4, serum TG and combined diagnosis in the differential diagnosis of benign and malignant thyroid tumors. ROC, receiver operating characteristic; T3, triiodothyronine; T4, thyroxine; FT3, free-triiodothyronine; FT4, free-thyroxine; TG, thyroglobulin.

the majority of the patients (31 cases, 36.05%). Meanwhile, 4A also had the poorest diagnostic accuracy (48.39%) compared to other grades (higher than 60%) (*Table 5*). After excluding 4A patients, the sensitivity and specificity of US increased to 92.86% and 74.07%, respectively. Considering that TI-RADS 4A patients accounted for the majority of US diagnoses and with the lowest diagnostic accuracy, a multi-biomarkers diagnostic model was adopted to improve the diagnostic effect of US in this subgroup.

In the diagnosis of 4A patients, salivary FT3 used alone had a sensitivity, specificity, and AUC of 100.00%, 66.67%, and 0.854%, respectively. Thereafter, SVM and KNN algorithms were used to develop a prediction model which enrolled salivary T3, FT3, FT4, and serum TG. Consequently, the sensitivity and specificity of the SVM model reached 87.50% and 86.67%, respectively. The sensitivity and specificity of the KNN model reached 87.50% and 100.00%, respectively (*Table 6*).

Multi-biomarkers model-assisted US differential diagnosis

Finally, US TI-RADS grades and a multi-biomarkers diagnostic model for 4A patients were combined as a multi-index diagnostic model. In this diagnostic model, the patients were first evaluated by the US, then, a multi-biomarker model was utilized to verify benign and malignant lesions in those with 4A grade. The results revealed that US + salivary FT3 in 4A patients exhibited a sensitivity of 95.45% and a specificity of 71.43%. The US+SVM prediction model had a sensitivity of 90.91% and a specificity of 78.57%. The US + KNN prediction model had the best sensitivity and specificity performance of

Table 4 Comparison of the diagnostic value of multiple biomarkers for benign and malignant thyroid tumors

Biomarkers	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden index (%)	AUC	Cut-off value	95% CI
Salivary T3	68.18	66.67	66.67	68.18	34.85	0.679	1.710	0.517–0.840
Salivary FT3	72.73	59.09	68.42	64.00	31.82	0.695	1.995	0.540–0.851
Salivary FT4	66.67	77.27	70.83	73.68	43.94	0.714	0.955	0.555–0.874
Serum TG	67.50	64.29	67.50	64.29	31.79	0.671	32.25	0.552–0.789
US	59.09	83.33	78.95	72.00	42.42	–	–	–
Combination	86.36	77.27	79.17	85.00	63.63	0.836	0.335	0.706–0.966

PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve; CI, confidence interval.

Table 5 The accuracy of different TI-RADS grades in judging benign and malignant thyroid tumors

Grades	No. (%)	BTT	PTC	Accuracy, %
3	22 (25.58)	20	2	90.91
4A	31 (36.05)	15	16	48.39
4B	24 (27.91)	5	19	79.17
4C	6 (6.98)	2	4	66.67
5	3 (3.49)	0	3	100.00

TI-RADS, thyroid imaging reporting and data system; PTC, papillary thyroid carcinoma; BTT, benign thyroid tumor.

Table 6 Combined diagnosis of US and biomarkers in predicting benign and malignant thyroid tumors

Biomarkers	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden index (%)
Traditional method					
US	59.09	83.33	78.95	72.00	42.42
US (without 4A)	92.86	74.07	66.93	78.79	90.91
Logistic	86.36	77.27	79.17	85.00	63.63
TI-RADS 4A group					
4A_FT3	100.00	66.67	66.67	76.19	100.00
4A_SVM	87.50	86.67	74.17	87.50	86.67
4A_KNN	87.50	100.00	87.50	100.00	88.24
Combination					
US + 4A_FT3	95.45	71.43	66.88	77.78	93.75
US + SVM	90.91	78.57	69.48	81.63	89.19
US + KNN	90.91	83.33	74.24	85.11	89.74

US, ultrasound; TI-RADS, thyroid imaging reporting and data system; PPV, positive predictive value; NPV, negative predictive value; SVM, support vector machine; KNN, K-nearest neighbour.

90.91% and 83.33%, respectively (*Table 6*).

Discussion

For the first time, the present study examined salivary tumor markers and thyroid hormone profiles in individuals with benign and malignant thyroid tumors. We found that salivary T3, FT3, and FT4 levels were significantly different in benign and malignant tumors. Subsequently, we performed univariate and multivariate logistic regression to evaluate salivary FT3 and US as independent risk variables for predicting benign and malignant cancers. Consequently, patients of 4A accounted for the greatest proportion (36.05%) among all participants; nevertheless, it had the lowest diagnostic accuracy (48.39%), which influenced the overall diagnostic efficiency. As a result, a multi-biomarker model primarily based on salivary indicators was constructed to verify the diagnosis of 4A patients, where several machine learning algorithms were employed. The diagnostic performance of the KNN model was efficient, with a sensitivity of 87.50% and a specificity of 100.00%. Finally, we combined US and 4A diagnostic models, where patients with 4A grades received multi-biomarkers diagnostic models for validation, and other patients directly received US diagnoses. As a consequence, the US + KNN model had the best diagnostic efficacy, with a sensitivity and specificity of 90.91% and 83.33%, respectively, compared to that of 59.09%, 83.33% for the US alone. In this regard, salivary biomarkers could improve the diagnostic efficacy of borderline thyroid nodules in US examination with a potential application in the differentiation of thyroid benign and malignant nodules.

Currently, US is the primary screening and diagnostic technique for the distinction of benign and malignant thyroid nodules. A meta-analysis examined US elastography for the detection of benign and malignant thyroid cancers. After pooling 5,481 nodules from 4,468 patients, the average sensitivity and specificity of US identification approached 0.79 and 0.77, respectively (15), suggesting the high potential of US in differentiating benign from malignant tumors. Notably, the TI-RADS classification is the extensively used evaluation method in ultrasonography. Horvath (16) and Kwak (17) found that TI-RADS may be used to increase diagnostic accuracy, decrease needle biopsies, and eventually improve thyroid nodule treatment. In the TI-RADS system, thyroid nodules are classified into 6 classes, among which the probability of malignancy was 0 in the category below 3; 5% in category 3; 5–10% in category 4A; 10–50% in 4B; 50–85% in 4C; higher than 85% in category 5; and 100% in category 6 (7).

In clinical practice, it is often difficult to distinguish benign from malignant tumors in patients with borderline nodules including 4A grade; this, therefore, requires further needle biopsies to confirm the diagnosis, hence increasing patient suffering and impairing the effective performance of the medical system. As a result, auxiliary strategies are essential to augment the diagnostic accuracy of US. Additionally, borderline patients account for a significant fraction of the population. Among the 86 patients who participated in this work, 4A patients as borderline cases accounted for 36.05% (the highest percentage). At the same time, the diagnosis of category 4A patients was also difficult with a malignancy probability of 51.61%. Other studies on the predictive accuracy of various TI-RADS classifications have reported similar findings (18) in a malignant rate of 9.5% in 4A patients. Nonetheless, herein, we found a malignancy rate of 48% for category 4B individuals. This disparity may reflect regional differences in the subjective diagnosis of US physicians. However, the US examination frequently exhibited low prediction accuracy among the boundary nodules between benign and malignant classifications. Notably, needle biopsy is a critical adjunct to the US with high diagnostic accuracy for the ultimate confirmation of high-risk patients. Nevertheless, it has a number of limitations, including high cost, risk of bleeding, and so on, which hamper its applicability in mass screening. Thus, a simple and non-invasive auxiliary detection tool is immediately required in clinical practice.

Serum biomarkers are used in the diagnosis and treatment of liver cancer, pancreatic cancer, lung cancer, and other malignancies (19,20), and also offer diagnostic potential in the detection of thyroid carcinoma. Giovanella *et al.* (21) discovered the value of serum TG in detecting differentiated thyroid cancer. Elsewhere, Gul *et al.* (22) discovered that low serum FT3, FT4, and TSH levels were linked to an increased risk of thyroid cancer. Many other indicators have also been uncovered including interleukin (IL)-8 (23), Leptin (24), and so on. Nonetheless, the application of serum biomarkers is hindered by the low diagnostic efficacity of PTC. Moreover, our findings of poor diagnostic value of serum biomarkers indicated similar outcomes. Therefore, we used salivary biomarkers for investigation.

Besides serum tumor indicators, studies on salivary biomarkers have intensified in recent years. Salivary biomarkers have been used to diagnose pancreatic cancer,

breast cancer, and other types of cancers (11,25,26). Several studies have shown that numerous salivary indicators have greater concentrations than those in serum. This is potentially attributed to a one-way filtering process between the salivary glands and blood, resulting in constant biomarker concentrations in saliva. Because thyroid cancer is less aggressive compared to other types of cancer, its blood biomarkers change insignificantly. In contrast, highly concentrated salivary biomarkers may have a significant diagnostic capability for thyroid nodule evaluation. Herein, salivary tumor biomarkers were also higher than serum tumor biomarkers. However, they lacked considerable diagnostic significance. This might be due to the small sample size; therefore, this should be confirmed in future research with a larger sample size.

Furthermore, previous research has reported a link between thyroid illness and the salivary glands. Saliva contains a greater quantity of iodine than that in serum (27). Salivary glands, stomach, and thyroid share a sodium iodide transporter (NIS) capable of concentrating iodine ions (28). As such, when I^{131} radiation is used as radiotherapy after thyroid surgery, the salivary glands usually develop additional damage (29). Consequently, salivary biomarkers were used in the detection of thyroid disorders. Guo *et al.* (30) noted a significant correlation between elevated salivary iodine levels and the presence of thyroid nodules. Nonetheless, no research has been performed to establish the correlation between salivary biomarkers and thyroid cancer. Therefore, we examined the expression of tumor markers and thyroid hormones in saliva and discovered saliva T3, FT3, FT4, and blood TG levels as potential biomarkers that distinguish benign from malignant thyroid nodules.

As previously stated, the major difficulty in US thyroid nodule identification is the low differential predictive value of benign-malignant borderline nodules. In this work, 2 machine learning algorithms, SVM and KNN, were used to improve differential diagnosis in category 4A. Researchers have previously employed machine learning to help in PTC diagnosis; however, most of them focused on the analysis of US images (2,31). Also, these investigations require labeling and identification of many US photographs by specialists, which demands high development costs. The objective of this study was to develop a low-cost, simple, and easy-to-promote saliva indicator program to supplement the US examination and consequently improve the prediction accuracy of thyroid nodules.

We confirmed that the distribution of salivary biomarkers is significantly different between benign and malignant nodules, hence making diagnosis feasible. By integrating with the US, a KNN-assisted salivary biomarker-based diagnostic model could distinguish PTC from BTT with a sensitivity and specificity of up to 90.91% and 83.33%, respectively, compared to 59.09% and 83.33%, respectively, for US analysis alone. Therefore, we believe that using salivary biomarkers combined with the US can significantly improve the accuracy of thyroid nodule identification, specifically in borderline cases. As a convenient, rapid, and non-invasive detection method, salivary biomarkers have the potential to significantly improve the prediction accuracy in the differential diagnosis of thyroid tumors, reduce the number of fine-needle aspiration biopsy needed, thereby reducing patient suffering, and increasing the efficiency. This minimizes the medical resource consumption, making them a feasible option for thyroid nodule screening.

This research has compelling limitations. First, it was a single-center study, suggesting a possibility of sample bias. Secondly, the sample size was inadequate, with 86 overall cases and 31 cases in 4A grade. In the future, we will enroll additional cases and centers in this project. Moving forward, we will focus on 4A borderline patients and assess the predictive value of salivary biomarker-based diagnostic models.

Conclusions

In conclusion, our findings indicated that salivary biomarkers showed great potential for identifying benign and malignant thyroid cancers, which might enhance the US prediction accuracy considerably. Moreover, we found that salivary FT3 is an independent risk factor for PTC and may be utilized as a diagnostic biomarker. This means that salivary biomarkers have extensive application potential; therefore, additional research into their diagnostic value is required.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The experimental protocol was approved by the Shanghai Ruijin Rehabilitation Hospital Ethics Committee. Informed consent was waived for this non-interventional, observational study by the Ethics Committees, in which the patient data used were kept strictly confidential.

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