Correlation analysis between the pre-operative contrast-enhanced ultrasound parameters and biological characteristics of papillary thyroid carcinoma and associated risk factors for prognosis after radiofrequency ablation

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Abstract. The present study aimed to assess the correlation between the contrast-enhanced ultrasound (CEUS) parameters and biological characteristics of papillary thyroid carcinoma (PTC), and identify risk factors of recurrence for the prognosis after radiofrequency ablation. A total of 72 patients with PTC were included. Disease recurrence and metastasis were evaluated based on the imaging performance and laboratory tests. Perfusion parameters were recorded and compared. After biopsy, the tumor tissues were subjected to hematoxylin and eosin staining, and the expression levels of P53 and Ki67 were detected using immunohistochemistry. The association between the CEUS parameters and the expression of P53 and Ki67 was analyzed. Risk factors for disease recurrence were analyzed using logistic regression. Compared with normal tissues, PTC tissue was characterized by a slow perfusion pattern with no obvious enhancement. The peak time and intensity were lower, while the initial increasing time and mean transit time (MTT) were longer in the PTC tissue. The expression levels of P53 and Ki67 in PTC tissues were associated with age, local infiltration, lymph node metastasis and the number of lesions, but not with gender, tumor size, histological type or degree of differentiation. The initial increasing time, peak time and MTT were negatively correlated, while the peak intensity was positively correlated with the expression of P53 and Ki67 in PTC tissues. Multivariate logistic regression analysis suggested that lymph node metastasis, age, local infiltration, peak intensity and MTT were risk factors for disease recurrence. In conclusion, quantitative CEUS parameters are closely linked to the biological characteristics of PTC and may serve as indicators for the prognosis of patients.

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Introduction

Papillary thyroid carcinoma (PTC) is a common type of thyroid malignancy, accounting for 74-80% of all cases of thyroid cancer, and the incidence has kept increasing in recent years (1). In addition to PTC, other malignant tumors of the thyroid gland include follicular carcinomas (FTC; moderate malignancy; prevalence of 11-15%), undifferentiated carcinomas (ATC; high malignancy; prevalence of 1-2%) and medullary carcinomas (MTC; prevalence of 3-8%). PTC is clinically characterized by obscure symptoms, slow disease development and relatively low mortality (2). With the application of color Doppler ultrasound imaging, the time-intensity curve of the tumors may be obtained using quantitative parameter analysis of ultrasound contrast dynamic imaging, as well as the association between the parameters and the expression levels of P53 and Ki67 in the tumors (3). Information on the blood supply of tissues and organs, together with the evidence of vascular invasion or metastasis, is important for tumor staging, recurrence analysis and prognostication (4,5). On the other hand, FTC is a type of thyroid cancer that occurs relatively rarely, which may be divided into invasive and non-invasive subtypes. The clinical manifestations, diagnostic methods and treatment methods are basically similar to those of PTC. Furthermore, ATC accounts for 1-2% of thyroid tumor cases, with low disease incidence but high degree of malignancy. The growth and development of ATC is rapid, featured by strong local invasiveness and a high distant metastasis rate. The prognosis of ATC is dismal, and at present, no effective treatment is available. Furthermore, MTC is derived from thyroid follicular cells (also known as C cells) that secrete calcitonin. The major cause of MTC is mutation of the RET proto-oncogene and the disease prognosis is generally poor, while it is frequently closely associated with the blood

calcitonin level. The present study reported on the clinical incidence, disease manifestations and examination results of patients with PTC and determined prognostic factors.

Over the past years, relatively less invasive methods that do not cause excessive scarring have gradually replaced routine surgical resection as the clinical application. Ultrasound-guided radiofrequency ablation (RFA) is a type of minimally invasive treatment, which causes local hyperthermia to induce necrosis in the tumor tissue, not only representing an effective disease treatment but also meeting the patients' aesthetic requirements (6). However, although it is a minimally invasive treatment method, ultrasound-guided RFA may still cause certain damage to the body and tissue.

Recently, with the rapid development of molecular biology, the molecular biological indicators for PTC have gradually become a research focus (7). P53 and Ki67 are the major indicators for malignant tumors (8,9). Therefore, the establishment of specific tumor markers for PTC are of important significance in the clinic to improve the diagnosis and treatment of the disease.

A high incidence and therapeutic value of PTC have been observed in the clinic, and compared with other types of thyroid tumor, the prognosis for PTC is relatively favorable. In the present study, the association between the features of the contrast-enhanced ultrasound (CEUS) and the biological indicators for PTC were further analyzed. In addition, the ultrasound performance of PTC, as well as the disease recurrence after RFA, were analyzed and discussed. Furthermore, possible influencing factors on the prognosis of PTC were determined.

Materials and methods

Study subjects. A total of 72 patients with PTC (22 males and 50 females; average age, 48.7±4.5 years; age range, 28-70 years), who received treatment at Taihe Hospital, Hubei University of Medicine, from February 2016 to January 2018, were included in the present study. The follow-up visits lasted for 18 months. The inclusion criteria were as follows (10): i) Patients receiving ultrasound-guided RFA for PTC at our hospital. The indications should be in accordance with at least one of the following conditions: a) Patients having a lesion with a diameter of <2 cm or aesthetics-affecting tumor convexity; b) patients having multiple tumors (<3 lesions) with mild infiltration and lymph node metastasis around the tumor, who would not consider surgical resection; ii) patients with complete pre-operative ultrasound examination data; iii) patients with complete follow-up data; and iv) patients who were able to be staged according to the TNM Clinical Staging Criteria of thyroid papillary carcinoma from the American Joint Committee on Cancer (AJCC; 7th edition, 2017) (11). Based on TNM staging, the patients at the T1 stage were included and subjected to clinical treatment. The exclusion criteria were as follows (5): i) Patients with a previous history of thyroid disease or abnormal vocal cord function on the contralateral side of the lesion; ii) patients with a history of serious diseases that required long-term medication; iii) patients with unclear pathological findings or previous history of psychosis or neuropathy; iv) patients having received relevant treatments that may affect the observation indicators; and v) patients with severe heart, liver and/or kidney damage that affected the metabolism, or severe coagulation mechanism disorders. The present study was approved by the ethics committee of Taihe Hospital, Hubei University of Medicine, and all patients had provided written informed consent.

Tumor puncture biopsy. All patients underwent the routine ultrasound examination of the neck and thyroid prior to treatment. The lesions were located on the body surface and the needling depth was determined. For the conventional disinfection, local infiltration anesthesia and puncturing, following local anesthesia, a disposable 7# needle was inserted to the subcutaneous positioning point. The puncturing direction of the 18G needle was observed by and adjusted according to the real-time ultrasound, until the needle tip was located in the lesion area at the sagittal and coronal section. The 2-3 tissue samples (1-2 cm in length) were obtained, which were placed in formalin solution, followed by pathological analysis.

CEUS. For the CEUS, the SonoVue contrast agent was used (Bracco), diluted with 5 ml 0.9% NaCl solution, which was administered through the anterior elbow vein as a bolus injection (2.4 ml). The whole process of the contrast perfusion (including peaking and withdrawal) was dynamically observed. Furthermore, the lesion's enhancement level, enhancement pattern and its association with surrounding tissues, were observed. The time-intensity curve was drawn and the parameters, including the peak value (PEAK), mean transit time (MTT), initial increasing time and peak time, were obtained. The image data were read and analyzed by two physicians with ultrasound experience of >10 years.

Ablation treatment. RFA was performed with the Celon AG RFA system (Olympus Corp.), equipped with an 18G bipolar RFA needle with a 9- or 15-mm electrode, and the output frequency was 5-8 W. Under the guidance of ultrasound, 1% lidocaine hydrochloride was injected subcutaneously and around the thyroid anterior capsule for local anesthesia. Furthermore, 20-40 ml NaCl (0.9%) was injected in the area around the thyroid capsule. After isolating the thyroid from the surrounding tissues (including large blood vessels, nerves, trachea, esophagus and muscles), the 18G RFA needle was placed inside the mass under the guidance of color Doppler ultrasound, with the Philips IU22 color Doppler ultrasound imaging system (Philips Ultrasound), with the line array probe of L3-9 and frequency of 3-9 MHz, or L5-12 and frequency of 5-12 MHz. The mobile ablation technique was applied to ablate the mass, with the from-inside-to-outside and from-shallow-to-deep mode, until the lesion was completely covered by the hyperechoic gasification zone. After the ablation, CEUS was performed during the operation to evaluate whether the ablation was sufficient.

Pathological analysis. The obtained tissues were fixed with conventional neutral formalin (at room temperature for 12-24 h), dehydrated, and embedded in paraffin. For immunohistochemical staining for P53 and Ki67, the tissue section was dewaxed and treated with xylene and alcohol (for 3 rounds, 5 min per round), followed by washing with distilled water and PBS. According to standard protocols,

after antigen retrieval, the section was incubated with 3% hydrogen peroxide at room temperature for 5 min, and then washed with PBS for 3 times (2 min each time). The sections were then incubated with mouse anti-human P53 monoclonal antibody (1:100 dilution; Zhongshang Goldenbridge-BIO) and mouse anti-human Ki-67 monoclonal antibody (1:100 dilution; Zhongshang Goldenbridge-BIO), respectively, at room temperature for 1-2 h. The sections were subsequently treated with Reagents 1 and 2 within the kit (REF.10128105P-G, P/N80302-3101; Hangzhou Shitai Biotech Co., Ltd.), at room temperature for 20 min each time. Diaminobenzidine chromogenic droplets were used to treat the tissue section, followed by the hematoxylin counterstaining for 5 min. After treatment with alcohol and xylene, the sections were observed under a microscope. The data were analyzed and scored according to the following criteria: Ratio of cells with positive staining <10%, negative (-), and ≥10%, positive (+), where 10-25% was considered as weakly positive (1 point), 25-50% as moderately positive (2 points) and >50% as strongly positive (3 points). The staining was performed and the results were analyzed by two pathologists (Lu Jian, Department of Pathology, Taihe Hospital, Hubei University of Medicine, Shiyan City, Hubei, China; and Dandan Zou, Attending Physician, Department of Pathology, People's Hospital of Longhua District, Shenzhen, Guangdong, China), with senior professional titles.

Statistical analysis. SPSS 17.0 software (SPSS, Inc.) was used for statistical analysis. Count data were expressed as % and were analyzed with the χ^2 test. Measurement data were expressed as the mean ± standard deviation. The measurement data fitting a normal distribution (The ratio of the sample median to the arithmetic mean, and the relationship between the arithmetic mean and the standard deviation, were used for the normal distribution), were compared with the Student's t-test or analysis of variance (with the Bonferroni post-hoc test), while the measurement data with an anomalous distribution were compared with the rank-sum test followed by the Conover post-hoc test. Multivariate analysis of parameters associated with recurrence was performed using logistic regression analysis with the stepwise backward method. Non-normally distributed measurement data and non-parametric grading data were analyzed by Spearman correlation analysis. The non-parametric data were compared using the χ^2 test. $P<0.05$ was considered to indicate statistical significance.

Results

Imaging performance and time-intensity curve analysis of PTC on CEUS. The clinicopathological characteristics of the patients were provided in Table SI. For PTC, the CEUS indicated that the lesion had an irregular shape and no clear boundary, with no obvious or uneven enhancement, as well as the focal perfusion defect regions (Figs. 1 and 2A and B). Compared with the normal surrounding thyroid tissues, the carcinoma lesion exhibited a slow perfusion pattern. In the lesion tissue, the initial increasing time was prolonged and the enhancement level was lower compared with that in the surrounding thyroid tissue. For the carcinoma lesions, the rising slope of the curve was relatively flat, slowly reaching the peak, which then gradually regressed. During the whole

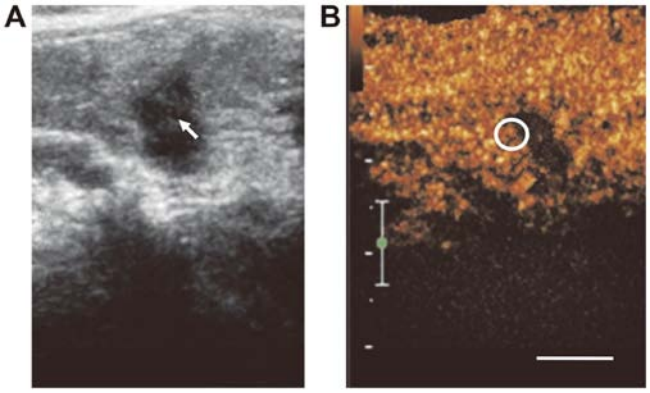

Figure 1. Contrast-enhanced ultrasound imaging analysis. (A) Two-dimensional ultrasound of papillary thyroid carcinoma (indicated by the white arrow). (B) Thyroid ultrasound contrast image with uneven and low enhancement, unclear boundary, irregular shape and local perfusion defect region (indicated by the white circle; scale bar, 1 cm).

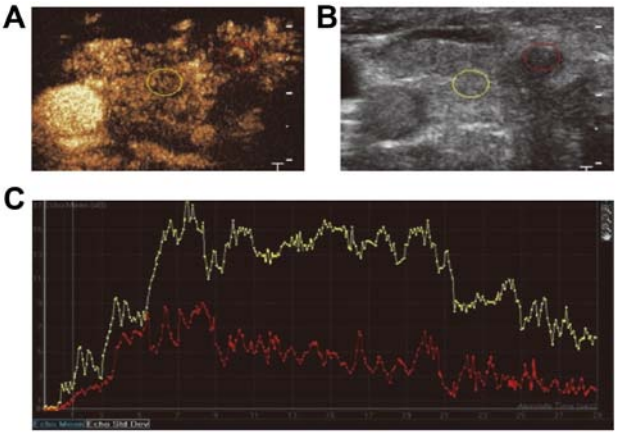

Figure 2. Time-intensity curves of PTC and normal surrounding thyroid tissues. (A) Contrast-enhanced imaging of PTC. (B) Two-dimensional image of PTC. The red circle indicated the ROI in lesions, while the yellow circle indicated the ROI in the normal tissues. (C) Time-intensity curves of PTC (red curve) and normal surrounding thyroid tissues (yellow curve). PTC, papillary thyroid carcinoma; Std Dev, standard deviation.

enhancement process, the enhancement level for the PTC was lower than that for the normal thyroid tissue. Furthermore, the peak intensity for the normal thyroid tissue was higher than that for the PTC tissue, and the MTT for the PTC was significantly longer than that for the normal tissue (Fig. 2C; Table I).

Correlation between biological indicators and clinical features of PTC. For the PTC group, the positive rates of P53 and Ki67 were significantly associated with age (<45 years), local infiltration, lymph node metastasis, and lesion numbers ($P<0.05$). However, the positive rates were not related to the gender, tumor size, histological type, or differentiation degree ($P>0.05$; Table II; Fig. 3).

Correlation between CEUS parameters and expression of P53 and Ki67 in PTC. For the patients with PTC, the initial increasing time, peak time and MTT were significantly negatively associated with the expression levels of P53 and Ki67

Table I. Time-intensity curve analysis of CEUS for papillary thyroid carcinoma.

CEUS	PTC tissue	P-value	Normal surrounding tissue
Initial increasing time	15.32±2.73	<0.05	11.44±1.26
Peak time	11.23±3.01	<0.05	21.16±3.53
Regression time	30.0±4.24		29.06±3.98
Mean transit time	54.14±14.26	<0.05	53.63±13.27
Peak intensity	37.06±9.07	<0.05	40.6±9.45

P<0.05 vs. compared with the normal surrounding tissue. CEUS, contrast-enhanced ultrasound.

Table II. Comparison of P53- and Ki67-positive rates in patients with papillary thyroid carcinoma with different clinicopathological features.

Parameter	N	P53 (+)	χ^2	P-value	Ki67 (+)	χ^2	P-value
Age (years)			4.47	0.03		5.51	0.01
≥45	24	22 (91.66)			20 (83.33)		
<45	48	19 (39.58)			15 (31.25)		
Sex			0.137	0.711		0.16	0.68
Male	22	16 (72.72)			18 (81.81)		
Female	50	42 (84.0)			35 (70.0)		
Tumor size (cm)			0.12	0.72		0.13	0.71
≥1, <2	19	14 (73.68)			15 (78.94)		
<1	53	45 (84.9)			36 (67.92)		
Local infiltration			4.13	0.04		4.85	0.02
Yes	32	31 (96.87)			30 (93.75)		
No	40	18 (45.0)			16 (40.0)		
Lymph node metastasis							
Yes	25	23 (92.0)	3.89	0.04	22 (88.0)	6.79	0.00
No	47	20 (42.55)			14 (29.78)		
Multiplicity of lesions							
Multiple	31	10 (32.25)	6.32	0.01	8 (25.8)	7.57	0.00
Single	41	38 (92.68)			36 (87.80)		
Degree of differentiation							
Well	57	47 (82.45)	8.31	0.36	40 (70.17)	1.16	0.28
Poor	15	8 (53.33)			6 (40.0)		

Values are expressed as n or n (%).

in the PTC tissues (P<0.05). In addition, the peak intensity was positively associated with the expression of P53 and Ki67 (P<0.05). Furthermore, the regression time was negatively associated with the expression of P53 and Ki67 in the PTC tissues, but with no statistical significance (P>0.05; Table III).

Analysis of risk factors associated with recurrence after RFA. Multivariate logistic regression analysis was performed to determine the association of CEUS parameters and clinicopathological features (used as independent variables) with recurrence at 18 months after RFA (used as the dependent variable). The results revealed that the presence of lymph node metastasis [odds ratio (OR): 0.010, 95% CI:<0.001-0.063], age (OR: 0.853, 95% CI: 0.757-0.960), local infiltration (OR: 0.080, 95% CI: 0.007-0.895), peak intensity (OR: 0.828, 95% CI: 0.701-0.979) and MTT (OR: 0.755, 95% CI: 0.649-0.879) were significant factors for predicting recurrence (P<0.05), with negative partial regression coefficients indicating a negative association based on the risk factor assessment form (Table IV). These results suggest that lymph node metastasis, age, local infiltration, peak intensity and MTT are major risk factors for recurrence after RFA.

Discussion

Thyroid cancer is one of the most common malignant tumor types of the endocrine system, accounting for 1-5% of such

Table III. Correlation between contrast-enhanced ultrasound parameters and expression of P53 and Ki67 in papillary thyroid carcinoma.

	P53		Ki67	
Parameter	r	P-value	r	P-value
Initial increasing time	-0.28	0.01	-0.52	<0.01
Peak time	-0.32	<0.01	-0.26	0.02
Peak intensity	0.26	0.02	0.39	0.01
Mean transit time	-0.40	<0.01	-0.25	0.02
Regression time	-1.64	0.16	-0.01	0.87

Table IV. Analysis of risk factors for recurrence after radiofrequency ablation.

						Exp (β) 95% CI	
Parameter	Regression coefficient β	SE	Wald χ^2	Sig.	Odds ratio Exp (β)	Lower limit	Upper limit
Mean transit time	-0.281	0.077	13.239	<0.001	0.755	0.649	0.879
Peak intensity	-0.189	0.085	4.895	0.027	0.828	0.701	0.979
Lymph node metastasis	-6.722	2.016	11.116	0.001	0.001	<0.001	0.063
Age	-0.159	0.061	6.908	0.009	0.853	0.757	0.960
Local infiltration	-2.525	1.232	4.201	0.040	0.080	0.007	0.895
Constant	33.410	9.140	13.362	<0.001	$3.236x10^{14}$		

SE, standard error; CI, confidence interval.

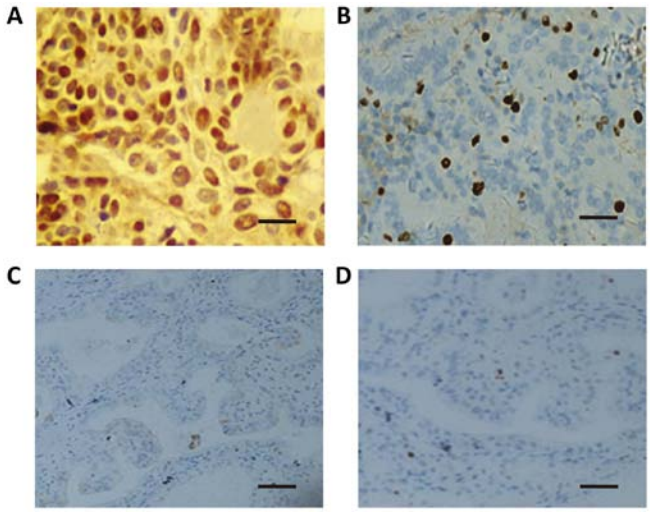

Figure 3. Representative figures for positive staining in PTC tissues. (A) P53-positive PTC tissue, (B) Ki67-positive PTC tissue, (C) P53-positive thyroid papillary hyperplasia tissue and (D) Ki67-positive thyroid papillary hyperplasia tissue (scale bar, 5 μm). PTC, papillary thyroid carcinoma.

cases (12). The total incidence has kept increasing rapidly worldwide (13,14). At present, the diagnosis of most thyroid tumor cases depends on the histopathological results. The tumor staging was performed based on the results of the pathological puncture biopsy, according to the staging criteria from the AJCC (7th edition, 2017). However, the diagnosis of PTC is relatively difficult based on the imaging technique, in terms of distinguishing between benign and malignant papillary nodular hyperplasia. CEUS, also known as ultrasound microcirculation angiography, is a non-invasive imaging technique, which may be used to dynamically observe the microvascular perfusion of tumors in real-time and sensitively reflect the status of blood vessels and microvessels in the tumor tissues, contributing to the identification of malignant tumors and diagnosis of the disease. Ultrasound imaging has been widely applied in the detection of abdominal organs, including the liver and kidney (15,16), and has also been applied in the detection of small organs, including the thyroid and breast. Considering the detection and prognosis prediction of PTC, there are still no effective specific molecular markers (17). Due to the enhanced tumor cell growth activity, the tumor grows rapidly. Therefore, P53 and Ki67 are considered to be reliable indicators for the detection of the proliferation activity of tumor cells (18). Previous studies have reported that P53 and Ki67 are associated with the development, metastasis and prognosis of various malignant tumor types (19,20).

In the RFA treatment, high-heat energy is transferred to destroy the tumor tissues, while inducing relatively less damage to normal tissues surrounding the lesion. When applied to patients with PTC, RFA treatment may preserve thyroid function. Compared with traditional surgery, RFA treatment induces less inhibitory effects on the immune response, which

is beneficial for disease recovery (21,22). Therefore, in the clinic, it is of great significance to develop a more sensitive, efficient and convenient diagnostic method for PTC, which, together with the clinical features based on cytology and pathology, provides effective markers for the disease.

It has been reported that CEUS of thyroid carcinoma is always characterized by a fast-in and fast-out pattern, mainly due to the increased and/or dysfunctional internal vessels, uneven distribution and uneven vessel diameters in the thyroid carcinoma, which may lead to the formation of a large number of arteriovenous fistula. However, the present results suggested that the initial increasing time in the CEUS for PTC was later, while the enhancement level and PEAK were lower than those of the surrounding normal tissues. However, there were no significant differences in the regression time between the PTC and normal surrounding tissues. The CEUS of PTC exhibited the slow-in and low-enhancement pattern and the initial increasing time, peak time and MTT in the PTC lesions was significantly negatively correlated with the expression levels of P53 and Ki67 in the tumor tissues. Furthermore, the regression time in PTC was also negatively associated with the expression levels of P53 and Ki67 in the tumor, but with no statistical significance. This phenomenon was probably due to the fact that there were plenty of newly generated blood vessels in the tumor tissue (23-25), and the malignant growth destroyed various tissue structures (including the blood vessels), resulting in different degrees of cirrhosis, necrosis and liquefaction. At the same time, the blood vessels are chaotic and irregular, and the peripheral blood vessels are finer, leading to increased blood flow resistance and, subsequently, inefficient arrival of the contrast agents. Furthermore, at the early stage, the tumor vascular bed and arteriovenous fistula are not formed and the blood supply is not sufficient, which may be associated with the delayed contrast perfusion. The delayed contrast perfusion in the lesion tissue, together with the sufficient blood supply in the normal surrounding thyroid tissue, contributes to the obvious contrast in the perfusion patterns.

P53 and Ki67 participate in the regulation of the cell cycle and apoptosis through various pathways, which are also involved in the proliferation, infiltration, and metastasis of tumors. They are important indicators reflecting the activity of tissue cells. Studies have indicated that the positive expression rates of P53 and Ki67 in the PTC are 88.4 and 66.7%, respectively (26-28). Furthermore, it has been suggested that the expression levels of P53 and Ki67 are able to reflect the proliferative activity of thyroid tumor cells, which may contribute to the determination of the biological behavior of thyroid carcinoma. In the present study, the results suggested that P53 and Ki67 were positively expressed in PTC tissues, which were significantly associated with age (<45 years; which has been updated to 45-55 years in the 8th edition of the Clinical Staging Criteria of TNM from the AJCC), local infiltration, lymph node metastasis and number of lesions, rather than the gender, tumor size, histological type or degree of differentiation. These results provide evidence for the pathogenesis, development and prognosis of PTC in the clinic. Analysis of recurrence after RFA indicated that the presence of lymph node metastasis, age <45 years, local infiltration, peak intensity and MTT were major factors influencing post-treatment recurrence, which was in line with the results from a previous study (28). Early recurrence is closely associated with the biological behavior of tumors, including tumor cell proliferation, as well as structural changes within the tumors. Along with the tumor growth, the vessels within the tumor lesions are destroyed and re-constructed, with an increased proportion of nourishing arteries within the tumor, which is linked to higher recurrence at a later stage (25).

In the present study, 72 patients were followed up for 18 months. During the follow-up period, no recurrence was reported after RFA. The relatively short follow-up period and limited sample size represent limitations for the prediction of disease recurrence. Furthermore, no stratification analysis was performed based on the disease staging, which may have affected the results. Previous studies concerning the ultrasound examination of PTC are mainly clinical studies or basic research articles. As another limitation, the TNM stage may have had an impact on the disease prognosis. As the conventional RFA ablation range was 2-4 cm, only T1 cases were included in the present study and the inclusion of N\M was moderately adjusted. The prognosis for cases of other TNM stages after RFA treatment will be investigated in further in-depth studies in the future.

P53/Ki67 are common tumor markers, which have, however, not been suggested as indicators for the clinical diagnosis according to the clinical guidelines for thyroid cancer, and have not been implemented in hospitals. The present study was also in line with previous basic studies on the clinical application of P53/Ki67 in diagnosing this type of tumor. At the same time, due to the numerous types of thyroid cancer, further in-depth studies should be performed focusing on the analysis of P53/Ki67 contents in different thyroid cancer types in the future. In addition, in fact, exact tumor markers for PTC still remain to be determined. Most of the markers are still at the clinical research stage. It may be possible that better markers will be available in the future. These issues should be addressed in further in-depth studies.

In conclusion, the present results indicated that CEUS provided additional information for the diagnosis of PTC. CEUS was characterized by delayed enhancement, low perfusion, weak development or uneven perfusion performance. The slow-in, low enhancement pattern (rather than the fast-in and fast-out, high enhancement pattern) may provide additional information and quantitative analysis for the diagnosis of malignant thyroid tumors and distinguishing them from benign lesions. The present results confirmed that P53 and Ki67 were highly expressed in PTC tissues, which may contribute to the current knowledge on the pathogenesis and development of PTC and may be utilized for the prognostication of patients. Furthermore, the ultrasound-guided RFA was able to effectively control PTC lesions and the quantitative parameters from the ultrasound and clinicopathological features proved to be effective predictive factors for disease recurrence after RFA. The present results provide further knowledge on gene mutations associated with PTC based on immunohistochemistry and in the future, genetic analysis may provide evidence to benefit the clinical treatment. Based on the performance of the ultrasound imaging, the estimation of biological characteristics and associated risk factors may provide an accurate evaluation and powerful evidence for the clinical diagnosis and treatment of PTC.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

DW, XZ and ML contributed to the study design, experiment performance, data collection and analysis, and manuscript preparation. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the ethics committee of Taihe Hospital, Hubei University of Medicine, and all patients had provided written informed consent.

Patient consent for publication

All patients had provided written the informed consent and consent for publication.

Competing interests

All authors declare that they have no competing interests.

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