

Clinical value of matrix metalloproteinase-2 and -9 in ultrasound-guided radiofrequency ablation treatment for papillary thyroid carcinoma

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Abstract

Objective: This study aimed to investigate serum matrix metalloproteinase (MMP)-2 and MMP-9 levels in patients with papillary thyroid carcinoma (PTC).

Methods: Forty-one patients with PTC undergoing ultrasound-guided radiofrequency ablation (RFA) and 56 controls were included. Serum MMP-2 and MMP-9 levels were determined by enzyme-linked immunosorbent assay before and after surgery. Potential affecting factors were evaluated by logistic regression analysis.

Results: Serum MMP-2 and MMP-9 levels were significantly higher in PTC patients compared with controls, and decreased significantly after surgery. According to receiver operating characteristic curve analysis, diagnostic values for preoperative serum MMP-2 and MMP-9 levels were 82.4% and 86.6%. There was no contrast-agent perfusion in the ablation zone in 88.5% of lesions, and enhancement within or at the lesion edge in 11.4%. The volume reduction at 3 months' follow-up was >40%. Age, microcalcification, irregular shape, and lesion diameter and number were influencing factors for PTC. Age, and lesion diameter and number were independent risk factors, while calcification and morphology were protective factors.

Conclusion: Serum MMP-2 and MMP-9 levels have important clinical values for the diagnosis and treatment of PTC by RFA. Preoperative serum MMP-2 and MMP-9 levels, combined with other affecting factors, contribute to disease prognosis.

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Keywords

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Introduction

Papillary thyroid carcinoma (PTC) is a common thyroid malignancy, accounting for about 1% of systemic malignancies.¹ PTC is currently characterized by a high clinical incidence and low mortality.² Recent improvements in high-frequency ultrasonic diagnostic technologies and the application of ultrasound-guided puncture techniques have led to an apparent increase in the incidence of PTC year by year.³⁻⁵ Regarding the diagnosis of thyroid tumors, the palpation detection rate for thyroid micro-tumors in the general population is about 4%, compared with as high as 20% to 50% for high-frequency ultrasound,⁶ which has thus greatly improved disease diagnosis.

Surgical resection is a routine treatment for thyroid tumors, but the recurrence rate is usually high, and the consequent reduction in thyroid function can seriously affect the patient's quality of life.⁷ Associated with the increasing detection rate of thyroid tumors and the pursuit of minimally invasive treatments, radiofrequency ablation (RFA) has been gradually applied in the clinic. RFA uses local hyperthermia to cause tissue necrosis. The thermal effects do not depend on the tissue type, and most lesions can be completely eliminated by RFA.^{8,9} RFA has thus become a novel local treatment for tumors.

The clinical diagnosis of benign and malignant thyroid tumors currently depends on the clinical manifestations and pathological examinations. However, the clinical manifestations are mostly derived from

subjective empirical analysis, while a final pathological diagnosis involves invasive procedures, with less-satisfactory specificity. It is therefore necessary to identify appropriate diagnostic and predictive tumor markers.

The collagenases matrix metalloproteinase (MMP)-2 and MMP-9 can degrade type IV collagen in the basement membrane, with important implications for tumor angiogenesis, and tumor cell invasion and metastasis.¹⁰ MMP-2 and MMP-9 expression levels were found to be up-regulated in thyroid cancer tissue;¹¹ however, these studies mostly examined pathological tissues after invasive surgery, and less-invasive measures, such as serum levels of MMP-2 and MMP-9, have been less-well considered. In this study, we detected serum levels of MMP-2 and MMP-9 in patients with PTC before and after ultrasound-guided RFA. We also determined the therapeutic effects of RFA during the follow-up period, and investigated the relevant prognostic factors.

Materials and methods

Study subjects

Patients who underwent ultrasound-guided RFA in our hospital from May 2017 to October 2018 were included in this study. The inclusion criteria were as follows: (1) patients diagnosed by preoperative fine needle aspiration cytology; (2) no history of neck surgery; and (3) patients requiring minimally invasive treatment for aesthetic reasons and because of neck oppression,

with anxiety. The exclusion criteria were as follows: (1) benign lesions confirmed by fine needle aspiration cytology; (2) history of neck surgery; and (3) severe coagulopathy. Peripheral venous blood samples were obtained from the included patients before and at 1, 3, 6, and 12 months after the operation, and serum levels of MMP-2 and MMP-9 were determined. Additional subjects with confirmed benign thyroid nodules without RFA were included as a control group. Prior written informed consent was obtained from all patients and the study was approved by the ethics review board of our hospital.

Preoperative preparation

The number, size, nature, echo, boundary, morphology, calcification, surrounding halo, and nodular blood flow distribution of the tumors were assessed before the operation. After skin disinfection, local anesthesia was performed with 2% lidocaine solution. A total of 2 mL Sonovue (Bracco, Milan, Italy) was injected via the elbow vein and the blood-supply characteristics were then evaluated by contrast-enhanced ultrasound (CEUS) of the ablation-targeted lesions using a Mylab90 ultrasonic device with 10-MHz probe (Esaote, Shenzhen, Guangdong, China).

According to the location of the thyroid nodules, the thyroid and carotid space, thyroid and tracheal space, thyroid and esophageal space, and posterior thyroid space (recurrent laryngeal nerve) were separated using a saline and lidocaine mixture, based on the intraoperative conditions, to form a liquid separation zone to protect these structures from thermal damage.

Ablation treatment

Under ultrasound guidance, the tip of the RFA needle (rated power 250 W, output frequency 470 kHz) was accurately

penetrated into the nodule, and RFA was performed using an Olympus-Celon Power RFA System (Germany) in mobile mode,¹² following the from-deep-to-shallow principle. The lesions were subjected to multi-pointed and multi-faceted ablation until the thyroid tissue layer with the nodules was completely covered by the strong echo generated by heat accumulation. The whole process was carried out under continuous ultrasound monitoring. A high-echo area was produced in the ablation zone during the ablation treatment. The position of the electrode needle was gradually adjusted according to the lesion size. After ensuring that there was no residual enhancement in the ablation zone, the ablation needle was removed and the ablation was completed. After the operation, an ice compress was applied for 24 h to avoid skin burns.

Serum MMP determination

For all subjects, 6 mL venous blood was collected from the elbow vein under fasting conditions before and after the operation, respectively. Blood samples in the control group were collected after ultrasound contrast examination. The blood samples were placed at room temperature for 30 minutes, and then subjected to centrifugation at $2000 \times g$ for 15 minutes. The serum was harvested and serum levels of MMP-3 and MMP-9 were determined using enzyme-linked immunosorbent assay (ELISA) kits (Boster Bioengineering, Wuhan, Hubei, China).

Follow-up and efficacy evaluation

Immediately after the operation, the ablation range was evaluated by CEUS. If residual tissues were detected, ablation was repeated immediately. Ultrasound detection was performed at 1, 3, 6, and 12 months after surgery to determine the nodule sizes and volumes. The volume-reduction rate

was calculated according to the following formula: volume reduction rate = (preoperative volume – follow-up volume)/preoperative volume \times 100%. Echo and blood-flow changes in the ablation zone were also observed and analyzed. The efficacy was determined based on the criteria for RFA for treating tumors:¹³ disappearance of nodules indicated by complete disappearance of blood flow confirmed by ultrasonography indicated complete cure; nodule volume reduced by $\geq 50\%$ indicated marked effect; and nodule volume reduced by 25% to 50% indicated improvement.

Clinicopathological features

Information on ultrasound-based clinicopathological features, including number, size, and calcification of the lesion, were obtained.

Statistical analysis

Data were expressed as mean \pm standard deviation. Statistical analysis was performed using IBM SPSS Statistics for Windows, version 19.0 (IBM Corp., Armonk, NY, USA). Comparisons between groups were performed using χ^2 tests. Potentially related factors were analyzed by univariate or multivariate logistic regression. The prognostic predictive effects of serum MMP-2 and MMP-9 levels were evaluated by receiver operating characteristic (ROC) curve analysis. $P < 0.05$ was considered statistically significant.

Results

Patients

Forty-one patients with PTC (96 lesions) were enrolled, including 30 women and 11 men (mean age 41.5 ± 16.8 years; range 20 to +65 years). The control group included 56 patients with confirmed benign thyroid nodules, including 39 women and

17 men (mean age 45.8 ± 15.3 years, range 20–65 years).

Serum MMP-2 and MMP-9 levels before and after treatment

The characteristics of the ultrasound images in the included subjects are shown in Table 1. Serum levels of MMP-2 and MMP-9 were measured before and after treatment. Serum levels of MMP-2 and MMP-9 were significantly higher in patients with PTC compared with the control subjects ($P < 0.05$). Serum levels of MMP-2 and MMP-9 had declined at 1 month after the operation compared with before surgery, but the difference was not significant. However, serum levels of MMP-2 and MMP-9 had declined significantly in the PTC patients at 3, 6, and 12 months (all $P < 0.05$) (Table 2). These results suggest that changes in serum MMP-2 and MMP-9 levels between before and after surgery may have significant implications for the therapy of PTC.

ROC curve analysis of preoperative serum MMP-2 and MMP-9 levels

Preoperative serum MMP-2 and MMP-9 levels were used as potential diagnostic indicators. In the 41 patients with PTC, the predictive probability from the regression model was used as the diagnostic results, and the gold standard classification criteria were used as the pathological results. ROC curves were obtained accordingly. The area-under-curve (AUC) values for serum MMP-2 and MMP-9 levels were 82.4% and 86.6%, respectively (Figure 1). These results suggest that serum levels of MMP-2 and MMP-9 could contribute to the disease diagnosis.

Evaluation of RFA efficacy

We also evaluated the efficacy of RFA. CEUS of the 96 lesions before ablation showed hypo-enhancement in 18 nodules, iso-enhancement in 67 nodules, and slight hyper-enhancement

Table 1. Characteristics of thyroid ultrasound images.

	PTC patients		Normal control	
	n	%	n	%
Lesion number				
One	10	24.3	10	17.8
Two	8	19.5*	18	32.1
Multiple	23	56.0	28	50.0
Lesion size				
≤2 cm	27	65.8*	11	19.6
>2 cm	14	34.1	45	80.3
Calcification				
Microcalcification	19	46.3*	25	44.6
Coarse calcification	7	17.0	18	32.1
Morphology				
Regular	14	34.1	38	67.8
Irregular	27	65.8*	18	32.1
Age				
<45 years	29	70.7*	34	60.7
≥45 years	12	29.2	22	39.2

* $P < 0.05$ compared with the control group.

Table 2. Serum matrix metalloproteinase-2 and -9 levels in controls and in patients with papillary thyroid carcinoma before and after treatment.

	MMP-2	P	MMP-9	P
Controls	547.32 ± 98.36	–	126.62 ± 19.26	–
PTC patients				
Before surgery	794.54 ± 152.59	0.04	299.98 ± 70.48	<0.01
1 month after surgery	481.21 ± 102.87	0.06	223.25 ± 68.00	0.13
3 months after surgery	404.36 ± 88.53	0.04	201.65 ± 65.31	0.03
6 months after surgery	281.85 ± 67.65	0.02	184.64 ± 64.82	0.01
12 months after surgery	171.60 ± 72.75	<0.01	169.07 ± 64.16	<0.01

MMP, matrix metalloproteinase; PTC, papillary thyroid carcinoma.

in 11 nodules. Ultrasound examination after ablation showed no contrast-agent perfusion in the ablation zone in 85 lesions (88.5%), and enhancement of different degrees at the edge or inside the lesion in the other 11 lesions (11.4%), and the ablation area was gradually reduced with prolonged ablation (Figure 2). There was no significant change in ablation volume in any patients at 1 month after surgery, compared with before surgery. However, the volume was reduced

by >40% at 3 months of follow-up, compared with before surgery ($P < 0.05$) (Table 3). These results showed that RFA treatment could effectively reduce the tumor volume in patients with PTC.

Influence of relevant factors on disease prognosis

The clinical data of patients before RFA were retrospectively analyzed by

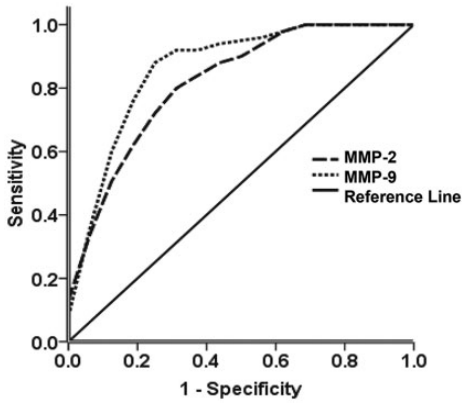
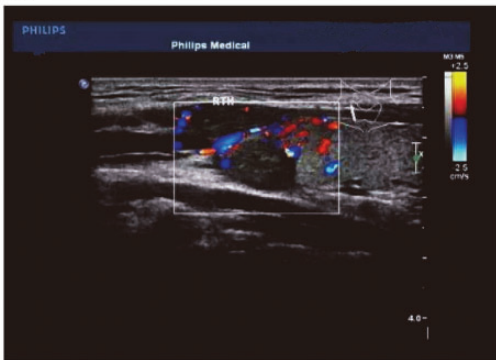


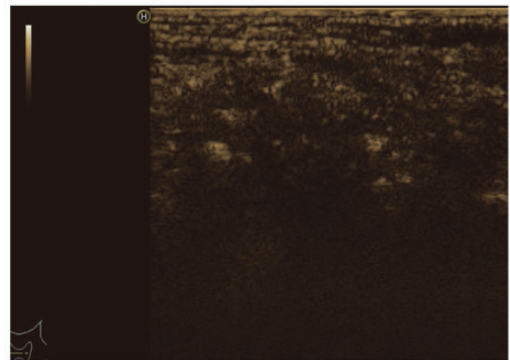
Figure 1. ROC analyses of serum MMP-2 and MMP-9 levels. MMP, matrix metalloproteinase.

logistic regression to identify factors that may affect the disease prognosis. Age, microcalcification, irregular shape, and diameter and number of lesions were significant influencing factors for PTC ($P < 0.05$). The hazard ratios (HRs) for age, and lesion diameter and number were >1 , indicating that these represented independent risk factors. In contrast, the HRs for microcalcification and irregular shape were negative, indicating that a greater degree of calcification and regular shape were associated with lower risks of developing the disease, and were thus protective factors (Table 4).

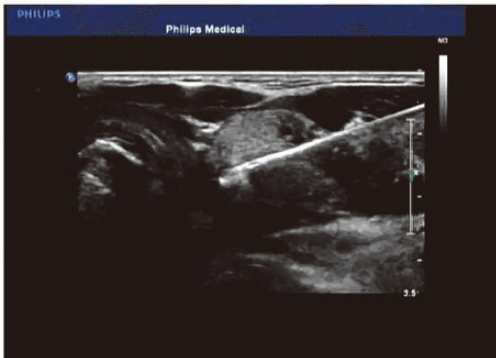
(a)



(b)



(c)



(d)

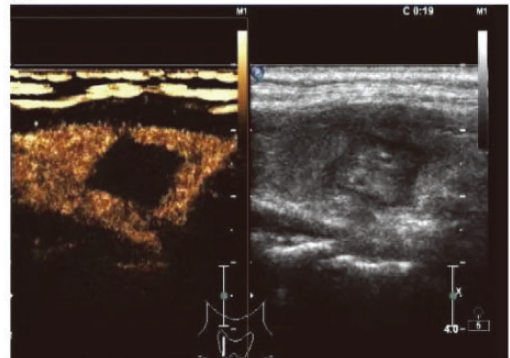


Figure 2. Efficacy evaluation of radiofrequency ablation. (a) Two-dimensional (2D) ultrasound showing obvious blood-flow signals around the tumor and fewer signals within the tumor. (b) Preoperative contrast-enhanced ultrasound showing no obvious enhancement in the lesion, with low-perfusion performance. (c) In the 2D imaging localization, the ablation needle was inserted into the tumor for ablation. (d) The tumor was completely ablated, with no blood-flow signal, at 1 year after ablation.

Discussion

PTC is a type of thyroid tumor with a high incidence,¹⁴ which has been increasing rapidly worldwide.^{15,16} Most thyroid tumor cases are currently diagnosed by histopathological or cytological detection. However, it is difficult to distinguish between benign and malignant papillary hyperplastic nodules, and it is therefore difficult to diagnose PTC. There is also a lack of effective and specific prognostic molecular markers for PTC.¹⁷ The relationship between MMPs and tumors is a current hotspot of modern cancer research. MMPs play important roles in pathophysiological processes, such as the dynamic extracellular matrix balance, as well as in tissue remodeling and repair.¹⁰ Tumor cells may induce the matrix to secrete MMPs via a series of signaling pathways, thus providing

favorable conditions for tumor cell invasion and metastasis.

Surgical resection is a traditional method for the treatment of thyroid nodules.¹⁸ However, the extensive surgical trauma, unsightly neck scars, and risks of laryngeal nerve injuries, postoperative recurrence, and multiple operations, mean that increasing numbers of patients are opting for minimally invasive ablation methods. RFA is a thermal ablation therapy that uses high-frequency alternating electromagnetic waves generated by the radiofrequency instrument inserted into the tumor tissue to accumulate heat by rapid friction of positive and negative ions within the cells, causing local coagulation in the tumor tissue, which is then removed by the body's immune system.¹⁹ Reduction rates for benign thyroid nodules of 33% to 58% after 1 month of ablation and 51% to 92% after 6 months of ablation have been reported.²⁰ In this study, ultrasound performed immediately after ablation of 96 lesions showed no perfusion of contrast agents in 88.5% lesions, and enhancement to varying degrees at the edge or inside the lesion in 11.4% of lesions. Although there were no significant changes in lesion volume at 1 month after the operation, the lesion volumes were reduced by 40% to 80% at 3 months after surgery. Considering that the ablation effects might be associated with the heat and the ablation needle, a

Table 3. Volume reductions after radiofrequency ablation of papillary thyroid carcinomas.

	Lesion volume (cm ³)	Reduction rate (%)
Before surgery	2.95 ± 0.84	—
1 month after surgery	2.45 ± 0.69*	19.23
3 months after surgery	1.95 ± 0.57**	46.25
6 months after surgery	1.66 ± 0.49**	68.36
12 months after surgery	1.07 ± 0.24**	81.57

* $P > 0.05$, ** $P < 0.05$ compared with before surgery

Table 4. Logistic regression analysis of relevant risk factors for disease prognosis.

	B	HR	95%CI		P
			Lower limit	Upper limit	
Age	0.174	1.190	1.006	1.408	0.042
Microcalcification	-4.874	1.008	0.000	0.494	0.022
Irregular morphology	-2.694	2.068	0.005	0.990	0.049
Lesion diameter	2.001	7.393	1.623	33.676	0.010
Lesion number	1.289	3.629	0.182	72.516	0.033

HR, hazard ratio; CI, confidence interval.

fine needle is good for mobile conformal ablation; the high-frequency alternating electromagnetic wave only circulates in the effective region between the two needle tips, thus allowing accurate control of the ablation zone. The ablation safety zone around the PTC was relatively small in the current study, and the nodule-reduction rate after ablation was thus relatively greater.

We analyzed the serum levels of MMP-2 and MMP-9 in PTC patients by ELISA. Preoperative serum levels of both enzymes were significantly higher in patients with PTC compared with patients with benign thyroid nodules. Regarding changes in serum MMP-2 and MMP-9 levels, the AUC values based on the ROC curves were 82.4% and 86.6% for MMP-2 and MMP-9, respectively, suggesting satisfactory clinical diagnostic and prognostic values. In the present study, serum levels MMP-2 and MMP-9 were lower in the first month after surgery compared with before surgery, but the difference was not significant. This might be because, before ablation, the tumor parenchyma induced the tumor stroma to produce larger amounts of MMP-2 and MMP-9, which were released into the blood. These results suggest that failure to completely ablate the tumor or tumor recurrence may result in the secretion of high levels of MMP-2 and MMP-9 into the blood. However, serum levels of MMP-2 and MMP-9 were significantly decreased at 3, 6, and 12 months after surgery compared with before surgery, suggesting that serum MMP-2 and MMP-9 were secreted by the tumor. The lesions disappeared after PTC ablation, thus reducing the secretion of MMP-2 and MMP-9 and thereby reducing the degradation and destruction of type IV collagen, protecting the basement membrane of normal tissues and inhibiting the growth and metastasis of tumor cells. The current results also showed that age, microcalcification, irregular shape, and lesion diameter and number

were risk factors for PTC. Logistic regression analysis showed that age (≤ 45 years) was an important risk factor for PTC, in line with the findings of Yu et al.²¹ Microcalcification is caused by the deposition of calcium salts at the tip of the nipple or the secretion of calcium salts by the tumor itself, and has been considered to be the most specific sign of PTC. In this study, the incidence of microcalcification was higher in PTC patients compared with the control group, and logistic regression identified it as an independent risk sign for thyroid PTC. Our results also identified irregular morphology, two nodules, and a nodule diameter ≤ 2 cm as danger signs for PTC, largely consistent with previous findings.²²

This study had some limitations. It was a single-center study with a relatively small number of cases. Moreover, the relevant thyroid hormone analysis and other serum indicators could not be followed up for a longer period.

In summary, the results of this study showed that RFA could shrink or eliminate thyroid lesions, thus representing a minimally invasive, safe, and effective method for treating PTC. Furthermore, serum levels of MMP-2 and MMP-9 before RFA could provide a valuable reference for the diagnosis of PTC. These serological indexes, combined with relevant risk factors, may also help to predict the prognosis of PTC after ablation.

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Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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