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Initial Report of the Results of Percutaneous Laser Ablation of Benign Cold Thyroid Nodules: Evaluation of Histopathological Changes After 2 Years

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Abstract Percutaneous laser ablation (PLA) is a new method to be used for reducing the volume of symptomatic cold benign thyroid nodules. We aimed to introduce the long-term histopathological effects of PLA procedure in thyroid nodules. A 42-year-old male patient with 18.7-mL nodule volume was transferred a total of 720 J of energy with 3-W power. A 50-year-old female patient with 14.1-mL volume nodule was transferred a total of 3,300 J with 5-W power. A 61-year-old female patient with 49.7-mL volume nodule was transferred a total of 4,200 J with 5-W power. The thyroidectomy materials were examined histopathologically 2 years later. In histopathologic examination, it was observed that the periphery of ablated area was organized with hyalinized connective tissue and the center was organized with necrosis and carbon particles. Concurrently, in the center, we detected calcifications and colloid and foreign-body giant cells against colloid in one patient. We did not encounter any pathologic findings in the tissue adjacent to ablated area. In the cold benign thyroid nodules taken out by thyroidectomy 2 years after PLA procedure, necrosis and hyalinization were detected histopathologically. PLA procedure in cold benign thyroid nodules seems to be safe according to long-term histopathological findings.

Keywords thyroid · nodule · percutaneous laser ablation · thyroidectomy · histopathology

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Introduction

Percutaneous laser ablation (PLA) procedure was first performed in animal model by Bown et al. [1] and in liver tissue in an animal model by Pacella et al. [2]. Pacella et al. [3] carried out the PLA procedure on thyroid tissue in in vitro thyroidectomy material. In this study, effects of PLA on thyroid tissue with various energies and durations were detected. This initial study has been a guide to in vivo experimental studies on human thyroid tissue in benign thyroid nodules. Imaging-guided percutaneous thermal ablation procedure was shown to be successful among non-surgical methods targeting cellular destruction in malignant and benign thyroid lesions [4–6].

Infrared diode laser or neodymium–yttrium aluminum garnet laser was used as laser power supply in associated studies [5–11]. PLA has been shown to be quite successful in reducing nodule volume in cold benign thyroid nodules [7, 8, 11]. Valcavi et al. carried out the most extended study on cold benign thyroid nodules on 119 patients and by the end of the first year they provided a nodule volume reduction of more than 50% [12]. Results of two randomized studies have demonstrated that satisfactory responses were obtained in most of the patients with PLA on cold benign thyroid nodules [13, 14].

The first study to investigate the cytopathologic effects of PLA procedure on cold benign thyroid nodules 1 year after the procedure was reported by us [11]. Now, we represent the histopathological findings of three cases that had cold benign thyroid nodules and underwent thyroidectomy 2 years after the procedure.

Patients and Method

Patients

Case 1 A 42-year-old male consulted with compression symptoms. A nodule was detected in the right lobe. A total of 720-J energy was transferred to the nodule, 18.7 mL in volume, within 240 s with 3-W power. The procedure was planned in two stages, first the inferior half of the nodule and then the superior half. But a subcapsular hematoma developed during the first stage [15]. By 1 month after the procedure, the hematoma had completely resorbed. The initial nodule volume of 16.8 mL was measured as 16 mL at the end of the second year. Compression symptoms continued, and he was asked to undergo surgery. After his agreement, by the second year, he underwent right total–left subtotal thyroidectomy and isthmectomy.

Case 2 A 50-year-old female consulted with compression symptoms. She had undergone thyroidectomy three times previously. After each operation, nodules recurred large enough to cause compression symptoms again. The surgeon had refused to operate her for the high risk of laryngeal nerve paralysis at the fourth operation. She admitted to our clinic especially to undergo PLA treatment. She had multiple nodules on both sides and the predominant nodule on left side was 14.1 mL in volume. Procedure was carried out on this nodule twice. A total of 3,300 J of energy was transferred as 1,500 J of energy within 300 s with 5-W power in the first one and 1,800 J of energy within 360 s with 5-W power in the second one. Although there was a decrease to 8.8 mL in nodule volume, an increase to 14 mL was observed again at the end of the 24th month. After this stage, she left her follow-up in our clinic. We learned that another surgeon performed left lobectomy on the nodule side as fourth operation. But, complications like laryngeal nerve paralysis and dysphonia developed in this case. Then, she returned to our clinic to keep on her follow-up. She brought the pathology specimens for further evaluation. Specimens were examined histopathologically in our center.

Case 3 A 61-year-old female consulted with compression symptoms. A nodule, 49.7 mL in volume, was detected on the left lobe of the thyroid gland. The procedure was planned in two stages, first the inferior half of the nodule and then the superior half. A total of 4,200 J of energy was transferred as 3,000 J of energy within 600 s with 5-W power in the first one and 1,200 J of energy within 240 s with 5-W power in the second one. Although there was a decrease to a 20.8 mL in nodule volume, an increase to 35.8 mL was observed again at the end of the 24th month. She stated that she had an increase in symptoms and agreed to undergo surgery. Then, she underwent left total lobectomy, right subtotal lobectomy, and isthmectomy.

A 24-month follow-up of laboratory, scintigraphy, fine-needle aspiration biopsy (FNAB), and volume values of cases are presented on Table 1.

Ultrasonography

All the patients were evaluated by ultrasonography (US) for detection of nodule textures and volumes before PLA procedure. Pro 200 Scanner (GE Medical Systems, Kyunggido, Korea) was used for these ultrasonographic measurements and all the procedures. Volume of an ellipsoid object is calculated by the formula $V=(a\times b\times c)\times\pi$ / 6 (mL), where a, b, and c are dimensions of the object.

Laboratory

Serum thyrotropin (TSH; normal range, 0.4–4 µIU/mL, BIO-DPC Immulite 2000 Third generation kit, Los Angeles, CA, USA), serum-free triiodothyronine (fT3; normal range, 1.57–4.71 pg/mL, BIO-DPC Immulite 2000 competitive chemiluminescent enzyme immunoassay, Los Angeles, CA, USA), serum-free thyroxine (fT4; normal range, 0.85–1.78 ng/dL, BIO-DPC Immulite 2000 competitive chemiluminescent enzyme immunoassay, Los Angeles, CA, USA), serum antithyroid peroxidase antibodies (anti-TPOAb, normal range, 0–35 IU/mL, BIO-DPC Immulite 2000 chemiluminescent sequential immunometric assay Los Angeles, USA), serum antithyroglobulin antibodies (anti-TgAb, normal range, 0–40 IU/mL, BIO-DPC Immulite 2000 chemiluminescent sequential immunometric assay Los Angeles, CA, USA), thyroglobulin (Tg; normal range, 0–55 ng/mL, BIO-DPC Immulite 2000 Third generation kit, Los Angeles, CA, USA), and calcitonin (normal range, 5.0–11.5 pg/mL, BIO-DPC Immulite 2000 chemiluminescent immunometric assay, Los Angeles, CA, USA) tests were used in laboratory studies.

The nodules were cold by technetium-99m (^{99m}Tc) scintigraphy. As the result of US-guided FNAB of nodules, benign thyrocytes were observed. Patients were asked to undergo surgery for their compression symptoms. But all three refused surgical treatment. So PLA procedure was suggested for thyroid nodules. Patients accepted the suggestion and informed consent forms were obtained. Also, the

Table 1 Twenty-four-month follow-up of FNAB, laboratory, scintigraphy, and volume results of cases

Cases	Before PLA			1st month			3rd month			6th month			12th month			24th month		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
TSH (μIU/mL)	1.11	0.79	0.44	0.73	1.08	0.51	0.80	0.92	0.48	1.2	0.53	0.46	1.09	0.62	0.44	1.01	0.80	0.55
f T3 (pg/mL)	3.50	3.30	2.1	3.20	3.40	2.4	3.0	3.20	2.3	3.1	3.60	2.6	2.8	3.50	2.3	2.9	2.90	2.2
f T4 (ng/dL)	1.3	1.2	1.2	1.5	1.4	1.6	1.4	1.5	1.7	1.6	1.8	1.8	1.3	1.9	1.4	1.4	1.5	1.9
Anti- TPO (IU/mL)	10	10	35	55	10	82.9	70	12.4	145	30	10	74	20	10	25	20	10	20
Anti-Tg (IU/mL)	20	20	20	60	25.4	25.4	94	47	47	20	21.4	21.4	20	20	20	20	20	20
Tg (ng/mL)	15	20	35	128	30	40	75	80	20	30	20	20	20	20	20	10	5	20
Scintigraphy	C	C	C															
FNAB	B	B	B										B	B	B			
Calcitonin (pg/mL)	5	7	9													5	5	7
Volume (mL)	16.8	14.1	49.7	16.7	11.7	36.3	14.1	10.4	22.2	14.9	8.8	20.8	15.9	13.9	31.8	16.0	14	35.8

Normal laboratory ranges: TSH 0.4–4 μIU/mL, fT3 1.57–4.71 pg/mL, fT4 0.85–1.78 ng/dL, anti-TPOAb 0–35 IU/mL, anti-TgAb 0–40 IU/mL, thyroglobulin 0–55 ng/mL, calcitonin 5.0–11.5 pg/mL

B benign, *C* cold

approval of the local ethics committee was obtained (Ataturk Education and Research Hospital, Ankara; date November 9, 2004, record no. 2004/10/009).

PLA Procedure

Twenty-one-gauge (G; 0.8 mm) needles prepared especially for each nodule were used. Sedation was obtained by 7.5 mg oral diazepam (SABA Pharmaceuticals, Istanbul, Turkey). After a careful sterilization on neck area where PLA would be applied, 1 to 2 mL 2% lidocaine (Adeka Pharmaceuticals, Samsun, Turkey) was injected slowly through subcutaneous tissue. Then, the custom-produced needle was inserted with US guidance, into inferior part of nodule along its long axis. Proper position of needle tip within the nodule was confirmed by two-plane US images. Then, a bare flat-end fiber, 400 μm in diameter, was inserted through needle lumen. The fiber tip was aligned with the needle tip. Then, the fiber tip was advanced 5 mm to provide direct contact with the tissue. Two-plane US images were obtained to confirm that the fiber tip was at least 2 cm from surrounding vital structures. Laser ablation was performed by an electronic infrared diode laser at 810-nm wavelength using a gallium–aluminum–arsenide power source (model 15 plus; Diomed, Cambridge, UK) [6, 7, 9, 11, 14–16]. According to nodule volume, 3–5-W power was used for the treatment.

In the first case, no changes in nodule sizes and symptoms were detected. In the two other cases, despite the nodules becoming smaller after PLA, they started to expand again. Because they did not have any attenuation in their compression symptoms 2 years after the procedure, cases 1 and 3 were asked for surgical treatment again. They accepted the surgery. The second case underwent surgery externally by leaving our follow-up despite of us not thinking of any surgery. They underwent surgical procedures appropriate for their clinical characteristics.

Nodule, localization of PLA application, and area to be examined especially were demonstrated to the pathologist (SU) by US images before thyroidectomy and on macroscopic postthyroidectomy material.

Results

The changes in thyroid nodule 24 h after PLA procedure detected by sonography were demonstrated in Fig. 1.

In macroscopic examination, multiple tissue samples were obtained primarily from ablated area and its surrounding tissue and from other sides. All the samples of the thyroidectomy material were fixed in 10% buffered formalin prior to the routine processing of the paraffin-embedded block. Four-micrometer-thick sections were cut and stained

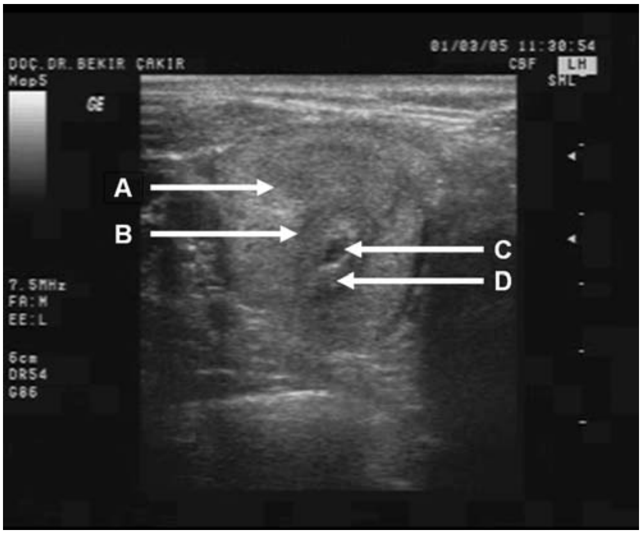

Fig. 1 *A* Nodule areas not affected by PLA procedure; *B* demarcation line around the debris (circular part sonographically seen as a hypoechoic halo around area *D*); *C* coagulation necrosis at the point of application site of the nodule (sonographically; hypoechoic area); *D* hyperechogenic debris area at the periphery of area *C*

with hematoxylin and eosin. All glass slides were examined under light microscope.

Similar histopathologic results were obtained from the nodules of these three patients operated on the second year after laser application. In histopathological examination, a triangular-shaped lesion with a wide base was observed in thyroid gland of the first patient. The periphery of this lesion was organized with hyalinized connective tissue and the center with necrosis and carbon particles (Fig. 2a, b). In the other patient, calcifications and a round-shaped lesion with hyalinization in the periphery and necrosis–carbon particles in the center were detected (Fig. 2c, d). In the third patient, hyalinization, carbon particles, necrosis, colloid, and foreign-body giant cell against colloid were observed (Fig. 3a, b). There were no significant findings within the surrounding thyroid tissue in all three patients.

Discussion and Commentary

Various physical, chemical, and biological agents (e.g., hypoxia, mechanical trauma, burns, and deep cold) causing

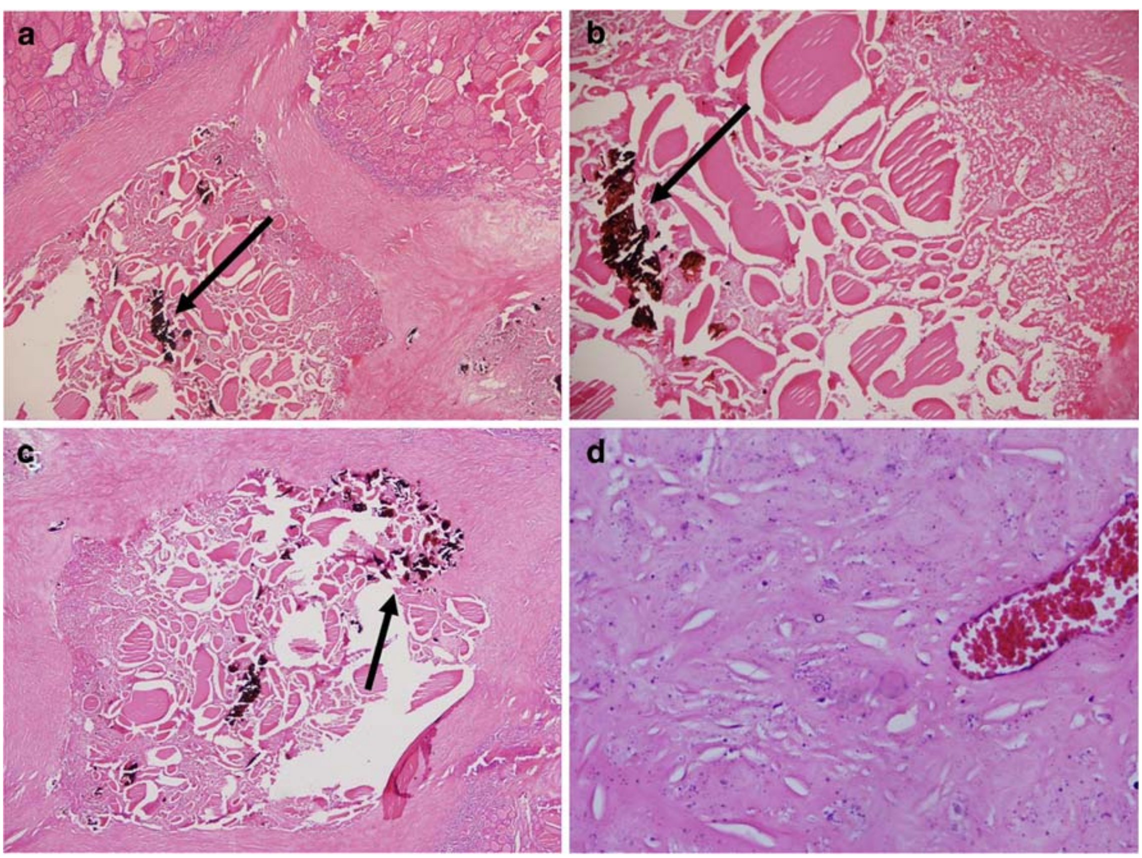

Fig. 2 **a** Necrosis and carbon particles in the center of the lesion surrounded by thyroid follicules and some hyalinized areas (*arrow*; hematoxylin–eosin stain, original magnification ×40); **b** necrotic follicules, colloid, and carbon (*arrow*; hematoxylin–eosin stain, original magnification ×100); **c** hyalinization, necrosis, and carbon particles (*arrow*; hematoxylin–eosin stain, original magnification ×40); **d** hyalinization and calcifications (*arrow*; hematoxylin–eosin stain, original magnification ×40)

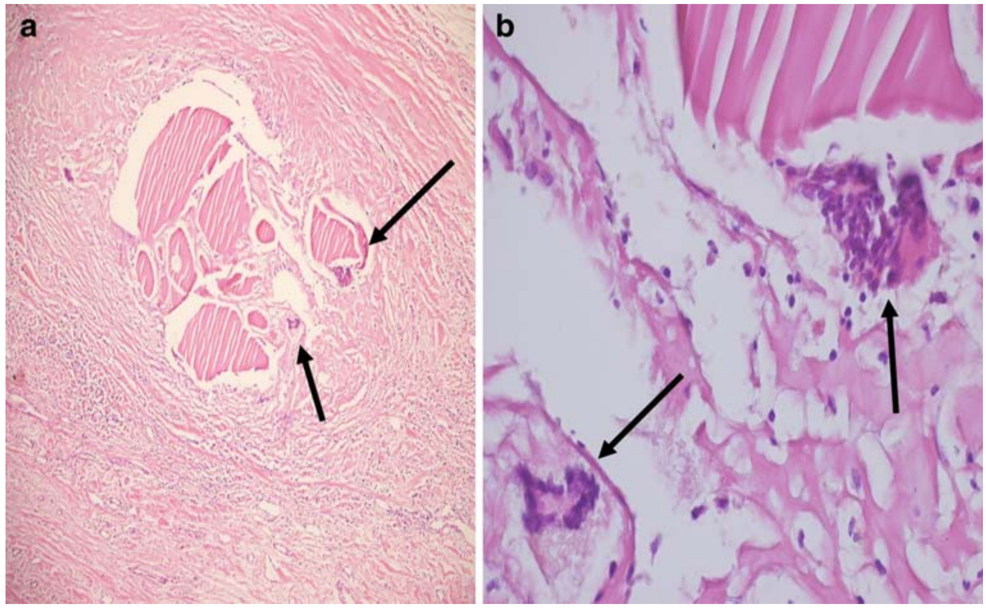

Fig. 3 **a** Hyalinized connective tissue, mononuclear cell infiltration, necrosis, colloid, and foreign body cells (*arrows*; hematoxylin–eosin stain, original magnification ×400); **b** colloid and foreign body cells (*arrows*; hematoxylin–eosin stain, original magnification ×600)

reversible and irreversible cell injury may develop hydropic change, fatty change, necrosis, and inflammation response in tissues [17]. Among these, the thermal effects vary according to the temperature. In the cellular homeostasis, cells become sensitive to damages when the temperature reaches to 42–45°C (hyperthermia). In the point of 60–100°C, a rapid protein coagulation arises by the irreversible damages of cytosolic and mitochondrial enzymes. With a temperature over 105°C, tissue vaporization and carbonization start [4]. It is expected that PLA procedure on thyroid gland, causing tissue warming over 105°C, should give rise to necrosis abolishing tissue skeleton accompanied by vaporization and carbonization.

If, by any cause, necrosis abolishes the tissue skeleton or affects the permanent cells, scar formation develops in the affected tissue. Firstly, necrotic debris, exudate, and fibrin in necrotic tissue are removed by the inflammatory responses. Then, endothelial cells and collagen fibers try to fill the cavity up by migrating to the area and after a while fibrous connective tissue (scar) develops. The scar is now permanent at this area [17]. As in any region of the body, necrosis in thyroid gland, which is formed by laser and abolishes the tissue skeleton, also causes the permanent scar formation in this organ after a while [16].

PLA-induced thermal injury in human thyroid tissue was first described by Pacella et al. They reported the histopathology of single nodules in two patients whose nodules were excised 1 week after PLA. They found central cavitation zone surrounded by zones of carbonization and coagulation necrosis [5]. The reason for observing no hyalinization in this case of Pacella et al. is their making the excision only 1 week after PLA procedure because we detected fibroblasts in the periphery of the lesion by the FNAB 1 year after PLA application [11]. In addition, we determined scar formation in the periphery of the lesion within thyroidectomy material obtained 2 years after PLA [16]. Also, we observed scars in the periphery of the lesion in all three patients included in our study.

In our previous study, we determined necrosis, histiocytes, and nuclear debris in the center, macrophages and multinuclear giant cells in the surrounding area, and fibroblasts in the outer layer of the laser-applied area in FNAB taken 1 year after the procedure [11]. In our only case with hot thyroid nodule which showed growth in sizes and was resected 2 years after PLA, despite the observation of hyalinization and vaporization, necrosis was not detected [16].

In all three patients, among which one had no changes in nodule volume and two others had increase after a decrease trend, similar histopathological findings were observed in cold nodules resected 2 years after PLA. These histopathologic findings were hyalinized connective tissue, necrosis, carbon particles, calcifications, and foreign-body giant cell against colloid. We did not observe any cellular atypia, frequent mitotic structures, or neoplastic changes after PLA.

The main effect of PLA procedure in scintigraphically hot and cold nodules is to provide a decreasing trend in nodule sizes. PLA procedure makes a lasting impact like hyalinization, after the coagulation necrosis it caused initially. Not only did we detect this histopathologically but we also observed that the decreasing trend still keeps going among our patients who were treated with PLA and were still in follow-up. On the other hand, the possibility of a reenlargement in some parts of the thyroid nodules was first reported by us in a hot nodule beginning from the sixth month after PLA application. There can be several reasons for this: (1) owing to the application technique, the formation of the irreversible change, hyalinization, in only a small part of the nodule and reenlargement as a result of an autonomy gained by the thyroid follicule epithelial cells localized at the remainder areas (Fig. 1 (A)) or their proliferation due to endocrine effects, (2) the expected results of natural progress of the thyroid nodules which did not have laser application, no volume changes in 30%, decrease in 30%, and increase in 30% [18]. An enlargement of a nodule that has an increasing trend and had PLA procedure applied on a small part is quite normal.

For a permanent and clear effect on nodules, namely for no reenlargement, we believe that the laser application on a wide part of nodule by a controlled and unharmful method is needed. However, in a randomized controlled study of Døssing H et al., it is reported that the increase rate of the nodules having overall three applications at three different parts of the nodule is 13% more than the ones which had only one application. Also, they had demonstrated that this 13% more decrease in nodule volume was not big enough to make changes in compression symptoms. Therefore, they claimed that it is suitable to perform PLA as a solitary application [9].

Throughout all the control, US and Doppler examinations were performed before and right after the procedure, at the 48th hour, first week, first month, and third month of the procedure; any blood flow increase or edema findings were detected in the nodule parts which did not have PLA application.

Pacella et al. applied low doses of PLA on large (>40 mL) autonomous functional thyroid nodules of two women aged 45 and 65 years old, one week before the thyroidectomy (for each patient, until the total energy of 600 or 1,800 J is reached, with 5-W power and for once). In the US imaging performed 7 days after PLA procedure which was carried out to evaluate the effects of laser prior to surgery and different conditions (cavitation and coagulation necrosis), the lesion was monitored in transverse, longitudinal, and anteroposterior axis. They had demonstrated that Doppler blood flow had entirely disappeared in the application areas, and no edema or blood flow increase was detected by sonography in the areas that were not exposed to laser effects [5]. The results of these two cases give rise to the thought that an enlargement could not be formed by an acute occasion right after the procedure.

Necrosis and hyalinization are detected in PLA-applied thyroid nodules by the end of the first and the second years of the procedure. While necrosis is not seen in hot and growing nodules, it is detected in cold and growing ones. But because we only have one case with a hot and growing nodule, we do not believe that it would be significant to compare hot and cold nodules. Further studies including adequate number of hot and cold nodules are needed to come to a conclusion. We believe that laser application to a bigger part of the nodule in a controlled and unharmful way is needed for a significant and permanent effect, meaning no development again.

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