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Low-Level Laser Therapy in Chronic Autoimmune Thyroiditis: A Pilot Study

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Background and Objectives: Chronic autoimmune thyroiditis (CAT) remains the most common cause of acquired hypothyroidism. There is currently no therapy that is capable of regenerating CAT-damaged thyroid tissue. The objective of this study was to gauge the value of applying low-level laser therapy (LLLT) in CAT patients based on both ultrasound studies (USs) and evaluations of thyroid function and thyroid autoantibodies.

Study Design/Materials and Methods: Fifteen patients who had hypothyroidism caused by CAT and were undergoing levothyroxine (LT4) treatment were selected to participate in the study. Patients received 10 applications of LLLT (830 nm, output power 50 mW) in continuous mode, twice a week, using either the punctual technique (8 patients) or the sweep technique (7 patients), with fluence in the range of 38–108 J/cm^2. USs were performed prior to and 30 days after LLLT. USs included a quantitative analysis of echogenicity through a gray-scale computerized histogram index (EI). Following the second ultrasound (30 days after LLLT), LT4 was discontinued in all patients and, if required, reintroduced. Triiodothyronine, thyroxine (T4), free T4, thyrotropin, thyroid peroxidase (TPOAb) and thyroglobulin (TgAb) antibodies levels were assessed before LLLT and then 1, 2, 3, 6, and 9 months after LT4 withdrawal.

Results: We noted all patients' reduced LT4 dosage needs, including 7 (47%) who did not require any LT4 through the 9-month follow-up. The LT4 dosage used pre-LLLT (96 ± 22 μg/day) decreased in the 9th month of follow-up (38 ± 23 μg/day; $P < 0.0001$). TPOAb levels also decreased (pre-LLLT = 982 ± 530 U/ml, post-LLLT = 579 454 U/ml; $P = 0.016$). TgAb levels were not reduced, though we did observe a post-LLLT increase in the EI (pre-LLLT = 0.99 ± 0.09, post-LLLT = 1.21 ± 0.19; $P = 0.001$).

Conclusion: The preliminary results indicate that LLLT promotes the improvement of thyroid function, as patients experienced a decreased need for LT4, a reduction in TPOAb levels, and an increase in parenchymal echogenicity. Lasers Surg. Med. 00:1–8, 2010.

Key words: autoimmunity; autoantibodies; Hashimoto's thyroiditis; hypothyroidism; laser therapy (LLLT); thyroid; treatment; ultrasonography

INTRODUCTION

Chronic autoimmune thyroiditis (CAT) is the most common cause of hypothyroidism in iodine-replete geographic areas [1]. An autoimmune dysfunction causes humoral and cellular responses that lead progressively to thyroiditis [2]. There is no effective therapy available that can change the natural history of CAT, which presents with a high incidence of hypothyroidism [1] and requires treatment with levothyroxine (LT4). Few reports exist of patients going into remission and thus no longer needing LT4 therapy [1]. An ideal therapy would be efficient in reestablishing immunological tolerance and regenerating

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damaged thyroid tissue so that the patient could regain normal thyroid function and cease using LT4.

LT4 replacement and selenium treatment lower autoimmune activity by decreasing the patient's serum concentration of thyroid peroxidase antibodies (TPOAb) [3]. Neither steroid nor non-steroid anti-inflammatory drugs are recommended for minimizing the destruction of thyroid follicular cells [3]. In this context, laser light can be valuable because the local and systemic actions of low-level laser therapy (LLLT) have been shown to be effective in treating autoimmune diseases, such as rheumatoid arthritis and Sjogren's syndrome [4–6]. There is also evidence to suggest that LLLT can facilitate the regeneration of various tissues [4–7] and, in animal thyroids, can lead to improvements in microcirculation and increases in serum triiodothyronine (T3) and thyroxine (T4) levels [8–10]. Irradiation of the thymus, thyroid, and supraclavicular fossa (vascular irradiation) in patients with CAT has been found to lead to systemic immunomodulation [11]. Evaluating LLLT with respect to CAT is particularly interesting for the following reasons: (1) the thyroid is superficial, and the laser can easily access it transcutaneously; (2) the procedure is non-invasive and painless; (3) the procedure is low-risk because it does not involve the use of ionizing radiation [12]; and (4) the procedure is low cost. With these considerations in mind, the objective of this pilot study was to evaluate the effectiveness of LLLT in patients with hypothyroidism associated with CAT, based on patients' thyroid function, their concentration of thyroid autoantibodies, and the parameters of their ultrasound studies (USs).

MATERIALS AND METHODS

Patients

This research was designed as a pilot study. Fifteen patients (13 women and 2 men, with a mean age of 44.87 years and a mean disease duration of 4.57 years) previously diagnosed with CAT-induced hypothyroidism (Table 1) were recruited from the Thyroid Outpatient Clinic of the Endocrinology and Metabolism Department at the Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo (HC-FMUSP). LLLT was carried out at the Laser Medical Center of HC-FMUSP's Heart Institute. US were performed at the HC-FMUSP's Radiology Institute. The selection, treatment, and tracking of patients took place between January 2006 and March 2007.

The presence of hypothyroidism, a range of laboratory measurements, and particular US criteria were applied to diagnose CAT. The patients included in the study had significantly elevated concentrations of thyroid peroxidase and/or thyroglobulin (TgAb) antibodies (> 100 U/ml, except for one patient), US results consistent with CAT, and a prior diagnosis of hypothyroidism established by the thyroid clinic. The patients were being treated with LT4 and therefore showed normal (or almost normal) levels of T3, T4, free T4, and thyrotropin (TSH). The only patient with undetectable thyroid autoantibodies was included in the study because, in addition to presenting hypothyroidism, her US revealed the typical characteristics of CAT (i.e., parenchyma with heterogeneous texture, hypoechogenicity, hypervascularization, and fibrous strands), and we were interested in studying her response to therapy. Thyrotropin receptor antibody (TRAb) was undetectable in all of the patients. HC-FMUSP's Research Ethics Committee approved this study and the patient consent forms. All patients signed their consent forms of their own free will.

Exclusion Criteria

The criteria for excluding potential research subjects included: (1) the use of immunosuppressants, immunostimulants, or other drugs that could interfere with the production, metabolism, and transport of thyroid

TABLE 1. Patient Characteristics, Fluence, SAEF, and LLLT Application Mode

Patient	Age	Gender	Disease endurance (years)	Body mass index	Fluence (J/cm^2)	SAEF (J/cm^2)	Application mode
1	47	Female	15	31.02	38	1.0	Sweep
2	32	Female	6	24.80	50	1.4	Sweep
3	51	Female	2	33.71	50	1.4	Sweep
4	42	Female	4	46.88	70	2.0	Sweep
5	30	Female	5	24.14	70	2.0	Sweep
6	53	Female	4	35.62	70	2.0	Sweep
7	28	Female	2.5	26.13	92	2.6	Sweep
8	59	Male	2	22.92	108	3.0	Sweep
9	54	Female	2	35.92	70	2.0	Punctual
10	30	Male	2	25.84	70	2.0	Punctual
11	41	Female	5	34.96	70	2.0	Punctual
12	34	Female	3	20.62	70	2.0	Punctual
13	58	Female	10	36.10	70	2.0	Punctual
14	60	Female	2	26.83	70	2.0	Punctual
15	54	Female	4	36.02	70	2.0	Punctual
Mean CI (95%)	44.87 (39.16–50.58)		4.57 (2.75–6.39)	30.77 (27.32–34.22)	69.2 (60.93–77.47)	1.96 (1.73–2.19)	

CI, confidence interval.

hormones; (2) a history of any other autoimmune disease; (3) CAT with normal thyroid function; (4) CAT with subclinical hypothyroidism; (5) thyroid nodules; (6) hypothyroidism stemming from postpartum thyroiditis (up to 18 months after gestation); (7) a history of Graves' disease; (8) prior treatment with radioiodine; (9) tracheal stenosis; (10) pregnancy; (11) a history of ionizing irradiation and/or neoplasia in the cervical area; (12) previous surgical intervention in the thyroid; (13) thyroid hypoplasia; (14) ectopic thyroid; and (15) serious illness (cancer, ischemic coronary artery disease, stroke, kidney or liver failure, etc.).

Low-Level Laser Therapy

A continuous wave (CW) diode laser (GaAlAs—830 nm) with a beam area of 0.02827 cm^2 (Thera Lase™, DMC®, São Carlos-SP, Brazil) was used under the following specifications: *emission mode*: continuous; *application method*: sweep in eight patients and punctual in seven patients, irradiating the whole thyroid; *dosimetry*: output power always at 50 mW. The sweep application method was used with fluence in the range of 38–108 J/cm^2 and spatial average energy fluence (SAEF) in the range of 1.0–3.0 J/cm^2 (Table 1), with the tip gliding along the skin's surface gently, continuously, and perpendicularly. The quantity of SAEF was used to better estimate energy distribution in the whole treated area. SAEF was calculated as the total energy delivered divided by the area of the lesion that was illuminated. The use of SAEF is based on the fact that as light diffuses through the tissue, the treated area will be larger than the tip area; at this wavelength, 1 cm^2 around the laser tip is a good estimation of the total illuminated area. The punctual application was performed with a fluence of 70 J/cm^2 for 40 seconds at the point of application, with the tip held in soft contact with and perpendicular to the skin surface (SAEF = 2.0 J/cm^2, Table 1). *Treatment*: a total of 10 applications of LLLT were given on a twice-weekly schedule. The laser was applied to the thyroid, the boundaries of which were defined by a surgical pen aided by ultrasonography. For treatments using punctual application, an adhesive plastic mask (mold) was made for each patient, defining the boundaries of the gland (containing perforations 3 mm in diameter separated by 1 cm) between the jugular notch of the sternum and the patient's thyroid cartilage prominence. Because these two structures are easy to detect, affixing the mask to the correct skin area was easy to repeat in subsequent treatments, delineating the exact location of the gland every time. For patients treated with sweep irradiation, the contours of the gland were marked using permanent black ink. The laser equipment was calibrated prior to each application. The presence of adverse effects and the use of drugs during LLLT and the follow-up period were evaluated. A single researcher (D.B. Höfling) performed all LLLT procedures and patient tracking.

Ultrasound Study

A single, independent, experienced investigator documented and compared the ultrasounds over the course of LT4 treatment prior to and 30 days after LLLT (M.C. Chavantes). The B-mode and real-time gray-scale computerized histograms (CH) were performed using a General Electric Voluson 730 PRO™ device (General Electric Company, Ultrasound Business of Milwaukee, WI). Pulsed and power Doppler US were performed using a Philips HDI-5000™ device (ATL/Philips Medical Systems®, Bothell, WA).

B-mode: In the B-mode ultrasound analysis, we estimated thyroid volume [13] and adopted reference values of 6–16 cm^3 [14]. The gland texture was subjectively classified as either homogeneous or heterogeneous. Echogenicity was evaluated using CH, a quantitative technique that gives numerical values to the 256 gray-scale B-mode ultrasound tones (White = 0, Black = 255; Fig. 1) and calculates the mean and standard deviation of the gray-scale in each region of interest (ROI), lending objectivity,

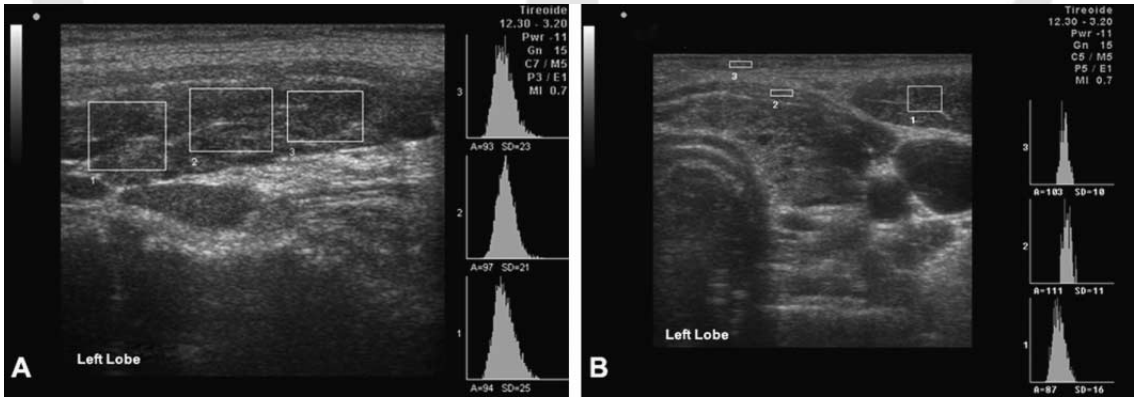

Fig. 1. Real-time gray-scale computerized histogram analysis of a longitudinal cut of the left lobe of the thyroid gland (**A**: region of interest—ROI—1, 2, and 3); transversal cut of the sternocleidomastoid muscle (**B**: ROI 1) and pre-thyroid musculature (B: ROI 2). The average and standard deviation of the computerized histogram (echogenicity) within each ROI are presented to the right of the respective images. This method transformed a qualitative variable into a highly sensitive and replicable quantitative variable.

sensitivity, and reproducibility to the method [15–18]. The average CH values of the upper, middle, and lower thirds of the longitudinal cut of the thyroid lobes were obtained (Fig. 1A; ROI 1, 2, and 3), as were the average CH values for the pre-thyroid muscles (Fig. 1B; ROI 1 and 2). The echogenic index (EI) was calculated as a ratio of the average CH of the thyroid to that of the adjacent muscles (with the same gain in brightness for both). *Power Doppler*: vascularization was divided into three categories: (A) normal; (B) increased vascularization; and (C) increased vascularization that is particularly marked and diffuse [19]. *Pulsed Doppler*: Maximal systolic peak velocity (MSPV) and resistance index (RI) values for the superior and inferior thyroid arteries were obtained.

Laboratory Measurements

Using AutoDELFIA® kits (PerkinElmer™ Wallac Oy, Turku, Finland), we determined patients' levels of TSH (0.4–4.5 μU/ml), T3 (70–200 ng/ml), T4 (4.5–12.0 μg/dl), free T4 (0.7–1.5 ng/dl), TPOAb (reference value: <35 U/ml), and TgAb (reference value: <35 U/ml) both prior to LLLT and in the first, second, third, sixth, and ninth months after the suspension of LT4 treatment. LT4 was maintained at the same dose until the 30-day post-LLLT US was performed, at which time it was discontinued for all patients. LT4 was gradually reintroduced to patients with clinical and laboratory hypothyroidism until their hormones reached normal levels. Pre-LLLT TRAb levels (reference value<8%) were determined using a radio-receptor assay (RSR, Cardiff, UK).

Statistical Analysis

Values of $P<0.05$ (*) were considered significant, while those ≥ 0.05 were considered non-significant. Data were expressed in terms of the average ± confidence interval (95%), and the main results were examined using the Wilcoxon signed rank test and Friedman's analysis of variance (ANOVA). The following statistical programs were used: Statistical Package for the Social Sciences (SPSS) release 16.0, 2007 (SPSS, Inc., IBM Company Headquarters, Chicago, IL) and Number Cruncher Statistical System (NCSS statistical software, 2004; NCSS LLC, Kaysville, UT).

RESULTS

All patients received 10 applications of LLLT and were followed for 9 months after LT4 withdrawal. All individuals enrolled in the study were included in the final analysis. No adverse effects were observed when LLLT was performed. Patients did not use drugs that might have interfered with the results of the study.

Thyroid Function

There was a reduction in the required LT4 dosage for all patients (100%), with 7/15 (47%) not needing LT4 at the time of the 9-month follow-up. The mean dose of LT4 used pre-LLLT (96 ± 22 μg/day) was significantly reduced 9 months post-LLLT (38 ± 23 μg/day; $P<0.0001$). The mean concentrations of T3 ($P=0.155$), T4 ($P=0.164$), free T4 ($P=0.268$), and TSH ($P=0.433$) were not statistically different before LLLT and 9 months after LT4 withdrawal (Table 2).

TABLE 2. Serum T3, T4, Free T4, TSH, TPOAb, and TgAb Concentrations Before LLLT (Pre-LLLT) and Following LLLT, 9 Months After Withdrawal of LT4 (Post-LLLT)

	T3		T4		Free T4		TSH		TPOAb		TgAb	
Patients	Pre-LLLT	Post-LLLT	Pre-LLLT	Post-LLLT	Pre-LLLT	Post-LLLT	Pre-LLLT	Post-LLLT	Pre-LLLT	Post-LLLT	Pre-LLLT	Post-LLLT
1	143	134	12.1	9.7	1.48	0.89	0.5	3.2	678	223	966	35
2	103	107	10.2	9.2	1.35	1.02	1.2	2.09	35	35	35	35
3	123	110	9.0	8.4	1.01	1.06	6.37	3.94	2299	1621	50	94
4	136	135	10.8	7.9	1.05	1.09	1.05	4.3	195	123	397	257
5	160	102	11.6	7.6	0.95	1.24	6.41	1.67	677	961	2587	3000
6	139	134	8.3	9.7	0.82	0.85	1.63	3.76	3000	3000	410	375
7	109	111	7.2	8.7	0.87	0.99	6.48	2.97	1621	426	35	35
8	109	95	9.8	10.7	1.1	1.66	6.42	0.24	1462	350	3000	3000
9	115	134	10.1	9.7	1.3	0.89	4.47	3.2	330	223	35	35
10	148	134	8.2	6.8	0.87	0.71	5.35	4.29	55	62	177	111
11	90	82	8.5	7.5	1.08	0.97	0.8	2.33	148	35	235	38
12	162	89	10.2	11.2	1.22	1.14	4.15	1.15	2354	135	78	35
13	108	135	13.5	13.2	1.42	1.21	3.42	1.65	1092	1175	1111	487
14	127	122	9.7	8.8	1.02	0.93	2.77	3.23	150	49	601	184
15	120	125	10.5	9.9	1.18	1.14	3.95	4.02	641	268	35	35
Mean (CI 95%)	126.13 (114.3–137.93)	116.6 (106.35–126.85)	9.98 (9.07–10.89)	9.27 (8.36–10.17)	1.11 (1.0–1.23)	1.05 (0.93–1.18)	3.66 (2.16–5.17)	2.8 (2.12–3.49)	982.47 (452.33–1512.6)	579.07 (124.83–1033.3)	650.13 (130.92–1169.4)	517.07 (–46.46–108.6)
P-value	0.155		0.164		0.268		0.433		0.016		0.074	

CI, confidence interval.
$P < 0.05$.

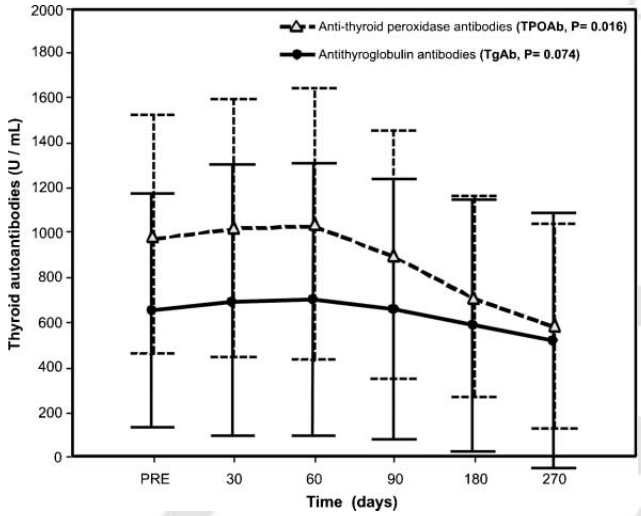

Fig. 2. Tracking of thyroid peroxidase and thyroglobulin antibodies pre- and post-LLLT. A significant reduction in TPOAb concentration was observed 9 months after LT4 withdrawal (*$P = 0.016$). There was no reduction in TgAb concentration ($P = 0.074$).

Thyroid Autoantibodies

Prior to treatment, elevated concentrations of TPOAb were observed in 14 of the 15 patients evaluated. A significant reduction in TPOAb (Fig. 2) was verified in the 9 months following LT4 withdrawal (pre-LLLT, 982 ± 530 U/ml and post-LLLT, 579 ± 454 U/ml; *$P = 0.016$; Table 2). Nine patients showed elevated concentrations of TgAb before treatment, with a trend toward reduction by the time of the examination 9 months post-LT4 withdrawal ($P = 0.074$; Fig. 2). In the patient with undetectable levels of TPOAb and TgAb prior to LLLT, these antibodies remained absent after LLLT.

Ultrasound Studies

In the US performed prior to commencing LLLT, 3/15 (20%) patients had a reduced thyroid volume, 4/15 (27%) had an increased volume, and 8/15 (53%) had a normal volume. The mean thyroid volume was not statistically different before and after LLLT, although a normalization of thyroid volume was observed in the post-LLLT US in 3/7 (43%) of the patients with abnormal thyroid volume (Table 3). Two other patients presented a considerable improvement in volume (patient nos. 12 and 14), reaching near-normal values subsequent to LLLT (Table 3). No alteration in the texture of the thyroid parenchyma was observed post-LLLT, which remained heterogeneous and showed no evidence of nodules.

LLLT led to an increase in EI in 13/15 (87%) patients (Table 3). Patient EI values were significantly higher 30 days post-LLLT (1.21 ± 0.19) relative to pre-LLLT values (0.99 ± 0.09; *$P = 0.001$; Table 3).

Prior to LLLT, thyroid analysis by power Doppler showed normal vascularization in 3 (20%) of the patients and hypervascularization in the other 12 (80%) patients. Following LLLT, 3/12 (25%) patients exhibited normal vascularization (Table 3) without significant variations in TSH concentrations as long as LT4 treatment was maintained (Fig. 3A,B). The pulsed Doppler variables (MPVS and RI) did not differ pre- and post-LLLT.

TABLE 3. Thyroid Volume, Echogenic Index, Vascularization and LT4 Dose Pre- and 30 Days Post-LLLT

	Volume reference values (6–16 cm^3)		Echogenic index		Vascularization		LT4 dose (μg/day)	
Patient	Pre-LLLT	Post-LLLT	Pre-LLLT	Post-LLLT	Pre-LLLT	Post-LLLT	Pre-LLLT	Post-LLLT
1	5.8	11.1	0.78	0.88	↑	↑	150	100
2	3.6	3.8	1.15	1.14	↑	↑	75	25
3	18.7	15	0.98	1.26	↑	↑	50	0
4	9.7	12	1.00	1.40	↑	↑	100	0
5	14.9	16	0.87	1.02	↑	↑	75	50
6	26.6	15.7	0.88	0.93	↑	Normal	50	0
7	11.6	10.9	0.93	0.99	↑	↑	125	50
8	9.5	10.1	0.95	1.05	↑	↑	150	112
9	12.1	9.7	1.10	1.06	↑	Normal	50	0
10	5.9	5.9	1.31	1.57	Normal	Normal	50	0
11	6.3	4.4	1.28	1.95	↑	Normal	150	0
12	29.3	19.7	1.15	1.84	↑	↑	75	0
13	13.7	12.2	0.84	0.93	Normal	Normal	150	88
14	25.2	16.7	0.83	0.96	↑	↑	100	75
15	6.2	9.2	0.81	1.16	Normal	Normal	100	75
Mean	13.27	11.5	0.99	1.21			96.67	38.33
(CI 95%)	(8.73–17.82)	(8.95–14.11)	(0.9–1.09)	(1.02–1.39)			(74.55–118.78)	(14.88–61.79)
P-value		0.272		0.001*				<0.0001*

↑, Increased vascularization (B and C pattern).
*$P < 0.05$.

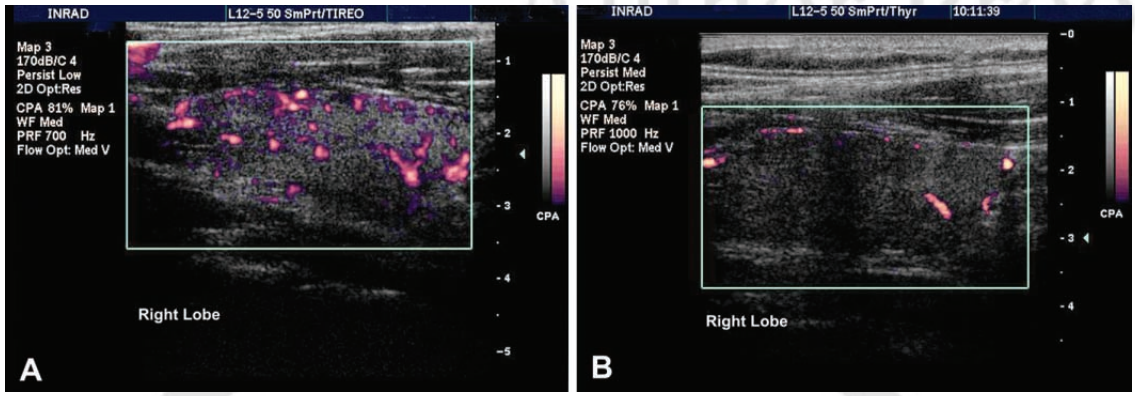

Fig. 3. Power Doppler of the right lobe of a thyroid gland. Note the visible normalization of vascularization post-LLLT. **Panel A** shows increased vascularization compatible with chronic autoimmune thyroiditis pre-LLLT, whereas **panel B** shows normal vascularization post-LLLT.

DISCUSSION

Accumulating evidence in the literature suggests that LLLT works through photochemical actions at a biomolecular level in animal and human tissues [12,20], and various studies have worked to clarify its mechanism of action. The laser light is absorbed by the photoacceptors of the mitochondrial respiratory chain via the cytochrome *c* oxidase enzyme and promotes an increase in reactive oxygen species (ROS), adenosine triphosphate (ATP), and/or cyclic adenosine monophosphate (cAMP). These effects initiate a complex signaling cascade, which is essential for the proliferation, regeneration, and protection of various cells and tissues [20–24], likely including the thyroid. Irradiation of the thyroid gland in healthy rats has demonstrated that an infrared laser can stimulate microcirculation [9,10], which may contribute to the regeneration of the gland's follicular cells. An increase in serum levels of T3 and T4 in healthy mice observed 7 days after irradiation suggests the treatment's role in stimulating thyroid function [8].

A significant decline was found in patients' need for LT4 even 9 months post-LT4 withdrawal, at which point free T4 and TSH concentrations were found to be similar to their baseline levels. This finding indicated a significant improvement in thyroid function, likely associated with the regenerative activity of LLLT. All patients exhibited a reduction in LT4 doses, with 7/15 (47%) not needing LT4 by the end of the study period. We attribute these improvements to the use of this simple, non-invasive, well-tolerated, and cost-effective therapy. Of those seven patients, three still do not require LT4, three needed to resume LT4 after 12 months of tracking (but with lesser doses than they were utilizing before LLLT), and one patient moved away and was unavailable for follow-up study.

Although replacement doses of LT4 can fluctuate in patients with hypothyroidism, it is unlikely that such fluctuations explain the present results, which were also corroborated by a drop in patients' TPOAb levels and an enhancement in echogenicity (Table 3). A future study using an appropriate control group would be able to confirm this outcome.

Thyroid autoantibodies are considered to be organ-specific markers of autoimmunity [2], and the reduction in their numbers suggests a decrease in autoimmune activity directed at the thyroid. The fall of TPOAb and TgAb levels was slow and progressive from the second month after LT4 withdrawal until the ninth month of follow-up, at which point TPOAb levels were significantly reduced (Fig. 2). TgAb levels did not exhibit a significant reduction, perhaps due to the fact that only 9 of the 15 study patients showed elevated levels of TgAb pre-LLLT. The observed decline in TPOAb levels suggests that LLLT had suppressive effects on thyroid-specific autoimmunity. The maximization of the antioxidant effects of the glutathione peroxidase enzymes promoted by LLLT may play a role in TPOAb suppression [25]; this possibility has also been suggested as an explanation for the action of selenium in the reduction of these antibodies [3]. In addition, in vitro and in vivo studies in rats have demonstrated that LLLT (red or near-infrared light) inhibits gene expression and/or reduces plasma levels of important pro-inflammatory cytokines (e.g., tumor necrotic factor-α (TNF-α), interleukin (IL)-1β, IL-2, IL-6, IL-8, and interferon-γ (IFN-γ)), while stimulating the expression of genes encoding growth factors with immunosuppressant actions (e.g., transforming growth factor-β—TGF-β) [20,26–29]. Elevated plasma levels of pro-inflammatory cytokines exhibiting the secretory profile of Th1 cells, such as IFN-γ, TNF-α, IL-2 [30,31], and IL-6 [30], as well as a decrease in TGF-β levels [32], may play a role in CAT pathogenesis. Hence, LLLT's direct or indirect actions on inflammatory and/or suppressor cytokines may result from the local and systemic immunoregulatory effects of laser irradiation.

The optimal time for post-LLLT ultrasonography has not been empirically determined. The choice to perform the

follow-up US 30 days after treatment was based on two factors. First, we surmised that the LLLT's peak effects would likely not be reached immediately after the final application [8], and we thus expected that some delay would be needed. Second, the ultrasound equipment used to perform the computerized histogram had limited availability, and the 30-day interval between the pre- and post-LLLT ultrasounds could be accommodated by the facility.

Echogenicity is inversely related to both lymphocyte infiltration [33] and the lesion size of the thyroid's follicle structure [34]. Normal follicle structure, characterized by a large colloid volume with a relatively small cellular portion, represents the main acoustic interface that provides the gland with high echogenicity. The lymphocyte infiltration and destruction of thyroid follicles found in CAT reduce echogenicity. Thus, it follows that an increase in EI, an objective and a quantitative variable that is highly sensitive and reproducible [15–18], suggests a decrease in lymphocyte infiltration and/or the regeneration of the follicle structure, allowing partial restoration of the cell-colloidal acoustic interface. Thirteen patients presented an increase in EI, and 6 (86%) of the 7 patients who remained off LT4 after LLLT exhibited EI increases (Table 3). No alteration in the texture of the parenchyma was observed after LLLT, though it should be noted that parenchymal texture is a subjective variable with low sensitivity to subtle differences.

LT4 was maintained at a steady dose until the post-laser treatment US to avoid an augmentation in TSH levels, which could induce enlargement of the thyroid gland and increased vascularization. We observed variations in thyroid volume in the direction of normality, independent of whether the patient's initial thyroid volume was abnormally high or low. This finding suggests that photobiomodulation promotes tissue homeostasis. It is noteworthy that two patients with goiters and one with reduced thyroid volume achieved normalization of gland volume in just 30 days post-LLLT. Two other patients with goiters exhibited significant volume reduction post-LLLT (Table 3). Of the 12 patients who had thyroid hypervascularization pre-LLLT, 3 returned to normal vascularization at the same doses of LT4 without significant changes in TSH concentrations (Table 3, Fig. 3).

The limitations of this initial study include its small sample size and the lack of a control group. We also used different dosimetry methods to determine the best response to LLLT, which was obtained with a fluence of 70.0 J/cm^2 and an SAEF of 2.0 J/cm^2. We found that the punctual method of treatment was more practical and reproducible, and it reduced LT4 by at least as much as the sweep method (Table 3). To confirm these findings, a randomized placebo-controlled clinical trial is currently underway, and the data obtained to date suggest that all of the results in this pilot study indicating the efficacy of LLLT in CAT will be corroborated.

In conclusion, these preliminary data imply that LLLT, a safe, cost-effective, and non-invasive procedure, is successful for improving thyroid function, reducing TPOAb levels, and increasing thyroid echogenicity. As a result, LLLT appears to promote a regeneration of the follicle structure and a reduction of the thyroid autoimmune response in patients with CAT-induced hypothyroidism.

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