Use of Color Doppler Ultrasonography to Measure Thyroid Blood Flow and Differentiate Graves' Disease from Painless Thyroiditis

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Key Words

Color Doppler ultrasonography · Thyroid blood flow · Graves' disease · Painless thyroiditis · Superior thyroid artery

Abstract

Backgrounds: Color Doppler ultrasonography (CDU) has not yet been established as a method to investigate the pathogenesis of thyrotoxicosis. **Objectives:** Our first objective was to determine whether the measurement of peak systolic blood-flow velocity in the superior thyroid artery (STV) and thyroid tissue blood flow (TBF) using CDU could differentiate Graves' disease (GD) from painless thyroiditis (PT). The second objective was to examine the factors mediating increased blood flow to the thyroid gland in GD. **Methods:** Recruited patients had untreated GD or PT and visited the Department of Internal Medicine (I), Osaka Medical College, between April 1, 2006 and May 31, 2010. Age, gender, blood pressure, pulse rate, thyroid-stimulating hormone, free thyroxine, tri-iodothyronine, TSH receptor antibody and thyroid volume were evaluated in patients. In addition, bilateral measurements of STV, TBF and peak systolic velocity in the common carotid artery (CCV) were also

performed. TBF was quantified by calculating the ratio of blood-flow pixels to total pixels in the region of interest using sagittal section images of the thyroid gland. Receiver-operating characteristic curve analysis was performed to determine the ability of STV and TBF measurements to differentiate GD from PT. **Results:** For the average of STV measured on both sides, the area under the receiver-operating characteristic curve (AUC) was 0.956. For the average of TBF measured on both sides, the AUC was 0.920. At an average STV cut-off value of 43 cm/s, the sensitivity to discriminate GD from PT was 0.87 and the specificity was 1.00. At an average TBF cut-off value of 3.8%, the sensitivity was 0.71 and the specificity was 1.00. In the GD group, neither blood pressure nor pulse rate correlated with the average STV or TBF. Moreover, there was no correlation between STV and CCV or between TBF and CCV on either side. However, STV was correlated with TBF (right side: $R = 0.47$; left side: $R = 0.52$). **Conclusions:** The results demonstrate that STV and TBF are useful for differentiating GD from PT. Furthermore, the increased STV and TBF found in GD are not related to hyperthyroidism-induced increases in systolic blood pressure, pulse rate or CCV.

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Introduction

In 1987, Ralls et al. [1] first used color Doppler ultrasonography (CDU) to show increased blood flow to the thyroid gland in patients with Graves' disease (GD). Since then, CDU has been used to investigate the pathogenesis of thyrotoxicosis. The methods included semiquantitative measurement of the blood-flow pattern in the thyroid gland parenchyma [2], thyroid blood-flow pixels per thyroid cross-sectional area [3] and blood-flow velocity in the thyroid artery [4]. However, the utility of these methods is still under investigation, and the best CDU method has not yet been established [5].

In this study, the first objective was to determine whether a differential diagnosis between patients with GD and those with painless thyroiditis (PT) could be achieved through the use of CDU to measure peak systolic blood-flow velocity in the superior thyroid artery (STV) and thyroid tissue blood flow (TBF). The second objective was to examine the factors mediating increased blood flow to the thyroid gland in GD. Using retrospective data, a comparative study was performed in GD and PT patients as well as normal controls using clinical parameters, laboratory data, STV, TBF and peak systolic velocity of the common carotid artery (CCV).

Patients and Methods

Patients

The study used data from patients with untreated GD or PT who first visited the Department of Medicine, Osaka Medical College, between April 1, 2006 and May 31, 2010. GD was diagnosed on the basis of clinical findings and laboratory tests showing high values of free thyroxine (FT₄) and free tri-iodothyronine (FT₃), low levels of thyrotropin-stimulating hormone (TSH), increased TSH receptor antibody (TRAb) titer and/or thyroid-associated ophthalmopathy. PT was diagnosed by increased FT₄ and FT₃ levels for less than 3 months, and/or later development of transient hypothyroidism without neck pain or tenderness. Clinical parameters, laboratory data, STV, TBF and CCV were sampled in untreated patients with thyrotoxicosis. The patient groups were matched for age and gender. Similar data were collected in a group of 25 normal controls. The study was approved by the ethics committee of Osaka Medical College.

Laboratory Tests

Serum concentrations of TSH, FT₄ and FT₃ were measured with an electrochemiluminescence immunoassay (ECLUSYS TSH, FT₄ and FT₃; Roche Diagnostics KK, Tokyo, Japan). The normal ranges for serum TSH, FT₄ and FT₃ were 0.340–5,000 mIU/L, 0.90–1.70 ng/dl and 2.30–4.30 pg/ml, respectively. Serum TRAb was measured by a radioreceptor assay (DYNO test TRAb human; Yamasa Co., Chiba, Japan). The normal range for TRAb was set below 1.0 IU/l. All blood samples were collected on the day of the first visit to our department.

Ultrasonography Methods

Diagnostic ultrasound imaging was performed with the Aplio XV (SSA-770A) or XG (SSA-790A) (Toshiba Medical Systems, Otawara, Tochigi, Japan) with linear probes, PLT-805AT (standard driving frequency: 8 MHz) and PLT-704SBT (standard driving frequency: 7.5 MHz). Measurement of thyroid blood flow was performed in the high-resolution power Doppler mode. STV was measured on both the right and left sides as follows. The STV image was obtained by moving the probes from the sagittal section of the upper pole toward the cranial side of the thyroid gland, while maintaining the sagittal plane position of the probe. Blood-flow velocity waveforms were measured at a stable site where the inclination between the beam and the STV was <60°, and the peak systolic velocity was determined from the velocity waveform (fig. 1a). TBF was also measured on both the right and left sides using sagittal section images of the thyroid gland obtained by CDU. The region of interest (ROI) was established by circling the thyroid tissue. TBF was quantified by calculating the ratio of blood-flow pixels to total pixels in the ROI using computer software (fig. 1b), as previously reported [3]. STV and TBF measurements had coefficients of variation <8%. The volume of the thyroid gland was calculated using the product of the maximum major axis, maximum depth and greatest transverse diameter multiplied by 0.70 on the right and left lobes, as previously reported [6].

Variables

Age, gender, blood pressure (BP), pulse rate (PR), TSH, FT₄, FT₃, TRAb, thyroid volume, STV, TBF, and CCV were evaluated. STV, TBF and CCV were measured bilaterally, and the average STV and TBF values were calculated for each subject. BP, PR and TBF measurements were initiated on August 1, 2008, and were performed in 38 GD and 11 PT patients.

Statistical Analysis

Comparisons between groups were conducted using the Mann-Whitney U test or Fisher's exact test. Receiver-operating characteristic (ROC) analysis was performed to evaluate the performance of STV and TBF measurements for differentiating GD from PT. Spearman's rank correlation coefficient was calculated to examine the correlation between each clinical parameter and STV or TBF.

JMP software (SAS Institute Japan Inc., Tokyo, Japan), version 5.0.1, was used for all statistical analyses.

Results

The patients' characteristics are shown in table 1. There were 68 patients in the GD group, 33 in the PT group, and 25 in the normal control group. Six out of 27 female patients with PT had postpartum thyroiditis. The time intervals for the parturition of those 6 patients were 1, 3, 3, 4, 4 and 7 months. There were significant differences in PR, TSH, FT₄, FT₃, TRAb, thyroid volume, STV, TBF, and CCV between the GD and PT groups. In addition there were significant differences in PR, TSH, FT₄,

Fig. 1. a Measurement of peak systolic velocity of the STV. **b** TBF was calculated on a sagittal image of the right thyroid lobe.

Table 1. Patient characteristics

	Graves' disease	Painless thyroiditis	Normal control
Cases	68	33	25
Age	41 (33–54.5)	37 (29–43)	34 (29–42)
Male/female	15/53	6/27	7/18
Systolic BP, mm Hg [§]	123 (113–134)*	111 (109–127)	108 (105–122)
Diastolic BP, mm Hg [§]	72 (67–79)	72 (67–79)	70 (68–76)
Pulse, beats/min [§]	89 (79–103)*, #	76 (71–83)*	65 (60–73)
TSH, μ U/ml	0.005 (0.005–0.008)*, #	0.006 (0.005–0.014)*	1.510 (1.330–2.280)
FT ₄ , pmol/l	56.76 (37.07–86.49)*, #	28.31 (23.42–60.88)*	15.70 (14.54–17.50)
FT ₃ , pmol/l	20.61 (14.02–30.54)*, #	11.35 (7.23–17.74)*	not determined
TRAb, IU/l	7.6 (4.7–17.6)*, #	1.0 (1.0–1.0)	1.0 (1.0–1.0)
Thyroid volume, ml	24.8 (17.9–32.7)*, #	17.1 (13.4–21.6)*	11.5 (8.2–13.4)
Rt STV, cm/s	59.9 (47.9–85.1)*, #	27.8 (18.7–35.4)	23.1 (17.6–29.6)
Lt STV, cm/s	60.0 (47.2–81.6)*, #	26.0 (17.8–34.2)	24.8 (20.8–28.7)
Average STV, cm/s	58.9 (49.3–79.5)*, #	25.6 (17.4–33.9)	24.1 (18.6–27.3)
Rt TBF, % [§]	6.5 (2.7–11.3)*, #	1.0 (0.4–3.7)	1.0 (0.4–2.2)
Lt TBF, % [§]	6.4 (3.1–9.7)*, #	0.7 (0.5–3.2)	0.9 (0.2–1.5)
Average TBF, % [§]	6.6 (3.5–10.4)*, #	0.9 (0.5–3.1)	1.0 (0.5–2.0)
Rt CCV, cm/s	125.1 (109.1–142.3)*, #	97.7 (82.5–128.6)	115.1 (92.4–125.8)
Lt CCV, cm/s	129.7 (115.9–151.3)*, #	105.1 (88.2–145.5)	106.3 (94.1–134.4)

[§] These parameters were collected in 38 patients with Graves' disease, 11 with painless thyroiditis and 25 normal controls. * Significant difference compared with normal controls, $p < 0.05$. # Significant difference compared with painless thyroiditis, $p < 0.05$.

FT₃, and thyroid volume between the PT and normal control groups. However, there were no significant differences in STV, TBF or CCV between these two groups. There were also significant differences in systolic BP, PR, TSH, FT₄, FT₃, TRAb, thyroid volume, STV, TBF and CCV between the GD and normal control groups.

The utility of STV measurements for differentiating GD from PT was examined with ROC curve analysis. STV measurements successfully differentiated GD from PT. For STV measured on the right and left sides, the area under the curve (AUC) was 0.959 and 0.941, respectively. For the average STV, the AUC was 0.956 (fig. 2a). At an average STV cut-off value of 43 cm/s, the sensitivity was 0.87, the specificity was 1.00, the positive predictive value was 1.00, and the negative predictive value was 0.79. TBF measurements also successfully differentiated GD from PT. For TBF measured on the right and left sides, the AUC was 0.851 and 0.913, respectively. For the average TBF, the AUC was 0.902 (fig. 2b). At an average TBF cut-off value of 3.8%, the sensitivity was 0.71, the specificity was 1.00, the positive predictive value was 1.00, and the negative predictive value was 0.50.

Table 2 shows the correlation coefficients between the color Doppler ultrasound parameters (average STV or TBF) and the other parameters (systolic BP, diastolic BP,

PR, TSH, FT₄, FT₃, TRAb, thyroid volume and average TBF or STV) within each group. In the GD group, neither BP nor PR was correlated with the average STV. This indicates that the elevated BP and PR caused by thyrotoxicosis did not contribute to the increased STV. Similarly, there was no correlation between the average TBF and BP or PR.

In the GD group, there were weak correlations between the average STV and FT₄, FT₃, TRAb and thyroid volume, with correlation coefficients (R) ranging from 0.26 to 0.48 (table 2). Similarly, there were weak correlations between the average TBF and FT₄, FT₃, and TRAb in the GD group.

In the PT and normal control groups, there were no significant correlations between the average STV or TBF and any of the clinical parameters (table 2).

Table 3 shows the correlation between the different blood-flow parameters measured by CDU in each group. The left- and right-sided CDU parameters are shown separately. In the GD group, there was no significant correlation between STV and CCV or between TBF and CCV. However, there was a correlation between STV and TBF ($R = 0.47$ on the right side, and $R = 0.52$ on the left side). There were no significant correlations found in either the PT or normal control group.

Fig. 2. **a** ROC curve analysis using the average STV to differentially diagnose GD and PT. **b** ROC assessment of the ability to differentially diagnose GD and PT by average TBF.

Table 2. Correlation coefficients (R) between color Doppler ultrasound parameters and the other parameters

	Graves' disease		Painless thyroiditis		Normal control	
	R		R		R	
	average STV cm/s	average TBF %	average STV cm/s	average TBF %	average STV cm/s	average TBF %
Systolic BP, mm Hg	0.15	0.17	0.16	-0.06	0.11	0.12
Diastolic BP, mm Hg	-0.01	-0.07	0.12	-0.31	-0.07	0.34
Pulse, beats/min	0.22	0.05	-0.23	-0.35	-0.14	0.10
TSH, μ U/ml	-0.03	0.06	0.19	-0.09	-0.22	-0.21
FT ₄ , pmol/l	0.44**	0.44**	0.12	0.15	0.11	-0.29
FT ₃ , pmol/l	0.48**	0.47**	-0.17	-0.08	not determined	
TRAb, IU/l	0.26*	0.32*	0.18	-0.11	all measured values are negative	
Thyroid volume, ml	0.43**	0.28	0.20	0.21	0.26	-0.29
Average STV, cm/s	-	0.55**	-	0.58 (p = 0.06)	-	0.35
Average TBF, %	0.55**	-	0.58 (p = 0.06)	-	0.35	-

* p < 0.05; ** p < 0.01.

Discussion

The results of this study indicate that STV and TBF measurements obtained by CDU are useful in differentiating GD from PT. All STV measurements on the right and left sides and the average of the two sides had a high precision for differentiating GD from PT, with an AUC

of ≥ 0.94 in ROC curve analysis. The advantages of measuring STV are that it is a simple procedure and results can be confirmed immediately. However, the disadvantages of STV measurement are that the STV is thin, and the measurement of blood-flow velocity is difficult without a high-performance ultrasound device. Furthermore, STV measurement requires technical proficiency.

Table 3. Correlation coefficients (R) between the two color Doppler ultrasound parameters

	R		
	Graves' disease	painless thyroiditis	normal control
Rt STV, cm/s/Rt CCV, cm/s	0.19	0.13	-0.04
Lt STV, cm/s/Lt CCV, cm/s	0.24	-0.19	-0.25
Rt TBF, %/Rt CCV, cm/s	0.17	0.11	0.06
Lt TBF, %/Lt CCV, cm/s	0.24	-0.29	-0.24
Rt STV, cm/s/Rt TBF, %	0.47**	0.51	0.29
Lt STV, cm/s/Lt TBF, %	0.52**	0.44	0.36

** $p < 0.01$.

The advantages of TBF measurement are that the test is easy to perform and does not require technical expertise. It does, however, require the availability of image analysis software. Right-sided TBF, left-sided TBF and the average TBF had sufficient precision to differentiate GD from PT, with an AUC of ≥ 0.85 in ROC curve analysis. The disadvantage of TBF measurement is that images must be captured, uploaded and processed after the test. Both STV and TBF measurements are minimally invasive and can be performed repeatedly. In addition, the lack of exposure to radioactivity and the low cost are major advantages over computerized tomography and scintigraphy [5].

It is important to note that there were no significant differences in STV or TBF between the PT and normal control groups, despite significant differences in thyroid function. This indicates that the increased STV or TBF is independent of high levels of thyroid hormone. In the PT group, the average STV was not correlated with FT_4 or FT_3 , nor was TBF associated with FT_4 or FT_3 .

In the GD group, there were significant increases in systolic BP, PR and CCV on both sides compared with the normal control group. Nevertheless, the average STV and average TBF in the GD group were not significantly correlated with either systolic BP or PR. In addition, there were no significant correlations between STV and CCV measured on the same side. Similarly, there were no significant correlations between TBF and CCV measured on the same side. These findings indicate that the increased cardiac output and CCV caused by hyperthyroidism are independent of elevated STV and TBF in GD.

The correlations between STV and TRAb and between TBF and TRAb in the GD group were significant,

albeit very weak ($R = 0.26$ and 0.32 , respectively). Bogazzi et al. [2] used CDU to measure blood-flow velocity in the thyroid gland parenchyma and found higher velocities in patients with GD than in those with other types of thyrotoxicosis. They hypothesized that TSH receptor antibodies contributed to the increased blood-flow velocity. On the other hand, in TSH-secreting pituitary adenoma, the intrathyroid blood flow and peak systolic velocity are elevated [7]. In the present study, only a weak correlation was found between TRAb and either STV or TBF. This indicates that there could be other unidentified factors regulating thyroid blood flow.

The establishment of methods to measure STV and TBF has allowed quantitative assessment of blood flow by CDU in patients with GD and PT. Thyroid blood flow pattern classifications [8] or semiquantitative methods have been used in several other reports. However, these methods are so subjective that it is difficult to maintain high diagnostic accuracy. Therefore, methods such as STV and TBF measurement can address these limitations. Indeed, our results and previous studies by Kurita et al. [9], Ota et al. [3] and Uchida et al. [10] have used these methods to accurately differentiate GD from PT. Still, there remains room for improvement in diagnostic accuracy. Future developments in ultrasonographic equipment and computer software will likely improve their performance and reduce interobserver variation.

In conclusion, the STV and TBF methods of assessing thyroid blood flow by CDU are useful for differentiating GD from PT. This study demonstrated that the increased STV and TBF found in GD were independent of increased systolic BP, PR and CCV caused by hyperthyroidism. In addition, this study suggested that a high level of thyroid hormone was not a major factor responsible for the increase in STV and TBF.

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Disclosure Statement

The authors have no conflicts of interest to disclose.

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