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The association between thyroid hormone balance and thyroid volume in patients with Hashimoto thyroiditis

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Abstract. While patients with large goitrous thyroid diseases often have a relatively high serum free triiodothyronine (FT_3)/free thyroxine (FT_4) ratio, athyreotic patients have a relatively low FT_3/FT_4 ratio. Here we investigated the relationship between thyroid hormone status and thyroid volume (TV) among a large number of euthyroid Hashimoto thyroiditis (HT) patients. We retrospectively enrolled 2,603 untreated HT patients who visited the Kuma hospital from 2012 to 2016, and divided them into four groups as per the TV: normal TV (<20 mL), slight goiter (20 ≤ TV < 50 mL), moderate goiter (50 ≤ TV < 80 mL), and the large goiter group (≥80 mL). Baseline characteristics and laboratory data of each group were compared to those of 1,554 control subjects. The association between FT_3/FT_4 ratio and TV among HT patients was then analyzed. We observed a change in laboratory parameters among 13 patients in the large goiter group who were prescribed levothyroxine (LT_4) for reducing TV. Compared to normal subjects, the moderate and large goiter groups exhibited significantly higher serum FT_3 levels, while all HT groups exhibited lower serum FT_4 levels. Serum FT_3/FT_4 ratios showed a positive correlation with TV ($r = 0.35$, $p < 0.01$), which was independent of age, sex, body mass index, and TgAb and TSH levels. LT_4 treatment lowered serum FT_3 levels and FT_3/FT_4 ratios significantly. Our results indicated that HT patients with increased TV tended to present with high serum FT_3, low FT_4, and high FT_3/FT_4 ratios. The elevation of deiodinase activity may be an important factor affecting thyroid hormonal balance in such patients.

Key words: Thyroid hormone, Thyroid volume, FT_3/FT_4 ratio, Deiodinase, Hashimoto thyroiditis

THE TWO MAJOR THYROID HORMONES in the body are triiodothyronine (T_3) and thyroxine (T_4). Approximately 20% of T_3 is produced from the thyroid gland through two pathways, as follows: coupling of monoiodotyrosine (MIT) and diiodotyrosine (DIT), and the conversion of T_4 to T_3 by type 1 and type 2 iodothyronine deiodinases (D1 and D2, respectively). The remaining 80% of T_3 is derived from the conversion of T_4 to T_3 in extrathyroidal tissues. In contrast, 100% of T_4 is secreted by the thyroid gland through the coupling of two DIT moieties [1, 2]. Therefore, the serum FT_3/FT_4 ratio primarily reflects deiodinase activity.

The elevation of thyroidal deiodinase activity in patients with several large goitrous thyroid diseases such as thyroglobulin gene mutation-based disease, McCune-Albright syndrome, and T_3-predominant Graves' disease is well documented; this elevation in activity is thought to be responsible for the relatively high serum FT_3 levels and FT_3/FT_4 ratios observed in these patients [3-5]. Athyreotic patients show relatively low serum FT_3 levels and FT_3/FT_4 ratios, because of the absence of thyroidal deiodinase and the resultant lack of T_3 production from the thyroid gland [6]. These facts imply the existence of a relationship between the serum FT_3/FT_4 ratio and thyroid volume (TV). Recently, we reported an increase in thyroidal D1 and D2 activities in seven patients with large goitrous Hashimoto thyroiditis (HT), which could be responsible for the relatively high serum FT_3/FT_4 ratios observed in these patients. We also documented that deiodinase activity correlated to TV [7]. Thus far, most research on this topic consists of case reports and

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Abbreviations: FT_3, Free Triiodothyronine; FT_4, Free thyroxine; TV, Thyroid volume; HT, Hashimoto Thyroiditis; LT_4, Levothyroxine; TgAb, Anti-thyroglobulin antibody; MIT, Monoiodotyrosine; DIT, Diiodotyrosine; D1, Iodothyronine deiodinase type 1; D2, Iodothyronine deiodinase type 2; TPOAb, Anti-thyroid peroxidase antibody

small studies on patients with very large goiter sizes.

In the present study, we investigated the thyroid hormone balance among a large number of patients with HT who presented with a variety of TVs and analyzed the relationship between these parameters in more detail.

Subjects and Methods

HT subjects

From hospital medical records, we retrospectively identified 4,175 consecutive adult patients with HT who visited the Kuma Hospital between January 2012 and December 2016. We based the diagnosis of HT on the existence of anti-thyroglobulin antibody (TgAb) positivity and/or anti-thyroid peroxidase antibody (TPOAb) positivity, and a heterogeneous hypoechoic pattern in a thyroid ultrasound examination.

The inclusion criteria for the present study were as follows: (1) the patient was free from thyroid hormone replacement therapy before diagnosis; (2) the patient had a thyroid stimulating hormone (TSH) level within the laboratory reference range (0.3–5.0 μIU/mL); and (3) the patient underwent an ultrasound examination and the TV was measured.

The exclusion criteria were as follows: (1) patients with follicular adenoma and thyroid malignancies; (2) patients with thyroid nodules more than 1 mL; (3) patients with thyroid dysfunction, thyroid dyshormonogenesis, autonomously functioning thyroid nodules or Graves' disease; (4) patients with chronic or serious diseases which tend to influence thyroid function tests, such as cardiac, pulmonary, hepatic, and renal diseases; (5) patients taking drugs known to affect thyroid function or thyroid hormone metabolism such as a steroids, estrogen, amiodarone, lithium, β-blockers, sucralfate, and iron or iodine-containing drugs; (6) pregnant or lactating women; or (7) patients who did not achieve normal serum TSH levels. Finally, 2,603 consecutive patients with HT were enrolled in the present study.

Control subjects

A consecutive series of 1,554 euthyroid adult subjects who were examined for possible thyroid abnormalities at the Kuma Hospital during the same period as the study subjects, and who did not have clinical or laboratory signs of thyroid diseases served as controls. Subjects with positive TPOAb or TgAb test results or with abnormal findings on ultrasound examination were excluded.

The present study was approved by the Ethical Committee at the Kuma Hospital, and written informed consent was waived because of the retrospective design.

Methods

We collected baseline data such as patient age, sex, height, weight, and body mass index (BMI). The serum levels of TSH, FT_4, and FT_3 were measured with a chemiluminescent immunoassay (ARCHTECT i2000; Abbott Japan, Tokyo). The intra-assay coefficients of variation and the inter-assay coefficients of variation were 1.1%–5.0% and 1.7%–5.3% for the TSH assay, 2.3%–5.3% and 3.6%–7.8% for the FT_4 assay, and 1.4%–4.2% and 2.3%–5.0% for the FT_3 assay, respectively. Normal ranges were 0.3–5.0 μIU/mL for TSH, 0.7–1.6 ng/dL for FT_4, and 1.7–3.7 pg/mL for FT_3. The serum levels of TgAb and TPOAb were measured using an electrochemiluminescence immunoassay (ECLusys 2010; Roche Diagnostics Japan, Tokyo; normal range: <39.9 IU/mL for TgAb, <27.9 IU/mL for TPOAb). A TgAb level less than 28 IU/mL was regarded as 28 IU/mL and that more than 4,000 IU/mL was regarded as 4,000 IU/mL, for the purpose of statistical calculations. TV was measured by ultrasound examination, as reported previously [8]. First, the maximum width (W), maximum thickness (T), and maximum length (L) were measured in the right lobe (r) and left lobe (l). Second, TV was calculated by the following equation: $TV = 0.70\ (Wr \times Tr \times Lr + Wl \times Tl \times Ll)$. We divided HT patients into four groups according to TV; normal TV (<20 mL), slight goiter ($20 \leq TV < 50$ mL), moderate goiter ($50 \leq TV < 80$ mL), and the large goiter group (>80 mL). We also analyzed the change in thyroid hormone balance among several patients in the large goiter group who were prescribed levothyroxine (LT_4) for the purpose of reducing the TV after the diagnosis of HT.

Statistical analysis

Parameters in each group were analyzed by unpaired *t*-test in case of normal distribution, Mann-Whitney U test in case of nonparametric distribution and chi-squared test in case of sex, using Bonferroni corrections for multiple comparisons. Correlation between the serum FT_3/FT_4 ratio and TV was analyzed by Pearson's correlation coefficient test and multiple regression analysis. Treatment effects of LT_4 were analyzed by paired *t*-test. When available, two-sided test was always employed. *P*-values <0.05 were considered significant. We performed statistical analyses using StatFlex software (version 6.0, Artech Co., Ltd.).

Results

Baseline characteristics and laboratory data of the HT groups and control subjects are listed in the Table 1. In comparison with control subjects, the serum FT_3 levels were significantly low in the normal TV group, equiva-

Table 1 Baseline characteristics and laboratory parameters subgrouped by thyroid volume in patients with Hashimoto thyroiditis and in normal subjects

	Control group (n = 1,554)	Hashimoto thyroiditis groups			
		Normal TV TV < 20 mL (n = 1,428)	Slight goiter 20 ≤ TV < 50 mL (n = 1,005)	Moderate goiter 50 ≤ TV < 80 mL (n = 118)	Large goiter TV ≥ 80 mL (n = 52)
TV (mL)	11.5 (9.1–14.5)	14.3 (11.5–17.2)*	26.1 (22.4–32.0)*	60.8 (56.1–70.3)*	99.9 (90.0–227)*
Age (year old)	37.3 ± 13.3	45.2 ± 15.9*	46.0 ± 15.3*	59.3 ± 12.7*	61.5 ± 12.4*
Male/female	273/1,281	64/1,364*	81/924*	10/108*	6/46
BMI (kg/ m^2)	21.6 ± 3.25	21.9 ± 3.11	22.8 ± 3.40*	23.5 ± 3.21*	23.9 ± 3.80
TSH (μIU/mL)	1.53 (1.02–2.31)	2.02 (1.29–2.95)*	1.82 (1.16–2.96)*	1.99 (1.21–3.24)*	1.88 (1.26–2.65)
FT_4 (ng/dL)	1.04 ± 0.12	1.02 ± 0.11*	1.02 ± 0.13*	0.93 ± 0.13*	0.87 ± 0.11*
FT_3 (pg/mL)	2.85 ± 0.33	2.82 ± 0.31*	2.88 ± 0.30	2.96 ± 0.30*	3.13 ± 0.33*
FT_3/FT_4	2.77 ± 0.37	2.79 ± 0.37	2.86 ± 0.40*	3.23 ± 0.53*	3.65 ± 0.56*
TgAb (IU/mL)	negative	282 (98.3–457)	380 (169–528)	447 (289–503)	503 (232–2,555)
TPOAb (IU/mL)	negative	36.5 (16.0–170.4)$^+$	72.9 (16.0–330.4)$^+$	90.6 (16.0–382.4)$^+$	84.5 (16.0–395.2)$^+$

Values shown are the means ± SD in case of normal distribution and the medians (25–75% tile) in case of nonparametric distribution. TV, Thyroid volume; BMI, body mass index; TSH, thyroid stimulating hormone; FT_4, free thyroxine; FT_3, free triiodothyronine; TgAb, Anti-thyroglobulin antibody; TPOAb, anti-thyroid peroxidase antibody; *$p < 0.05$ compared to control group. $^+$TPOAb data of 78, 60, 11, and 3 patients in normal TV, slight goiter, moderate goiter, and large goiter groups, respectively, are missing.

lent in the slight goiter group, and significantly high in the moderate and large goiter groups. The serum FT_4 levels in all HT groups were decreased, resulting in high serum FT_3/FT_4 ratios in the slight, moderate, and large goiter groups. Multiple comparisons between all HT groups revealed that the serum FT_3 levels and the FT_3/FT_4 ratios increased as the goiter size increased. The serum FT_4 levels decreased as the goiter size increased. There was no difference in TSH levels among the HT groups. Results of the Pearson's correlation coefficient test and multiple regression analysis among HT patients demonstrated that the serum FT_3/FT_4 ratios showed a positive correlation with TV ($r = 0.35$, $p < 0.01$; Fig. 1), and this correlation was independent of patient age, sex, BMI, TgAb, and serum TSH levels ($stdβ = 0.35$, $p < 0.01$; Table 2). Serum TSH levels also showed a positive correlation with serum FT_3/FT_4 ratio to a small degree ($stdβ = 0.25$, $p < 0.01$; Table 2).

Thirteen of 52 patients received LT_4 after diagnosis for the purpose of reducing the TV. After 1 year of follow-up, the serum FT_3/FT_4 ratio decreased significantly from 3.66 ± 0.51 to 2.86 ± 0.36 ($p < 0.01$); serum FT_3 also decreased significantly from 3.01 ± 0.30 to 2.79 ± 0.24 ($p = 0.03$, Table 3).

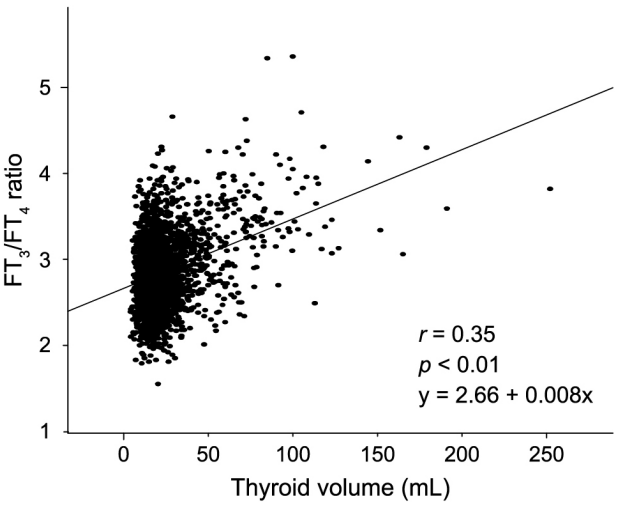

Fig. 1 The association between serum free triiodothyronine (FT_3)/free thyroxine (FT_4) ratio and thyroid volume (TV) by Pearson's correlation coefficient test among Hashimoto thyroiditis patients. The serum FT_3/FT_4 ratio showed a positive correlation with TV ($r = 0.35$, $p < 0.01$).

Discussion

Our present study revealed that HT patients with large goiters had relatively high serum FT_3 levels, low FT_4 levels, and a high FT_3/FT_4 ratio. We also demonstrated a positive correlation between the serum FT_3/FT_4 ratio and TV, findings consistent with those from our previous report [7]. Our study is the first to demonstrate these results in such a large number of patients.

It is well known that the serum FT_3/FT_4 ratio reflects activity of the deiodinase enzyme which converts T_4 to

Table 2 Associations between the serum free triiodothyronine (FT_3)/free thyroxine (FT_4) ratio and other parameters including thyroid volume by multiple linear regression analysis in patients with Hashimoto thyroiditis

Parameter	*β* (95% *CI*)	*Stdβ*	*p* value
Age (year old)	0.000010 (–0.00097 to 0.00099)	0.00020	0.99
TgAb (IU/mL)	–0.000013 (–0.000036 to 0.000010)	–0.017	0.33
BMI (kg/ m^2)	0.0059 (0.0013 to 0.0105)	0.046	0.012
Male (compared to female)	0.091 (0.029 to 0.153)	0.052	<0.01
TSH (μIU/mL)	0.090 (0.077 to 0.103)	0.25	<0.01
Thyroid volume (mL)	0.0080 (0.0072 to 0.0088)	0.35	<0.01

BMI, Body mass index; TSH, thyroid stimulating hormone; *CI*, confidence interval; TgAb, Anti-thyroglobulin antibody; *β*, Regression coefficient; *Stdβ*, Standardized regression coefficient.

Table 3 Comparison of thyroid hormone balance and thyroid volume between pre- and post-levothyroxine treatment among patients in the large goiter group. Comparisons were made using the paired *t*-test

Parameter	Pre-treatment	Post-treatment (after one year)	*p* value
Thyroid Volume (mL)	130.2 ± 50.6	125.4 ± 43.5	0.80
TSH (μIU/mL)	2.26 ± 0.99	1.21 ± 0.95	<0.01
FT_4 (ng/dL)	0.84 ± 0.12	0.99 ± 0.10	<0.01
FT_3 (pg/mL)	3.01 ± 0.30	2.79 ± 0.24	0.03
FT_3/FT_4	3.66 ± 0.51	2.86 ± 0.36	<0.01

Values shown are the means ± SD.
TSH, thyroid stimulating hormone; FT4, free thyroxine; FT3, free triiodothyronine.

T_3 by 5'-deiodination [2]. There are two types of deiodinases (D1 and D2) which contribute to T_3 production. D2 is reported to be expressed in the human thyroid gland and plays an important role as a source of plasma T_3 [9]. Athyreotic patients lack thyroidal deiodinase activity and T_3 production from the thyroid gland, resulting in a relatively low serum FT_3/FT_4 ratio [6]. Elevation of thyroidal deiodinase activity, predominantly that of D2 activity, was reported as the cause of serum FT_3/FT_4 ratio elevation in several large goitrous thyroid diseases such as those involving thyroglobulin gene mutations, McCune-Albright syndrome, and T_3-predominant Graves' disease [3-5]. Because the elevation of thyroidal D2 mRNA levels was subtle or not significant compared to those of the controls, it was concluded that the post-translational control played an important part in increased thyroidal D2 activity [3-5]. Recently, we reported the elevation of thyroidal deiodinase activity (especially D2 activity) at the post-translational level in seven HT patients with large goiters; this increase in enzyme activity may be responsible for the relatively high serum FT_3/FT_4 ratio observed in these patients. We also demonstrated a positive correlation between deiodinase activities and TV [7]. These findings suggest that intra-thyroidal T_3 production by increased deiodinase activity is a substantial factor influencing the relatively high serum T_3 levels in HT patients with large goiters.

This possible causal relationship may be targeted to restore the hormonal balance of patients. Interestingly, a relatively low T_4 content in the thyroid gland is reported to be one possible reason for the elevation of deiodinase activity [3]. D2 activity is known to be negatively regulated by T_4, its preferred substrate, at the post-translational level. The low T_4 content is reported to lower the ubiquitination of D2. As a result, ubiquitin-mediated proteasome degradation of the enzyme is suppressed, leading to an increase in D2 activity [10]. Although the mechanism is unknown, relatively low serum FT_4 levels in HT patients with large goiters were observed in our study; this observation implies that the T_4 content in thyroid tissues of these patients may have also been low, resulting in increased D2 activity. The fact that LT_4 therapy lowered not only serum FT_3/FT_4 ratios but also FT_3 levels suggests that a relatively low serum FT_4 level is presumably not the consequence but the origin of increased deiodinase activity. Although the decline of TSH might lower deiodinase activity to some degree, the recovery of T_4 content would accelerate the ubiquiti-

nation and ubiquitin-mediated proteasomal degradation of D2.

Serum FT_3/FT_4 ratio also reflects the proportion of T_3 and T_4 secreted by the thyroid follicular cells. When iodine is limited, the rate of coupling of MIT and DIT (*i.e.* T_3 production) increases relative to that of coupling of DITs (*i.e.* T_4 production), in order to suppress iodine consumption [1]. Although iodine intake is thought to be sufficient in Japan, it is theoretically expected that thyroidal iodine content per volume will decrease as goiter size increases. LT_4 therapy would thus restore the iodine content in patients with enlarged thyroid glands.

In the present study, TSH showed a positive correlation with serum FT_3/FT_4 ratio to a small degree. TSH is known to regulate D1 and D2 expression in the human thyroid gland at the transcriptional level, through the TSH receptor [11, 12]. However, increased deiodinase activity has been reported to be controlled predominantly at the post-translational level in large goitrous thyroid diseases such as those mentioned above [3-5, 7]. TSH is also known to induce enlargement of the thyroid gland. Although serum TSH levels in our study were within normal limits, goitrous HT patients may have had prior periods associated with a decline of T_4 and an elevation of TSH. It is thus theoretically possible that such periods increased as the goiter size increased, and this may have led to increased deiodinase activity; the influence of this elevated enzyme activity may have persisted beyond the period with elevated TSH. In order to restore the hormonal balances, LT_4 therapy may be considered for the patients with relatively low FT_4 and high FT_3 levels even if TSH is within normal limits. However, it is unclear whether the restoration of hormonal balances would improve clinical symptoms or prevent worsening of disease status. Though LT_4 therapy didn't change thyroid volume in our study, insufficient suppression of TSH could have affected the result. Further investigations are needed to clarify the effect and indication of LT_4 therapy.

Additionally, our study has several limitations. First, the study design is retrospective. Second, pathophysiological considerations such as measurement of deiodinase activity are lacking. Finally, investigations that include a large number of patients with other goitrous thyroid diseases such as adenomatous goiter and dyshormonogenesis are also needed.

In conclusion, we analyzed a large number of HT patients and demonstrated that they presented with relatively high serum FT_3 levels, low FT_4 levels and high FT_3/FT_4 ratios as their goiter size increased. Together with our previous report, these data suggest that elevation of deiodinase activity may play a significant role in the elevation of serum FT_3 and FT_3/FT_4 ratios in such patients. Knowing these characteristics will improve our care of patients with HT and other goitrous thyroid diseases.

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Disclosure Statement

The authors declare no conflict of interest in connection with this article.

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