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 (FT3) / free thyroxine (FT4) ratio, athyreotic patients have a relatively low FT3/FT4 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 FT3/FT4 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 treatment. Reduced TV. Compared to normal subjects, the moderate and large goiter groups exhibited significantly higher serum FT3 levels, while all HT groups exhibited lower serum FT4 levels. Serum FT3/FT4 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. LT4 treatment lowered serum FT3 levels and FT3/FT4 ratios significantly. Our results indicated that HT patients with increased TV tended to present with high serum FT3, low FT4, and high FT3/FT4 ratios. The elevation of deiodinase activity may be an important factor affecting thyroid hormonal balance in such patients.

Key words: Thyroid hormone, Thyroid volume, FT3/FT4 ratio, Deiodinase, Hashimoto thyroiditis

THE TWO MAJOR THYROID HORMONES in the body are triiodothyronine (T3) and thyroxine (T4). Approximately 20% of T3 is produced from the thyroid gland through two pathways, as follows: coupling of monoiodotyrosine (MIT) and diiodotyrosine (DIT), and the conversion of T4 to T3 by type 1 and type 2 iodothyronine deiodinases (D1 and D2, respectively). The remaining 80% of T3 is derived from the conversion of T4 to T3 in extrathyroidal tissues. In contrast, 100% of T4 is secreted by the thyroid gland through the coupling of two DIT moieties [1, 2]. Therefore, the serum FT3/FT4 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 T3-predominant Graves’ disease is well documented; this elevation in activity is thought to be responsible for the relatively high serum FT3 levels and FT3/FT4 ratios observed in these patients [3-5]. Athyreotic patients show relatively low serum FT3 levels and FT3/FT4 ratios, because of the absence of thyroidal deiodinase and the resultant lack of T3 production from the thyroid gland [6]. These facts imply the existence of a relationship between the serum FT3/FT4 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 FT3/FT4 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
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 subjects 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 thyroid hormone levels. Finally, 2,603 consecutive patients with thyroid nodules more than 1 mL; (3) the patient underwent an ultrasound examination and the TV was measured.

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**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, FT₄, and FT₃ were measured with a chemiluminescent immunoassay (ARCHTECT i2000; Abbott Japan, Tokyo). The intra-assay coefficients of variation 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 × Tr × Lr + Wl × Tl × Ll). We divided HT patients into four groups according to TV; normal TV (<20 mL), slight goiter (20 ≤ TV < 50 mL), moderate goiter (50 ≤ 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 (LT₄) 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 FT₄/FT₃ ratio and TV was analyzed by Pearson’s correlation coefficient test and multiple regression analysis. Treatment effects of LT₄ 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 FT₃ levels were significantly low in the normal TV group, equiva-
lent in the slight goiter group, and significantly high in the moderate and large goiter groups. The serum FT₄ levels in all HT groups were decreased, resulting in high serum FT₃/FT₄ ratios in the slight, moderate, and large goiter groups. Multiple comparisons between all HT groups revealed that the serum FT₃ levels and the FT₃/FT₄ ratios increased as the goiter size increased. The serum FT₄ 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 FT₃ levels and the FT₃/FT₄ 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 (r stdβ = 0.35, p < 0.01; Table 2). Serum TSH levels also showed a positive correlation with serum FT₃/FT₄ ratio to a small degree (r stdβ = 0.25, p < 0.01; Table 2).

Thirteen of 52 patients received LT₄ after diagnosis for the purpose of reducing the TV. After 1 year of follow-up, the serum FT₃/FT₄ ratio decreased significantly from 3.66 ± 0.51 to 2.86 ± 0.36 (p < 0.01); serum FT₃ also decreased significantly from 3.01 ± 0.30 to 2.79 ± 0.24 (p = 0.03, Table 3).

**Discussion**

Our present study revealed that HT patients with large goiters had relatively high serum FT₃ levels, low FT₄ levels, and a high FT₃/FT₄ ratio. We also demonstrated a positive correlation between the serum FT₃/FT₄ 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 FT₃/FT₄ ratio reflects activity of the deiodinase enzyme which converts T₄ to...
T₃ by 5'-deiodination [2]. There are two types of deiodinases (D1 and D2) which contribute to T₃ production. D2 is reported to be expressed in the human thyroid gland and plays an important role as a source of plasma T₃ [9]. Athyreotic patients lack thyroidal deiodinase activity and T₃ production from the thyroid gland, resulting in a relatively low serum FT₃/FT₄ ratio [6]. Elevation of thyroid deiodinase activity, predominantly that of D2 activity, was reported as the cause of serum FT₃/FT₄ ratio elevation in several large goitrous thyroid diseases such as those involving thyroglobulin gene mutations, McCune-Albright syndrome, and T₃-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 FT₃/FT₄ ratio observed in these patients. We also demonstrated a positive correlation between deiodinase activities and TV [7]. These findings suggest that intrathyroidal T₃ production by increased deiodinase activity is a substantial factor influencing the relatively high serum T₃ 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 T₄ 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 T₄, its preferred substrate, at the post-translational level. The low T₄ 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 FT₄ levels in HT patients with large goiters were observed in our study; this observation implies that the T₄ content in thyroid tissues of these patients may have also been low, resulting in increased D2 activity. The fact that LT₄ therapy lowered not only serum FT₃/FT₄ ratios but also FT₃ levels suggests that a relatively low serum FT₄ 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 T₄ content would accelerate the ubiquitin-

### Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>β (95% CI)</th>
<th>Stdβ</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year old)</td>
<td>0.000010 (–0.00097 to 0.00099)</td>
<td>0.00020</td>
<td>0.99</td>
</tr>
<tr>
<td>TgAb (IU/mL)</td>
<td>-0.000013 (–0.000036 to 0.000010)</td>
<td>–0.017</td>
<td>0.33</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.0059 (0.0013 to 0.0105)</td>
<td>0.046</td>
<td>0.012</td>
</tr>
<tr>
<td>Male (compared to female)</td>
<td>0.091 (0.029 to 0.153)</td>
<td>0.052</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TSH (μIU/mL)</td>
<td>0.090 (0.077 to 0.103)</td>
<td>0.25</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Thyroid volume (mL)</td>
<td>0.0080 (0.0072 to 0.0088)</td>
<td>0.35</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

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

### Table 3

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-treatment</th>
<th>Post-treatment (after one year)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid Volume (mL)</td>
<td>130.2 ± 50.6</td>
<td>125.4 ± 43.5</td>
<td>0.80</td>
</tr>
<tr>
<td>TSH (μIU/mL)</td>
<td>2.26 ± 0.99</td>
<td>1.21 ± 0.95</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>FT₄ (ng/dL)</td>
<td>0.84 ± 0.12</td>
<td>0.99 ± 0.10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>FT₃ (pg/mL)</td>
<td>3.01 ± 0.30</td>
<td>2.79 ± 0.24</td>
<td>0.03</td>
</tr>
<tr>
<td>FT₃/FT₄</td>
<td>3.66 ± 0.51</td>
<td>2.86 ± 0.36</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

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

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References


