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ORIGINAL ARTICLE

Type 1 and type 2 iodothyronine deiodinases in the thyroid gland of patients with huge goitrous Hashimoto's thyroiditis

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Abstract

Purpose The serum free triiodothyronine (FT_3)/free thyroxine (FT_4) ratio in patients with huge goitrous Hashimoto's thyroiditis (HG-HT) is relatively high. We investigated the cause of high FT_3/FT_4 ratios.

Methods We measured the serum FT_3, FT_4, and thyrotropin (TSH) levels of seven patients with HG-HT who had undergone a total thyroidectomy. Eleven patients with papillary thyroid carcinoma served as controls. The activities and mRNA levels of type 1 and type 2 iodothyronine deiodinases (D1 and D2, respectively) were measured in the thyroid tissues of HG-HT and perinodular thyroid tissues of papillary thyroid carcinoma.

Results The TSH levels in the HG-HT group were not significantly different from those of the controls. The FT_4 levels in the HG-HT group were significantly lower than those of the controls, whereas the FT_3 levels and FT_3/FT_4 ratios were significantly higher in the HG-HT group. The FT_3/FT_4 ratios in the HG-HT group who had undergone total thyroidectomy and received levothyroxine therapy decreased significantly to normal values. Both the D1 and D2 activities in the thyroid tissues of the HG-HT patients were significantly higher than those of the controls. However, the mRNA levels of both D1 and D2 in the HG-HT patients' thyroid tissues were comparable to those of the controls. Interestingly, there were significant correlations between the HG-HT patients' D1 and D2 activities, and their thyroid gland volume or their FT_3/FT_4 ratios.

Conclusions Our results indicate that increased thyroidal D1 and D2 activities may be responsible for the higher serum FT_3/FT_4 ratio in patients with HG-HT.

Keywords Thyroid hormone metabolism · Iodothyronine deiodinase · Hashimoto's thyroiditis · Thyroid gland

Introduction

Approximately 80% of the circulating 3,5,3'-triiodothyronine (T_3) in humans is produced by the 5'-monodeiodination of thyroxine (T_4) outside the thyroid gland. The quantitative contribution of different tissues in the body to the total production of T_3 from T_4 is unknown [1], but it is known that type 1 and type 2 iodothyronine deiodinase (D1 and D2, respectively) catalyze this reaction [2–4].

In humans, both D1 and D2 are expressed in the thyroid gland [5], and it is estimated that the intrathyroidal T_4 to T_3 conversion by D2 or D1 may be the major source of plasma T_3 in hyperthyroid Graves' diseases [5, 6]. The increased D2 activity in the thyroid tissues of patients with follicular thyroid carcinoma [7], Graves' disease during propylthiouracil (PTU) treatment [8], patients with thyroglobulin (Tg) gene mutations [9], and those with McCune-Albright syndrome [10] is thought to be responsible for the relatively high serum T_3 levels in these patients.

We reported that increases in thyroidal D1 and D2 activities may be responsible for the higher serum free triiodothyronine (FT_3)/free thyroxine (FT_4) ratio in patients with T_3-predominant Graves' disease, which is characterized by a persistently high serum T_3 level and a normal or even lower serum T_4 level during antithyroid drug therapy [11].

We recently observed that the serum FT_3/FT_4 ratio in patients with huge goitrous Hashimoto's thyroiditis (HG-HT) is relatively high, suggesting elevated 5'-deiodinase activity [12]. In the present study, we therefore investigated

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the cause of higher serum FT_3/FT_4 ratios in patients with HG-HT.

Materials, subjects, and methods

Materials

[^{125}I]T_4 and [^{125}I]3,3',5'-triiodothyronine (reverse T_3 or rT_3) were purchased from Perkin Elmer (Boston, MA). Sephadex LH-20 was purchased from Pharmacia Biotech (Uppsala, Sweden). All other chemicals were of the highest quality and were obtained from Sigma Chemical Co. (St. Louis, MO) or Nacalai Tesque (Kyoto, Japan) unless otherwise indicated.

Subjects

We studied seven patients with HG-HT who underwent total thyroidectomy at Kuma Hospital (Kobe, Japan) during the period 1999–2016. In accord with the guidelines issued by the Japanese Thyroid Society, all seven patients were diagnosed as having Hashimoto's thyroiditis (HT) based on clinical findings: diffuse swelling of the thyroid gland without any other cause (such as Graves' disease) accompanied by any one of the following laboratory findings: (1) positive for antithyroid peroxidase antibodies (TPOAb), (2) positive for antithyroglobulin antibodies (TgAb), and (3) lymphocytic infiltration in the thyroid gland confirmed by a cytological examination. For most of the patients, symptoms of compression caused by a huge diffuse goiter were the reason for surgery. No treatment was administered to the patients with HG-HT before surgery. As controls, normal thyroid tissue was obtained from perinodular tissue of glands containing a papillary thyroid carcinoma. All of the control patients had normal thyroid function with negative serum TPOAb and TgAb. This study was approved by the Kuma Hospital Ethics Committee, and all patients gave informed consent for their materials to be used and for their data to be published.

Thyroid function tests

We measured the control and HG-HT patients' serum concentrations of TSH, FT_4, and FT_3 with a chemiluminescent immunoassay (ARCHTECT i2000; Abbott Japan, Tokyo). The results for both TgAb and TPOAb were determined using commercially available hemagglutination assay kits (Fuji Rebio, Tokyo; normal ranges <100-fold, respectively). The volume of each patient's thyroid gland was measured by ultrasonography as described [13].

D1 assay

Human thyroid tissues were homogenized, and a microsomal fraction was prepared as described [5]. D1 activity was assayed as described [11]. In brief, the reactions contained microsomal protein, 0.2 nM [^{125}I]rT_3 purified by LH-20 chromatography, 1 μM rT_3, and 10 mM dithiothreitol (DTT) in the presence or absence of 1 mM PTU in 0.1 M potassium phosphate, and 1 mM EDTA, pH 6.9 (PE buffer). Incubations were for 60 min at 37 °C. $^{125}I^-$ was separated from unreacted substrate or iodothyronine products by trichloroacetic acid (TCA) precipitation. Separated $^{125}I^-$ was counted with a γ-counter (1480 Wizard, Perkin Elmer). The deiodinating activity is expressed as picomoles rT_3/min/mg protein. The deiodination of reverse T_3 produced equimolar concentrations of labeled I^- and 3,3'-diiodothyronine as assessed by the paper chromatographic separation of the reaction products [14].

D2 assay

D2 activity was assayed as described [5]. In brief, the reactions contained microsomal protein, 0.1 nM [^{125}I]T_4 purified by LH-20 chromatography, 2 nM cold T_4, 20 mM DTT, 1 mM PTU in PE buffer. Incubations were for 120 min at 37 °C. $^{125}I^-$ was separated by TCA precipitation and counted with a γ-counter as described above. The deiodinating activity is expressed as femtomoles T_4/min/mg protein. The deiodination of T_4 produced equimolar concentrations of labeled I^- and T_3.

RNA preparation and real-time quantitative PCR

Total RNA from thyroid tissues was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Real-time quantitative PCR assays were performed using an Opticon 2 apparatus (Bio-Rad, Hercules, CA). Briefly, 1 μg of total RNA was reverse-transcribed using the iScript cDNA synthesis kit (Bio-Rad), according to the manufacturer's instructions. The messenger RNA (mRNA) levels of human D1, D2, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA were analyzed using iQ SYBR Green Super MIX (Bio-Rad). The primers used are listed in Table 1. Real-time PCR experiments were performed in triplicate, and the mRNA levels are expressed as arbitrary units after correction for the GAPDH mRNA level.

Statistical analyses

Group data are expressed as the mean ± SD, and statistical significance was analyzed by the unpaired *t*-test, the

Table 1 Oligonucleotide sequences used for RT-PCR

Genes	Sequences (5′ to 3′)
DIO 1	Forward: TTAGTTCCATAGCAGATTTTCTTGTCA
	Reverse: CTGATGTCCATGTTGTTCTTAAAAGC
DIO 2	Forward: TCATTCTGCTCAAGCACGTG
	Reverse:ACCATTGCCACTGTTGTCAC
GAPDH	Forward: GCACCGTCAAGGCTGAGAAC
	Reverse: TGGTGAAGACGCCAGTGGA

Table 2 The basic characteristics of the patients with HG-HT and the controls

	HG-HT ($n = 7$)	Controls ($n = 11$)	*P*-value
Age, years	65 ± 9	55 ± 13	0.09
Females/males	7/0	10/1	
TSH (μIU/mL)	1.36 ± 0.92	1.32 ± 1.01	0.94
FT_4 (ng/dL)	0.88 ± 0.14	1.08 ± 0.15	0.01
FT_3(pg/mL)	3.53 ± 0.48	2.79 ± 0.23	< 0.01
FT_3/FT_4	4.13 ± 0.93	2.62 ± 0.32	< 0.01
Thyroid volume(cm^3)	240 ± 54	n.d.	

The normal ranges are 0.3–5.0 μIU/mL for TSH, 0.7–1.6 ng/dL for FT_4, 1.7–3.7 pg/mL for FT_3, and 1.8–3.3 (pg/mL ÷ ng/dL) for the FT_3-to-FT_4 ratio. A TSH concentration < 0.003 μIU/mL was regarded as 0, for the purpose of statistical calculation. Values shown are the means ± SD. The data of the HG-HT patients and controls were compared by unpaired *t*-test or the Mann–Whitney *U*-test, as appropriate

n.d. not done, *HG-HT* huge goitrous Hashimoto's thyroiditis

Mann–Whitney *U*-test, or the Wilcoxon test, as appropriate. Correlations were obtained by Pearson's correlation coefficient test. *P*-values < 0.05 were considered significant.

Results

Clinical findings

Basic characteristics of the seven patients with HG-HT who completed the study are listed in the Table 2. These measurements were made at the time of the thyroidectomy. The serum TSH levels in this HG-HT group were not significantly different from those of the controls. The serum FT_4 levels in the HG-HT group were significantly lower than those of the controls. The serum FT_3 and the FT_3/FT_4 ratio in the patients with HG-HT were significantly higher than those of the controls. The mean volume of the thyroid gland in the patients with HG-HT was more than tenfold higher than the mean volume of normal thyroid gland.

The serum FT_4, FT_3, and TSH levels in the HG-HT patients who had undergone a total thyroidectomy and levothyroxine (L-T_4) therapy were within the normal range.

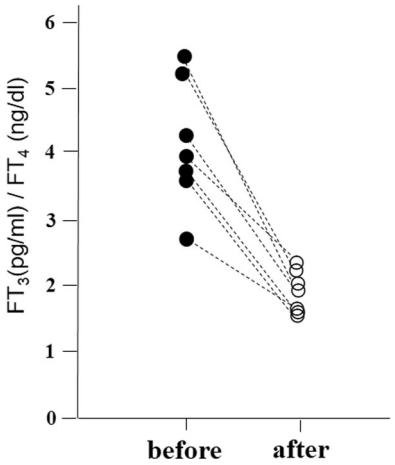

Fig. 1 Individual changes in the serum FT_3/FT_4 ratio of the patients with huge goitrous Hashimoto's thyroiditis (HG-HT) before and after a total thyroidectomy and levothyroxine therapy ($n = 7$)

After total thyroidectomy and L-T_4 therapy, the FT_3/FT_4 ratios in the patients were significantly decreased, and these FT_3/FT_4 ratios were comparable to the ratios in patients we have described [15] who had undergone both a total thyroidectomy for papillary thyroid carcinoma and L-T_4 therapy. Individual changes in the FT_3/FT_4 ratio from before to after total thyroidectomy are illustrated in Fig. 1.

D1 and D2 activities in the thyroid tissues

The D1 activity in the thyroid tissues of the HG-HT patients (5.0 ± 2.9 pmoles rT_3/min/mg prot) was significantly higher ($P < 0.01$) than that of the controls (1.1 ± 1.1 pmoles rT_3/min/mg prot) (Fig. 2a). The D2 activity in the thyroid tissues of patients with HG-HT (15.2 ± 7.5 fmoles T_4/min/mg prot) was also significantly and markedly higher ($P < 0.01$) than that of the controls (0.1 ± 0.1 fmoles T_4/min/mg prot) (Fig. 2b). In the HG-HT group, the patients' D1 activity was well correlated with their D2 activity ($r = 0.86$, $P < 0.01$; Fig. 3).

D1 and D2 mRNA levels in the thyroid tissues

We examined whether the increased D1 and D2 activities in the thyroid tissues of the patients with HG-HT are associated with increased mRNA levels. The mRNA levels of both D1 (Fig. 4a) and D2 (Fig. 4b) in the thyroid tissues of two of the HG-HT patients were comparable to those of the controls.

D1 and D2 activity and the thyroid volume

We examined whether the increased D1 and D2 activities in the thyroid tissues of the HG-HT patients were associated with the increased thyroid volume. As shown in Fig. 5a, the

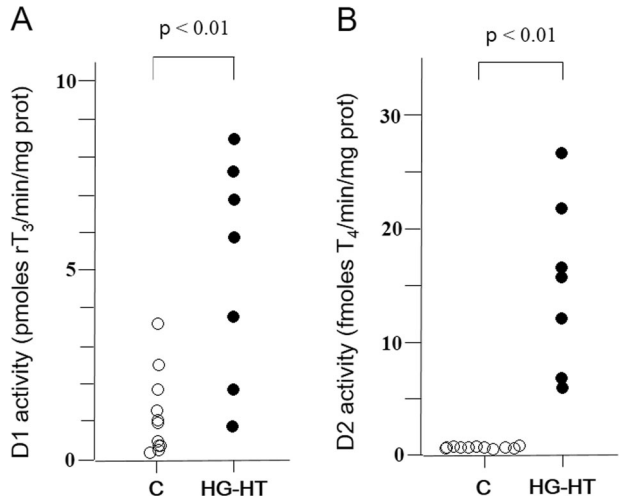

Fig. 2 D1 **a** and D2 **b** activities of thyroid tissues in the controls (C; open circles) ($n = 11$) and patients with huge goitrous Hashimoto's thyroiditis (HG-HT) (solid circles) ($n = 7$)

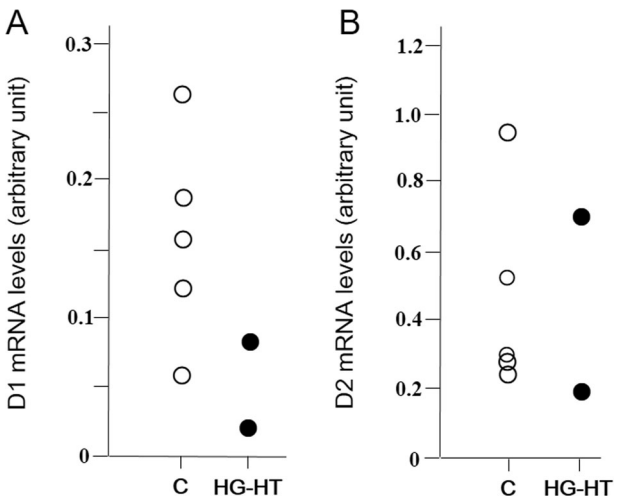

Fig. 4 The D1 **a** and D2 **b** messenger RNA (mRNA) levels of thyroid tissues in the controls (C; open circles) ($n = 5$) and patients with huge goitrous Hashimoto's thyroiditis (HG-HT) (solid circles) ($n = 2$)

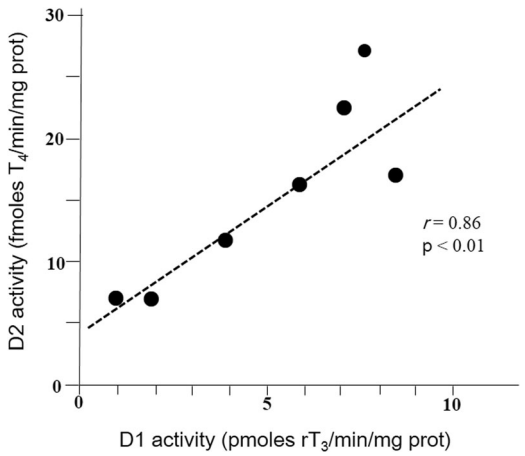

Fig. 3 The correlation between the D1 and D2 activities of the thyroid tissues in the huge goitrous Hashimoto's thyroiditis (HG-HT) patients ($n = 7$)

D1 activity in the HG-HT patients' thyroid tissues was well correlated with their thyroid volume ($r = 0.97$, $P < 0.01$). The D2 activity of the HG-HT patients' thyroid tissues was also well correlated with their thyroid volume ($r = 0.79$, $P < 0.03$) (Fig. 5b).

D1 and D2 activities and the serum FT_3/FT_4 ratio

To determine whether the D1 and D2 activities in the thyroid tissues of the individuals with HG-HT contribute to their serum FT_3/FT_4 ratios, we investigated the correlation between the serum FT_3/FT_4 ratio and the corresponding thyroidal D1 and D2 activities. As shown in Fig. 6a, the D1 activity of the HG-HT thyroid tissues was significantly correlated with the serum FT_3/FT_4 ratio ($r = 0.61$, $P < 0.01$). The D2 activity of the HG-HT thyroid tissues was also significantly correlated with the serum FT_3/FT_4 ratio ($r = 0.72$, $P < 0.01$) (Fig. 6b).

Discussion

Our present analyses revealed that the seven patients with HG-HT had relatively high-serum FT_3 concentrations along with disproportionately low FT_4 levels, resulting in a high serum FT_3/FT_4 ratio. The number of patients with HG-HT in the present study was small, since the number of these patients who did not receive L-T_4 therapy before total thyroidectomy was small. The serum FT_3/FT_4 ratios in the patients with HG-HT who had both undergone a total thyroidectomy and received L-T_4 therapy fell significantly to values comparable to those of the control patients with papillary thyroid carcinoma who had undergone a total thyroidectomy and received L-T_4 therapy. We also observed that both the D1 and D2 activities in the thyroid tissues of the patients with HG-HT were significantly higher than those of the controls. These results suggest that the higher D1 and D2 activities of the thyroid tissues of HG-HT patients may contribute to their higher serum FT_3/FT_4 ratios.

Interestingly, the D2 activity in the thyroid tissues of the HG-HT group was much higher than that of the control group, whereas the D2 mRNA levels measured in the thyroid tissues of two of the HG-HT patients were comparable to those of the controls. These results suggest that the increased D2 activity in the thyroid tissues of HG-HT patients is likely due to post-translational control. However, we cannot conclude that there was no significant difference between D2 mRNA levels in the thyroid tissues of patients with HG-HT and those of the controls. It is well known that

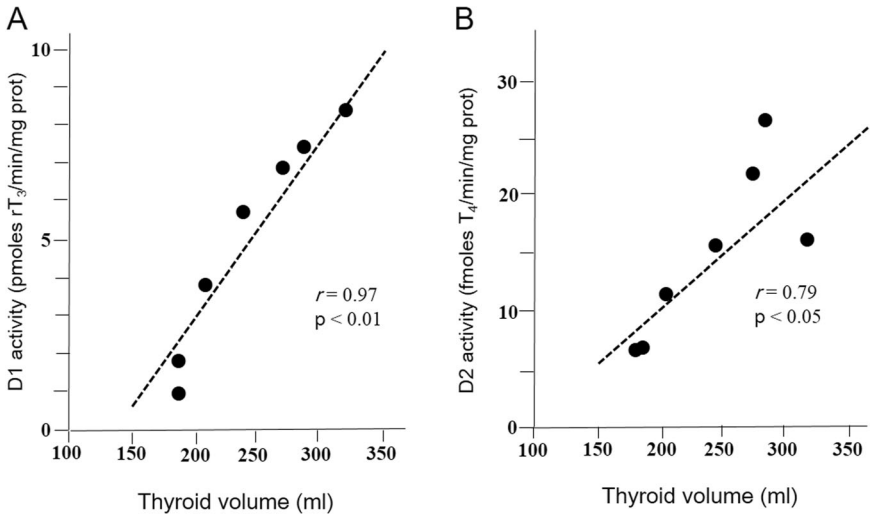

Fig. 5 The correlation between the D1 **a** and D2 **b** activities, and the thyroid volume of the huge goitrous Hashimoto's thyroiditis (HG-HT) patients ($n = 7$)

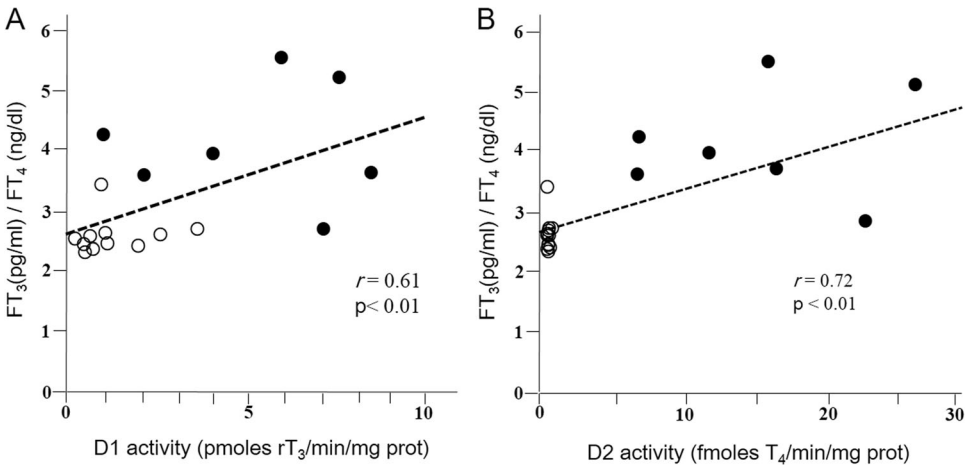

Fig. 6 The correlation between the D1 **a** and D2 **b** activities and the serum FT_3/FT_4 ratio in the controls (open circles) ($n = 11$) and the huge goitrous Hashimoto's thyroiditis (HG-HT) patients (solid circles) ($n = 7$)

D2 activity is negatively regulated by its preferred substrate, T_4, at the post-translational level via the stimulation of the ubiquitin-mediated proteasome degradation of the enzyme [16]. It is likely that the T_4 content in the thyroid tissues of patients with HG-HT is low, and the low T_4 content may decelerate the ubiquitination of D2, resulting in increased D2 activity. However, to the best of our knowledge there are no published data regarding the thyroid hormone contents in these thyroid tissues. Further investigations are necessary to test this hypothesis.

It was reported that the serum FT_3/FT_4 ratios were high in goitrous patients with Tg gene mutation and McCune-Albright syndrome [9, 10]. The D2 activity in the thyroid tissues of the patients with Tg gene mutation was significantly high but its mRNA level was not significantly different compared to those of the controls, suggesting that the increased D2 activity is likely due to the post-translational control [9]. The D1 activity in the thyroid tissues of those patients was not significantly different. On the other hand, not only the D2 activity and mRNA level but also the D1 activity and mRNA level were high in the thyroid tissues of patients with McCune-Albright syndrome. The increases of both D1 and D2 mRNA in thyroid tissues of the patients with McCune-Albright syndrome were presumably due to the ligand-independent cyclic AMP signaling activation by mutations of the α-subunit of the G stimulatory protein [10], since both D1 and D2 mRNA levels in human thyroid tissues were increased by the cyclic AMP signal [17].

We reported that the D2 activity in the thyroid tissues of patients with T_3-predominat Graves' disease is higher than that of patients with common-type Graves' disease, though the D2 mRNA levels in the thyroid tissues of these two patient groups were similar [11]. Of note is our finding that the volume of the thyroid gland in the patients with T_3-predominant Graves' disease was greater than that of the common-type group, and the mean value of the thyroid volume was compatible to that of the present HG-HT

patients. In addition, the D2 activity in the thyroid tissues of the present HG-HT patients was compatible to that of patients with T_3-predominant Graves' disease [11].

We also observed that the D1 activity in the HG-HT patients' thyroid tissues was higher than that of the controls, whereas the D1 mRNA level measured in the thyroid tissues of two of the HG-HT patients was comparable to those of the controls. We, therefore, suggest that the increased D1 activity in the thyroid tissues of HG-HT patients is also likely due to post-translational control, though we cannot conclude that there was no significant difference in D1 mRNA levels in the thyroid tissues between the HG-HT patients and controls. D1 has a long half-life (> 12 h) compared to D2 (< 1 h) [3]. D1 is inactivated but not ubiquitinated or degraded after exposure to its preferred substrate rT_3 [16, 18]. It is likely that the rT_3 content in the thyroid tissues of HG-HT patients is low, and the low rT_3 content may decelerate the inactivation and/or degradation of D1, resulting in increased D1 activity.

In the present study, we observed a strong correlation between D1 and D2 activities, and the thyroid volume in patients with HG-HT, and there was a strong correlation between the HG-HT patients' D1 and D2 activities. These findings suggest that lymphocytic infiltration, fibrosis, and thyroid-cell hyperplasia (which are thought to be the main causes of the thyroid enlargement in HG-HT) may participate in the higher D1 and D2 activities in these thyroid tissues [19]. Further investigations are necessary to clarify the mechanism(s) by which D1 and D2 activities become higher in thyroid tissues of individuals with HG-HT.

Maia et al. [20] estimated that D2 is the major contributor of extrathyroidal T_3 production in euthyroid subjects. In the present study, since the mean serum FT_4 level in the HG-HT group was within the normal range, we suggest that the higher D2 activity in the thyroid tissues of individuals with HG-HT may contribute to their higher serum FT_3/FT_4 ratios. It is necessary to clarify whether D1 and/or D2 activity in the thyroid tissues of HG-HG patients contributes to the patients' higher serum FT_3/FT_4 ratios.

In conclusion, our present findings demonstrated that the serum FT_3/FT_4 ratios in the patients with HG-HT were higher than those of the controls, and it appears that the increases in both D1 and D2 activities in the thyroid tissues of these patients may contribute to their higher serum FT_3/FT_4 ratios.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was approved by the Ethics Committee at Kuma Hospital, and all patients gave informed consent for their materials to be used and for their data to be published. This study was conducted in accordance with the principles of the Declaration of Helsinki.

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