Ratio of Serum Free Triiodothyronine to Free Thyroxine in Graves’ Hyperthyroidism and Thyrotoxicosis Caused by Painless Thyroiditis

JAEDUK YOSHIMURA NOH, NAOKO MOMOTANI*, SHUJI FUKADA**, KOICHI ITO, AKIRA MIYAUCHI** AND NOBUYUKI AMINO**

Abstract. The serum T₃ to T₄ ratio is a useful indicator for differentiating destruction-induced thyrotoxicosis from Graves’ thyrotoxicosis. However, the usefulness of the serum free T₃ (FT₃) to free T₄ (FT₄) ratio is controversial. We therefore systematically evaluated the usefulness of this ratio, based on measurements made using two widely available commercial kits in two hospitals. Eighty-two untreated patients with thyrotoxicosis (48 patients with Graves’ disease and 34 patients with painless thyroiditis) were examined in Kuma Hospital, and 218 patients (126 with Graves’ disease and 92 with painless thyroiditis) and 66 normal controls were examined in Ito Hospital. The FT₃ and FT₄ values, as well as the FT₃/FT₄ ratios, were significantly higher in the patients with Graves’ disease than in those with painless thyroiditis in both hospitals, but considerable overlap between the two disorders was observed. Receiver operating characteristic (ROC) curves for the FT₃ and FT₄ values and the FT₃/FT₄ ratios of patients with Graves’ disease and those with painless thyroiditis seen in both hospitals were prepared, and the area under the curves (AUC), the cut-off points for discriminating Graves’ disease from painless thyroiditis, the sensitivity, and the specificity were calculated. AUC and sensitivity of the FT₃/FT₄ ratio were smaller than those of FT₃ and FT₄ in both hospitals. The patients treated at Ito hospital were then divided into 4 groups according to their FT₄ levels (A: ≤2.3, B: >2.3~≤3.9, C: 3.9~≤5.4, D: >5.4 ng/dl), and the AUC, cut-off points, sensitivity, and specificity of the FT₃/FT₄ ratios were calculated. The AUC and sensitivity of each group increased with the FT₄ levels (AUC: 57.8%, 72.1%, 91.1%, and 93.4%, respectively; sensitivity: 62.6%, 50.0%, 77.8%, and 97.0%, respectively). The means ± SE of the FT₃/FT₄ ratio in the Graves’ disease groups were 3.1 ± 0.22, 3.1 ± 0.09, 3.2 ± 0.06, and 3.1 ± 0.07, respectively, versus 2.9 ± 0.1, 2.6 ± 0.07, 2.5 ± 0.12, and 2.3 ± 0.15, respectively, in the painless thyroiditis groups. In the painless thyroiditis patients, the difference in the FT₃/FT₄ ratio between group A and group D was significant (p<0.05). Thus, the FT₃/FT₄ ratio in patients with Graves’ disease likely remains unchanged as the FT₄ level rises, whereas this ratio decreases as the FT₄ level rises in patients with painless thyroiditis. In conclusion, the FT₃/FT₄ ratios of patients with painless thyroiditis overlapped with those of patients with Graves’ disease. However, this ratio was useful for differentiating between these two disorders when the FT₄ values were high.

Key words: FT₃/FT₄ ratio, Thyrotoxicosis, Graves’ disease, Painless thyroiditis

DESTRUCTION-induced thyrotoxicosis is often observed in patients with painless thyroiditis, postpartum thyroiditis, and subacute thyroiditis [1], and it is important to differentiate this type of thyrotoxicosis from the stimulation-induced thyrotoxicosis seen in patients with Graves’ disease because patients with Graves’ disease must be treated with antithyroid drugs, radioisotope therapy, or a subtotal thyroidectomy, whereas patients with destruction-induced thyrotoxicosis can be managed conservatively. Typical cases of subacute thyroiditis are relatively easy to diagnose, but it is often difficult to differentiate painless thyroiditis from Graves’ thyrotoxicosis without measuring radioactive
iodine uptake (RAIU). Thyrotoxicosis developing early during the postpartum period is usually destruction-induced thyrotoxicosis, but Graves’ thyrotoxicosis sometimes develops 3 to 6 months postpartum [2]; differentiating between these two conditions is difficult because the RAIU test is contraindicated in lactating patients. Another problem with measuring RAIU is that not all clinics are equipped to perform this test, and in Japan, patients must be kept on a low iodine diet for 2 weeks before undergoing RAIU. Thus, it is not practical to use the RAIU test to differentiate between these two conditions in all patients with thyrotoxicosis.

The detection of anti-TSH receptor antibodies (TBII) is generally a useful means of diagnosing Graves’ thyrotoxicosis, though 10–30% of patients with this disease are negative for TBII when tested using a conventional radioreceptor assay [3–5] and 15% of patients with painless thyroiditis are reportedly positive [6]. Thus, TBII is not a reliable marker for differentiating between the two types of thyrotoxicosis.

The serum T\textsubscript{3} to T\textsubscript{4} ratio has previously been reported to be helpful in differentiating destruction-induced thyrotoxicosis from Graves’ thyrotoxicosis [1, 7]. The measurement of FT\textsubscript{4} and FT\textsubscript{3}, instead of total T\textsubscript{4} and T\textsubscript{3}, was recently introduced, but Shigemasa et al. [8] found that the FT\textsubscript{3} to FT\textsubscript{4} ratio was not useful for differentiating between the two types of thyrotoxicosis. More recently, Izumi et al. [9] reported that the FT\textsubscript{3} to FT\textsubscript{4} ratio facilitates a differentiated diagnosis when a sophisticated assay system, unaffected by the concentration of thyroxine binding proteins, is employed.

In this study, we systematically evaluated the usefulness of the FT\textsubscript{3} to FT\textsubscript{4} ratio in making a differential diagnosis by using widely available commercial kits to measure serum FT\textsubscript{3} and FT\textsubscript{4} levels.

### Patients and Methods

#### Patients

Graves’ disease was diagnosed on the basis of clinical findings and laboratory tests showing high FT\textsubscript{4} and FT\textsubscript{3} levels, low TSH, increased TBII activity, and a high RAIU. Functioning tumors were excluded by confirming the diffuse uptake of radioactive iodine.

Painless thyroiditis was diagnosed on the basis of increased FT\textsubscript{4} and FT\textsubscript{3} values and a low RAIU. Postpartum painless thyroiditis cases were not included in this study, and the cause of the painless thyroiditis was unknown. Subacute thyroiditis was excluded based on the identification of a painful goiter. All serum samples were obtained during the thyrotoxic phase before treatment. At Kuma Hospital, 54 patients with untreated Graves’ hyperthyroidism, 48 of whom had FT\textsubscript{4} and FT\textsubscript{3} values below the upper detection limit (FT\textsubscript{3} <30.0 pg/ml, FT\textsubscript{4} <6.0 ng/dl) (41 women, 7 men; age, 38.6 ± 15.5 years), and 34 patients with painless thyroiditis (28 women, 6 men; age, 35.3 ± 14.2 years) were examined. No significant differences in sex or age distribution were observed between these two groups. At Ito Hospital, 138 patients with untreated Graves’ hyperthyroidism, 126 of whom had FT\textsubscript{4} and FT\textsubscript{3} values below the upper detection limit (FT\textsubscript{3} <30.0 pg/ml, FT\textsubscript{4} <10.0 ng/dl) (107 women, 19 men; age, 45.2 ± 14.6 years), and 92 patients with untreated painless thyroiditis (82 women, 10 men; age, 43.9 ± 14.2 years) were examined. Control samples were obtained from 66 normal individuals (55 women, 11 men; age, 33.7 ± 16.2 years). No significant differences in the sex distributions of the 3 groups or in the ages of the Graves’ hyperthyroidism group and the painless thyroiditis group were observed. Informed consent was obtained from all participants.

#### Detection of serum FT\textsubscript{4} and FT\textsubscript{3} levels

At Kuma Hospital, serum FT\textsubscript{4} and FT\textsubscript{3} values were measured using Architect FT\textsubscript{3} and FT\textsubscript{4} commercial kits (Abbott Japan Diagnostics, Tokyo, Japan); the reference ranges were 1.7–3.7 pg/ml and 0.7–1.6 ng/dl, respectively. At Ito Hospital, the serum FT\textsubscript{4} and FT\textsubscript{3} values were measured using Lumipulse FT\textsubscript{3} and FT\textsubscript{4} commercial kits (Fujirebio Inc., Tokyo, Japan); the reference ranges were 2.5–4.5 pg/ml and 0.75–1.75 ng/dl, respectively, with an upper detection limit of 30.0 pg/ml for FT\textsubscript{3} and 10.0 ng/dl for FT\textsubscript{4}.

#### Statistical methods

The FT\textsubscript{3}/FT\textsubscript{4} ratios were compared between the two groups using the Mann-Whitney U test and the Tukey-Kramer HSD test. A Spearman rank correlation was employed for simple correlation analysis. Differences were considered significant at p <0.05. Cut-off values were calculated using receiver operating characteristics (ROC) curves.
Results

The individual serum FT₃/FT₄ ratio values in the patients with Graves’ disease and those with painless thyroiditis at Kuma and Ito Hospitals are shown in Fig. 1. Significant differences between the Graves’ disease and painless thyroiditis groups were observed, but the values in the Graves’ disease group overlapped considerably with those in the painless thyroiditis group. The FT₃ and FT₄ values and the FT₃/FT₄ ratios of the patients with Graves’ disease and painless thyroiditis who were treated at Kuma and Ito Hospitals are presented in Table 1. At both hospitals, the FT₃ and FT₄ values and the FT₃/FT₄ ratios of the thyrotoxic patients with Graves’ disease were higher than those of patients with painless thyroiditis (P<0.001). The ROC curves for the FT₃ and FT₄ values and the FT₃/FT₄ ratios of patients with Graves’ disease and painless thyroiditis treated in both hospitals were plotted, and the area under the curves (AUC), the cut-off points for discriminating Graves’ disease from painless thyroiditis, the sensitivity, and the specificity were calculated (Table 2). The AUC and sensitivity values were smaller for the FT₃/FT₄ ratio than for the FT₃ and FT₄ levels in both hospitals. No correlations between the FT₃/FT₄ ratio and radioactive iodine uptake in either the patients with Graves’ disease or those with painless thyroiditis were observed among the patients treated at Ito Hospital.

The patients at Ito Hospital were then divided into 4 groups according to their FT₄ levels: group A: ≤2.3, group B: 2.3~≤3.9, group C: 3.9~≤5.4, and group D: >5.4 ng/dl. The AUC, cut-off points for discriminating patients with Graves’ disease from those with painless thyroiditis, the sensitivity, and the specificity of the FT₃/FT₄ ratios were calculated (Table 3). The individual serum FT₃/FT₄ ratios are shown in Fig. 2. No significant difference in age or sex were observed among the 4 groups of patients with Graves’ disease, but group C was significantly older than the other

Table 2. Differentiation of Graves’ disease from painless thyroiditis

<table>
<thead>
<tr>
<th>Hospital</th>
<th>AUC (%)</th>
<th>Cutoff point</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kuma Hospital</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FT₃ (pg/ml)</td>
<td>84.3</td>
<td>6.5</td>
<td>91.7</td>
<td>67.6</td>
</tr>
<tr>
<td>FT₄ (ng/dl)</td>
<td>83.8</td>
<td>2.5</td>
<td>91.7</td>
<td>73.5</td>
</tr>
<tr>
<td>FT₃/FT₄ ratio</td>
<td>71.0</td>
<td>2.8</td>
<td>77.1</td>
<td>58.8</td>
</tr>
<tr>
<td>Ito Hospital</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FT₃ (pg/ml)</td>
<td>84.5</td>
<td>10.5</td>
<td>77.8</td>
<td>82.6</td>
</tr>
<tr>
<td>FT₄ (ng/dl)</td>
<td>78.8</td>
<td>3.3</td>
<td>77.8</td>
<td>71.7</td>
</tr>
<tr>
<td>FT₃/FT₄ ratio</td>
<td>75.3</td>
<td>3.0</td>
<td>61.9</td>
<td>80.4</td>
</tr>
</tbody>
</table>

AUC: Area under the curve

Table 1. FT₃ and FT₄ values and FT₃/FT₄ ratios of Graves’ disease patients and painless thyroiditis patients at Kuma Hospital and Ito Hospital.

<table>
<thead>
<tr>
<th>Hospital</th>
<th>FT₃ (pg/ml)</th>
<th>FT₄ (ng/dl)</th>
<th>FT₃/FT₄</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>range</td>
<td>Median</td>
</tr>
<tr>
<td>Kuma Hospital</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Graves’ disease (n = 48)</td>
<td>11.5</td>
<td>3.2–28.0*</td>
<td>3.4</td>
</tr>
<tr>
<td>Painless thyroiditis (n = 34)</td>
<td>4.9</td>
<td>2.6–17.4</td>
<td>2.1</td>
</tr>
<tr>
<td>Ito Hospital</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Graves’ disease (n = 126)</td>
<td>13.3</td>
<td>3.6–29.5*</td>
<td>4.4</td>
</tr>
<tr>
<td>Painless thyroiditis (n = 92)</td>
<td>6.9</td>
<td>4.0–24.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Normal control (n = 66)</td>
<td>3.2</td>
<td>2.4–4.5</td>
<td>1.1</td>
</tr>
</tbody>
</table>

(): number of patients

* Significant difference from the value in the painless thyroiditis group and normal control group at P<0.001.
groups among the patients with painless thyroiditis (p<0.05). The FT$_4$/FT$_3$ ratios were significantly higher in groups B, C and D with Graves’ disease than in the corresponding groups with painless thyroiditis. The AUC and sensitivity of each group increased as the FT$_4$ levels rose. The mean ± SE of the FT$_3$/FT$_4$ ratios in the Graves’ disease groups were 3.1 ± 0.22, 3.1 ± 0.09, 3.2 ± 0.06, and 3.1 ± 0.07, respectively, versus 2.9 ± 0.1, 2.6 ± 0.07, 2.5 ± 0.12, and 2.3 ± 0.15, respectively, in the painless thyroiditis groups, and the difference in the FT$_3$/FT$_4$ ratios of groups A and D with painless thyroiditis was significant (p<0.05). The median and range of the FT$_3$/FT$_4$ ratio at 1 day, 2–30 days, 31–90 days, 91–180 days, 181–360 days, and >360 days after the initial diagnosis were calculated in each of the painless thyroiditis groups (Table 4). The values at 31–90 days after the initial diagnosis were the highest in all groups, and the degree of elevation corresponded to the FT$_4$ level at the time of the initial diagnosis. The median and range of the FT$_4$ levels at 31–90 days after diagnosis in each of the painless thyroiditis groups were 0.97 ng/dl (0.25–2.4), 0.84 ng/dl (0.15–3.24), 0.78 ng/dl (0.17–2.81), and 0.62 ng/dl (0.34–1.42), respectively. Those of TSH at 31–90 days after diagnosis were 1.67 µU/ml (0.01–71.1), 3.66 µU/ml (0.01–94.9), 3.81 µU/ml (0.01–45.3), and 20.2 µU/ml (6.8–43.9), respectively.

**Discussion**

Thyrotoxicosis in patients with painless thyroiditis and subacute thyroiditis is caused by thyroid destruction, and painless thyroiditis has been reported to account for 0–23% of all thyrotoxicosis cases [10–12]. Painless thyroiditis during the postpartum period, so-called postpartum thyroiditis, is 10 times more common in the general population than postpartum Graves’ disease [13]. The thyrotoxic symptoms in painless thyroiditis are usually milder than those associated with Graves’ disease, and as a result, many cases may be
missed. Because the symptoms are mild and nonspecific, most patients with painless thyroiditis probably consult a general practitioner, rather than a specialized endocrinology clinic. The prevalence of painless thyroiditis is thus probably higher than recognized. Destruction-induced thyrotoxicosis can be definitively differentiated from Graves’ disease using the RAIU test; however, this test is not available in the offices of general practitioners or in most clinics and it is contraindicated in lactating women. Thus, there has long been a need for a simple, practical test to differentiate between these two disorders.

The zinc concentration in erythrocytes has recently been reported to be a useful measure for making a differential diagnosis [4], but such measurements are not routinely performed. A highly sensitive third-generation TSH assay has also been reported to be useful [14], but a clear overlap in values for the two types of thyrotoxicosis limits its practical use.

A $T_3/T_4$ ratio (ng/µg) of less than 20 has been reported to indicate destruction-induced thyrotoxicosis [1, 7]. However, this ratio is affected by the thyroxine-binding globulin (TBG) concentration [15]. Several $FT_3$ and $FT_4$ assays have recently been introduced, and most of these assays are unaffected by the TBG concentration. Izumi et al. reported that the $FT_3/FT_4$ ratio was useful for differentiating between the two types of thyrotoxicosis, although some overlap between the values for Graves’ hyperthyroidism and destruction-induced thyrotoxicosis was observed [9]. In that study, $FT_4$ was measured using their analogue method and $FT_3$ was measured using a radioimmunoassay with a $^{125}$I-labeled anti-$T_3$ monoclonal antibody. Both of these assays are minimally influenced by binding proteins and autoantibodies to $T_4$ and $T_3$. The differences in the assay methods used in this previous study and the present study may account for the difference in the $FT_3/FT_4$ ratio results.

Which method is more useful for differentiating the two types of thyrotoxicosis? The $T_3/T_4$ ratio seems to be more useful than the $FT_3/FT_4$ ratio for differentiating Graves’ disease from painless thyroiditis because 85.5% (71/83) of the thyrotoxic patients with a $T_3/T_4$ ratio of $>20$ had Graves’ disease [16]. Moreover, the upper detection limit for $FT_3$ and $FT_4$ is a major disadvantage when using the $FT_3/FT_4$ ratio to diagnose in patients with severe thyrotoxicosis.

In this study, the $FT_3$ and $FT_4$ values and the $FT_3/FT_4$ ratios of the patients with Graves’ disease, were higher than those in patients with painless thyroiditis in both hospitals, but considerable overlap was noted. An analysis of the AUCs, the cut-off points for discriminating patients with Graves’ disease from those with painless thyroiditis, the sensitivity, and the specificity revealed that the $FT_3/FT_4$ ratio was no more useful as an indicator for the differential diagnosis of Graves’ disease from painless thyroiditis than the $FT_3$ and $FT_4$ values. We next divided the patients treated at Ito Hospital into 4 groups according to their $FT_4$ levels, and the AUCs, cut-off points, sensitivity, and specificity of the $FT_3/FT_4$ ratios for each group were calculated. Both the AUC and the sensitivity values increased with increasing $FT_4$ levels in all groups, suggesting that the $FT_3/FT_4$ ratio may be useful for diagnosing patients with high $FT_4$ levels. No significant differences between the $FT_3/FT_4$ ratios of the 4 Graves’ disease groups were observed, but the ratios in the painless thyroiditis groups decreased as the $FT_3$ levels increased. Type I and type II iodothyronine deiodinases are present in the thyroid [17, 18]. Type I iodothyronine deiodinase is increased by thyroid hormone, and Type II iodothyronine deiodinase is regulated by TSH receptors [17]. In painless thyroiditis, the $FT_4$ levels depend on the degree of thyroid destruction, and the activities of both deiodinases may also be affected by the degree of thyroid destruction. In patients with painless thyroiditis, the $FT_3/FT_4$ ratios and the $TSH$ levels increased over the course of the study, rising steadily during the 31-to-90-day period after the initial diagnosis in all groups. These results raise the possibility that changes in the $FT_3/FT_4$ ratio are regulated by both deiodinases in patients with painless thyroiditis.

The measurement of TBII is useful for the diagnosis of Graves’ disease, but some weakly TBII-positive patients with painless thyroiditis have also been observed. The cutoff value for TBII used by the Ito Hospital is $<10\%$, and 13 patients with TBII values between $>10$ to $<20\%$ were included in this study (Graves’ disease, $n = 12$; painless thyroiditis, $n = 1$ patient). The $FT_4$, $FT_3/FT_4$ ratio and TBII values of the patient with painless thyroiditis were 2.33 ng/dl, 2.45, and 15.9, respectively, and this patient was correctly diagnosed using the cutoff values. Among the patients with Graves’ disease, 66.7% (8/12) had an $FT_3/FT_4$ ratio $>0.2$ had Graves’ disease [16]. Moreover, the upper detection limit for $FT_3$ and $FT_4$ is a major disadvantage when using the $FT_3/FT_4$ ratio to diagnose in patients with severe thyrotoxicosis.

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In this study, the $FT_3$ and $FT_4$ values and the $FT_3/FT_4$ ratios of the patients with Graves’ disease, were higher
Graves’ disease from painless thyroiditis in weakly TBII-positive patients. Finally, the FT$_3$/FT$_4$ ratio was a useful indicator for differentiating painless thyroiditis from Graves’ disease in patients with high FT$_4$ values.

References


