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Poorly Differentiated Thyroid Carcinoma Coexisting with Graves' Disease Involving T3 Thyrotoxicosis due to Increased D1 and D2 Activities

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Background: Poorly differentiated thyroid carcinoma is rare and patients are typically euthyroid. We report a novel rare case of poorly differentiated thyroid carcinoma with triiodothyronine (T3) thyrotoxicosis.
Patient's Findings: A 77-year-old man presented to Kuma Hospital due to a neck tumor. A thyroid ultrasonography revealed a 220-mL mass in the right lobe. Laboratory data showed low serum thyrotropin (TSH), low free thyroxine (fT4), and high free T3 (fT3) levels. Anti-TSH receptor antibodies and thyroid-stimulating antibodies were positive. ^{131}I scintigraphy showed diffuse uptake only in the left thyroid lobe. The patient underwent a total thyroidectomy and histological examination identified as poorly differentiated thyroid carcinoma. He was diagnosed with poorly differentiated thyroid carcinoma coexisting with Graves' disease. The tumor showed elevated type 1 iodothyronine deiodinases (D1) and type 2 iodothyronine deiodinases (D2) activities compared with that of the left thyroid lobe.
Summary and Conclusions: Increased D1 and D2 activities in poorly differentiated carcinoma resulted in T3 toxicosis with a high serum fT3/fT4 ratio.

Keywords: Graves' disease, iodothyronine deiodinase, poorly differentiated thyroid carcinoma

Introduction

The normal human thyroid tissue expresses type 1 and type 2 iodothyronine deiodinases (D1 and D2), which are enzymes catalyzing the deiodination of thyroxine (T4) to an active form, 3,5,3′-triiodothyronine (T3) (1). Approximately 80% of the circulating T3 in humans is produced by the deiodination of T4 outside the thyroid gland (2). Some studies have reported that increased D2 activity in thyroid tissues causes relatively high serum T3 levels in patients with follicular thyroid carcinoma (3), Graves' disease during propylthiouracil (PTU) treatment (4), and thyroglobulin gene mutations (5). Our group previously reported that increased D1 and D2 activities might cause a higher serum free T3 (fT3)/free T4 (fT4) ratio in patients with T3-predominant Graves' disease (6) and huge goitrous Hashimoto's thyroiditis (7). We also reported cases of metastatic follicular thyroid carcinoma after total thyroidectomy wherein T3 thyrotoxicosis was observed. In these cases, we demonstrated that T3 production by the metastatic tumor did not occur but was due to the orally administered levothyroxine being converted to T3 due to increased D1 and D2 activities expressed in the tumor (8).

Herein, we describe a rare case of poorly differentiated thyroid carcinoma coexisting with Graves' disease. This case involved T3 thyrotoxicosis due to increased D1 and D2 activities expressed in the poorly differentiated thyroid carcinoma.

Materials and Methods

Measurement of D1 activity

Human thyroid tissues were homogenized, and the microsomal fraction was prepared as previously described (7). D1 activity was assayed as previously described (7). In brief, the reaction mixture comprised microsomal protein, 0.5 μM reverse T3 (rT3), and 10 mM dithiothreitol (DTT) in the presence or absence of 1 mM PTU in 0.1 M potassium phosphate, and 1 mM ethylenediaminetetraacetic acid, pH 6.9 (PE buffer). The mixture was incubated at 37°C for 60 minutes. The reaction was stopped by adding cold methanol at the same volume. The mixture was centrifuged at 14,000 × g

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at 4°C for 10 minutes. The supernatant was transferred to a new tube and stored at −25°C. Deiodination products were quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS) as previously described (9). Protein concentration was measured according to the Bradford method using bovine serum albumin as a standard. D1 activity was calculated as the number of picomoles of rT3/(min·mg) protein.

Measurement of D2 activity

D2 activity was assayed as previously described (7). In brief, the reaction mixture included microsomal protein, 0.1 nM [^{125}I] T4 purified by LH-20 chromatography, 2 nM cold T4, 20 mM DTT, and 1 mM PTU in PE buffer. The mixture was incubated at 37°C for 120 minutes. $^{125}I^-$ was separated by TCA precipitation and counted with a γ-counter as previously described (7). Deiodinating activity was expressed as the femtomoles of T4/(min·mg) protein.

RNA preparation and real-time quantitative polymerase chain reaction

Total RNA was isolated from thyroid tissues using TRIzol reagent (Invitrogen, CA) according to the manufacturer's protocol. Real-time quantitative polymerase chain reaction assays were performed as previously described (9). mRNA levels of human D1, D2, and GAPDH were analyzed using Rotor-Gene Q (Qiagen). mRNA levels were expressed as arbitrary units after correction for the GAPDH mRNA level.

Patient. A 77-year-old man presented to the Kuma Hospital for evaluation of his right-sided neck tumor with symptoms of excessive sweating and palpitations. Ultrasonography of the thyroid [the measurement method was as described previously (10)] revealed an estimated 220-mL highly vascular mass that occupied and totally replaced the right lobe and partly extended to the mediastinum. The volume of the left thyroid lobe was estimated to be 7 mL and was not atrophic. The tumor showed findings suggestive of a follicular tumor according to the results of fine needle aspiration cytology. Laboratory data showed a low serum thyrotropin (TSH) level of 0.187 μU/mL (reference range 0.5–5.0 μU/mL), low fT4 level (0.53 ng/dL; reference range 0.9–1.7), and high fT3 level (4.31 pg/mL; 2.3–4.0), resulting in an increased serum fT3/fT4 ratio. The serum rT3 level measured by LC-MS/MS was 19.0 ng/dL (reference range 4.0–38.0 ng/dL (11–13)). Titers of anti-TSH receptor antibodies (TRAb) and thyroid-stimulating antibodies (TSAb) were positive (16.3 IU/L; reference range <2.0 IU/L and 262%; <120%, respectively). The quantitative value of the ^{131}I uptake at 24 hours was 9.8%, with diffuse uptake only in the left thyroid lobe (Fig. 1A). No uptake was noted in the region of the tumor mass.

The patient underwent total thyroidectomy four months after the first visit. Before surgery, we carefully monitored the patient's thyroid function, but since thyroid function was unchanged and the symptoms of hyperthyroidism were not severe, we did not prescribe any antithyroid medication. In addition, the tumor size did not change between the first visit and the date of surgery. Histologically, the mass on the right side showed findings characteristic of poorly differentiated carcinoma: a high mitotic count and necrosis with solid, trabecular, and insular growth patterns. Grossly, the cut surface of the tumor was lobulated, which is a finding frequently observed in poorly differentiated carcinoma. Microscopically, however, the carcinoma cells were monotonous, even though they had different growth patterns. The entire tumor was a poorly differentiated carcinoma and did not include any areas of differentiated carcinoma (Fig. 1B). Thus, the patient was diagnosed with a poorly differentiated thyroid carcinoma coexisting with Graves' disease. A dose of levothyroxine to suppress TSH was administered after thyroidectomy to prevent tumor recurrence in addition to the substitution therapy. After adjusting the dose of levothyroxine to 150 μg/day, the laboratory data showed a low serum TSH level of 0.027 μU/mL, high fT4 level (2.02 ng/dL), and normal fT3 level (3.00 pg/mL), resulting in a normal serum fT3/fT4 ratio. The serum rT3 level increased to 44 ng/dL. Furthermore, thyrotoxicosis symptoms improved after surgery. The present case study was approved by the Ethical Committee at the Kuma Hospital (IRB:20200709-1).

D1 and D2 activities and mRNA levels. D1 activity in the poorly differentiated thyroid carcinoma tissue was 56.7 pmol rT3/(min·mg) protein, which was ∼13 times higher than that in the left lobe Graves' tissues (4.5 pmol rT3/(min·mg) protein). D2 activity in the poorly differentiated thyroid carcinoma tissue was remarkably high, showing 10.1 fmol T4/(min·mg) protein, which was ∼337 times higher than that of the left lobe Graves' tissue (0.03 fmol T4/(min·mg) protein) (Table 1).

However, the D1 mRNA levels of the poorly differentiated thyroid carcinoma tissue and left lobe Graves' tissue were 1.8 and 3.9 arbitrary units (a.u.), respectively. Furthermore, the D2 mRNA levels in the poorly differentiated thyroid carcinoma and the left lobe Graves' tissue were 2.7 and 1.9 a.u., respectively. These results suggest that D1 and D2 mRNA levels were expressed at similar levels in the poorly differentiated thyroid carcinoma tissue and the left lobe Graves' tissue in this study.

Discussion

Herein, we report a rare case of poorly differentiated thyroid carcinoma manifesting as T3 thyrotoxicosis due to increased D1 and D2 activities of the tumor tissue. In humans, both D1 and D2 are expressed in the thyroid gland, contributing to the plasma T3 pool (1). We (8) and Kim *et al.* (3) previously reported cases of massive metastatic or large primary follicular thyroid carcinoma, showing a decrease in serum T4 levels and an increase in serum T3 levels. In these cases, excessive conversion of T4 to T3 in the tumor tissues was suggested to be the reason for these laboratory findings. Similar to previous cases, the patient's laboratory data showed low fT4 and high fT3 levels. The preoperative fT3 (pg/mL)/fT4 (ng/dL) ratio was 8.13, which decreased to 1.49 after surgery. ^{131}I scintigraphy showed no iodine uptake in the region of the tumor mass, demonstrating that the tumor did not produce the thyroid hormone autonomously. D1 and D2 activity levels in the carcinoma were very high, which supports the hypothesis that excessive conversion of T4 to T3 in the tumor tissue resulted in these laboratory findings. In addition, the preoperative rT3 (ng/dL)/fT3 (pg/mL) ratio was 4.41, which increased to 14.7 after surgery upon taking 150 μg/day levothyroxine. This change was reasonable

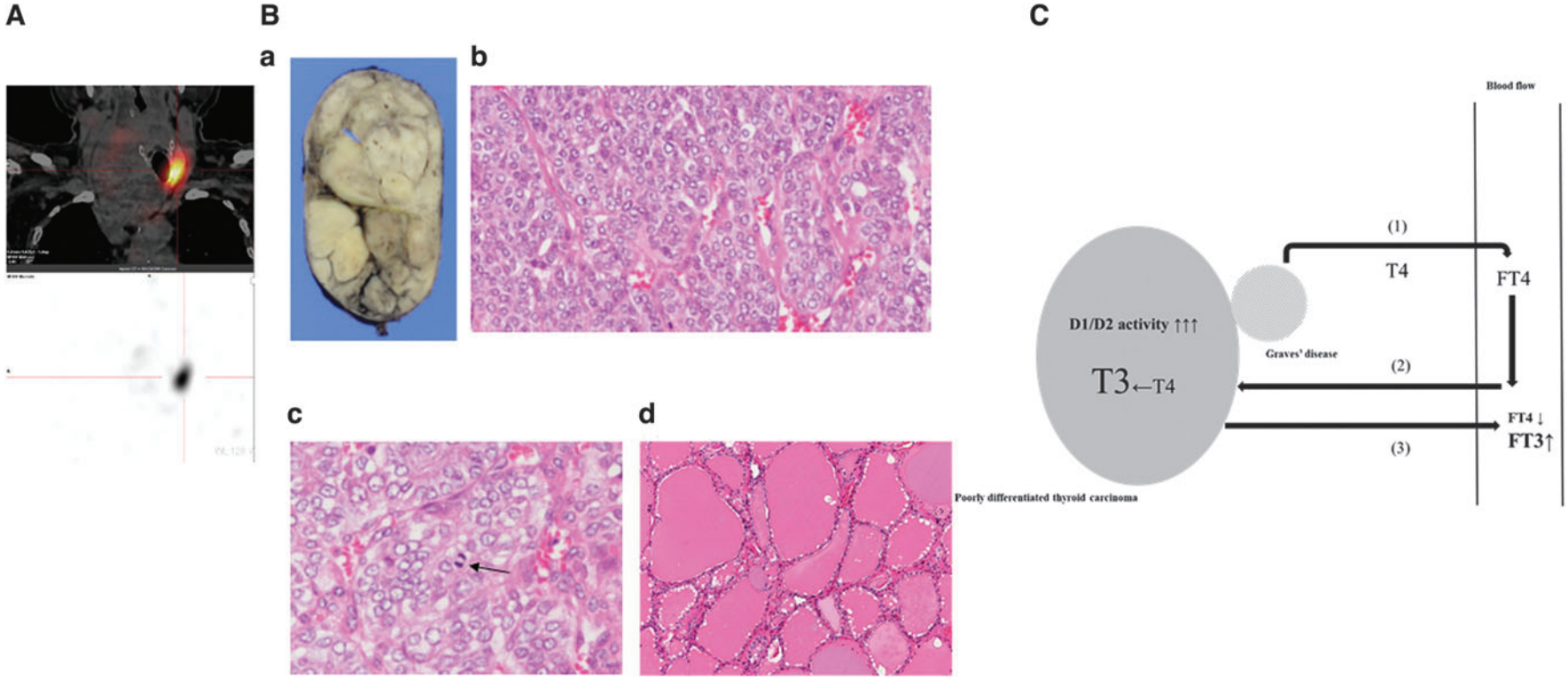

FIG. 1. (**A**) ^{131}I scintigraphy showing diffuse uptake in the left thyroid lobe. No uptake was noted in the region of the tumor. (**B**) Pathological findings of the resected thyroid specimen. (a) On gross examination, the tumor was well demarcated and lobulated. H&E shows a trabecular growth pattern (b), no nuclear features of papillary thyroid carcinoma (c), the arrow indicates the mitotic figure, and (d) non-neoplastic thyroid tissue showed slightly enlarged thyroid follicles associated with peripheral scalloping of the colloid, which is consistent with Graves' disease. (**C**) Schematic showing the posited cause of the T3 thyrotoxicosis in this case. D1, type 1 iodothyronine deiodinases; D2, type 2 iodothyronine deiodinases; fT3, free triiodothyronine; fT4, free thyroxine; H&E, hematoxylin and eosin; T3, triiodothyronine; T4, thyroxine.

Table 1. D1 and D2 Activities in the Present Case Comparing with the Previously Reported Cases

		Volume (mL)	*D1 (pmol rT3/min/mg protein)*	*D2 (fmol T4/min/mg protein)*
Present case	Poorly differentiated thyroid carcinoma	220	56.7	10.1
	Left lobe	7	4.5	0.03
Reported cases	Metastatic follicular carcinoma [data from Ref. (8)]	39.4–797.8	8.0–29.6	24.7–41.5
	Follicular carcinoma [data from Ref. (3)]	965	9.6	4.1
	Papillary carcionoma [data from Ref. (14)]	No data	0.08 ± 0.07	No data
	T3-predominant Graves' disease [data from Ref. (6)]	227 ± 106	8.2 ± 3.4	13.7 ± 9.9
	Graves' disease [data from Ref. (6)]	32 ± 23	5.3 ± 2.5	3.2 ± 2.2
	Huge goitrous Hashimoto's thyroiditis [data from Ref. (7)]	240 ± 54	5.0 ± 2.9	15.2 ± 7.5
	Normal tissue [data from Ref. (7)]	No data	1.1 ± 1.1	0.1 ± 0.1

D1, type 1 iodothyronine deiodinases; D2, type 2 iodothyronine deiodinases; rT3, reverse T3; T3, triiodothyronine; T4, thyroxine.

because the rT3 degradation increased and the T4 to T3 conversion was accelerated, due to the high D1 and D2 activity levels in poorly differentiated carcinoma, resulting in a decrease in the rT3/fT3 ratio. Subsequent resection of the poorly differentiated carcinoma resulted in an increase of the rT3/fT3 ratio.

De Souza Meyer *et al.* (14) and Ambroziak *et al.* (15) reported that D1 and D2 activities were low in the papillary thyroid carcinoma, while others reported higher D1 and D2 activities in follicular thyroid cancer tissue and follicular adenoma (16). Poorly differentiated thyroid carcinoma is described as a tumor with intermediate prognosis between differentiated thyroid carcinoma and anaplastic thyroid carcinoma. It was first reported as a distinct diagnostic entity in the World Health Organization diagnostic criteria in 2004 (17). The frequency of poorly differentiated carcinoma has been reported to be 2–15% of all thyroid carcinomas (18).

We compared D1 and D2 activity levels between this study and the previously reported levels in follicular thyroid carcinoma and Graves' disease tissues. Interestingly, D1 activity in the poorly differentiated thyroid cartinoma tissue was remarkably high compared with the contralateral Graves' disease tissue and compared with prior reports (Table 1). D2 activity in the carcinoma tissues was similar to that of T3-predominant Graves' disease. D1 activity levels in the left lobe Graves' tissue were higher than that of the normal tissue, which is consistent with previous findings in Graves' disease. While the D2 activity levels of the left lobe Graves' tissue were relatively low, it did not seem to be contradictory, as we previously reported low levels of D2 activity in some patients with Graves' disease but not in T3-predominant Graves' disease (6), as seen in the present case.

Interestingly, the mRNA levels of D1 and D2 in the thyroid tissues of the poorly differentiated carcinoma and Graves' thyroid were similar, although D1 and D2 activities in the thyroid tissues of the carcinoma were higher than those in the Graves' thyroid tissue. We suggest that the inactivation and/or degradation of D1 and D2 in poorly differentiated thyroid carcinoma tissue may be decelerated. Recently, we reported that there were significant correlations between D1 and D2 activities in the thyroid gland of patients with huge goitrous Hashimoto's thyroiditis and thyroid volume (7). The volume of the poorly differentiated thyroid carcinoma in this patient was large. Therefore, we also suggest that the same mechanisms by which the D1 and D2 activities in the thyroid tissues with large goitrous Hashimoto's thyroiditis are increased may be involved in poorly differentiated thyroid carcinoma. Further investigations are necessary to clarify the mechanisms by which the D1 and D2 activities in the poorly differentiated thyroid carcinoma are increased.

Of note, the characteristic finding of this case is that the initial laboratory data showed low fT4 levels despite having Graves' disease. Based on the results of thyroid scintigraphy, it is reasonable that T4 and T3 were excessively produced from the left lobe of the thyroid gland. However, we suggest that the increase in D1 and D2 activities in the poorly differentiated carcinoma tissue was so powerful that it outweighed the overproduction of T4, resulting in a low circulating fT4 level.

We hypothesize the mechanism of this case as follows (Fig. 1C): (1) T4 and some amount of T3 levels were overproduced from the left lobe of the thyroid gland because of Graves' disease. T4 and T3 production in the carcinoma and in the right lobe was negligible, as no uptake was visible in the scintigraphy. (2) T4 added to the blood, and when perfused into the tumor, it was excessively converted to T3 due to the overexpression of D1 and D2 in the tumor tissue. (3) The converted T3 was added to the circulating blood flow, thus resulting in high fT3/fT4 ratio and T3 thyrotoxicosis.

To the best of our knowledge, this is the first case reporting T3 thyrotoxicosis due to increased D1 and D2 activity levels in poorly differentiated thyroid carcinoma. In addition, this case is novel because there have been no cases in the past reporting Graves' disease associated with thyroid carcinoma that overexpresses D1 and D2 activities.

Authors' Contributions

All authors contributed to patient care. M.O.-H. drafted the article, and other authors made revisions. A.M. and N.T. performed the measurements of total RNA and D1 and D2 activities. All authors have read and approved the final article.

Author Disclosure Statement

No competing financial interests exist.

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