# Original Article Consumptive hypothyroidism due to a gastrointestinal stromal tumor expressing type 3 iodothyronine deiodinase

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Abstract: Context: Gastrointestinal stromal tumors (GISTs) are responsive to sunitinib (the tyrosine kinase inhibitor), this agent is widely used in prevention relapse of GISTs and neo-adjuvant chemotherapy in GIST patients without operation opportunity. The use of these agents has both advantages and disadvantages. On the one hand, it can improve the outcome for patient. On the other hand, it may lead to consumptive hypothyroidism, a rare syndrome caused by increased catabolism of T4 and T3 by increased type 3 iodothyronine deiodinase (D3) activity. D3 is the major physiologic inactivator of thyroid hormone, this selenoenzyme catalyzes the inner-ring deiodination of T(4) to reverse T(3) and T(3) to 3, 3;-diiodothyronine, both of which are biologically inactive [1]. Increased monitoring and supernormal thyroid hormone supplementation are required for affected patient. Objective: The aim of the study was to report the first case of consumptive hypothyroidism in an athyreotic patient after surgical resection of gastrointestinal stromal tumor. Design, setting, and patient: A 60-year-old athyreotic male was presented and he was euthyroid when receiving a stable therapeutic dose of thyroid hormone which was used to treat consumptive hypothyroidism resulting from the side effects of sunitinib, which is used for treatment of neo-adjuvant chemotherapy in gastrointestinal stromal tumor. With a discovery of large D3-expressing gastrointestinal stromal tumor, this patient suffered from marked Hyperthyrotropinemia, which instantly worsened after surgical resection of the gastrointestinal stromal tumor and then continued for 12 weeks after the surgical resection, in spite of further increases in levothyroxine therapy. The patient also had low serum T3 and elevated serum reverse T3 (rT3). Intervention: The patient's consumptive hypothyroidism caused by marked overexpression of the thyroid hormone-inactivating D3 within the gastrointestinal stromal tumor and adjacent normal gastrointestinal tissue. Main outcome measures and results: D3 immunostaining of the patient's gastrointestinal stromal tumor was positive, with no significant immunoreactivity in adjacent normal gastrointestinal tissue. The expression levels of CD34, CD117, and DOG1 in peri-tumor tissue samples was lower than that in tumor tissue. The mRNA expression level of KIT exon17 in peri-tumor tissue was higher than that in tumor tissue. Conclusions: This is the first case report of consumptive hypothyroidism in an adult after surgical partial resection of the gastrointestinal stromal tumor. This case demonstrates that hyperthyrotropinemia may worsen after surgical resection of the gastrointestinal stromal tumor.

Keywords: Gastrointestinal stromal tumors, sunitinib, type 3 iodothyronine deiodinase, hyperthyrotropinemia

#### Introduction

Two large abdominal tumors (endoscopy and biopsy examination led to the diagnosis of a GIST) were found in a 60-year-old man, who had received electronic gastroscopy because of sudden hematemesis, and one of which was in the stomach, and the other was in the colon. In the following examination we found that the size and location of the tumors was unfavorable for surgical excision immediately. This finding prompted the initiation of treatment with sunitinib, which is used for neo-adjuvant chemotherapy. One month before the patient's referral to the tumor hospital of Guangxi medical university (sunitinib has been used for two months), his primary care physician noted a sudden increase of serum thyrotropin (TSH) to 10.2  $\mu$ U per milliliter (normal range, 0.5 to 5.0 mU/liter), and in order to deal with hypothyroidism, the patient was treated with levothyroxine LT4. After the LT4 treatment, he was to be clini-

cally stable and to have a TSH level of 2.15 mU/ liter (normal range, 0.5 to 5.0 mU/liter) on an oral LT4 dose of 200 µg (2 µg/kg) daily. After 3 months' continuous sunitinib treatment, the patient received laparoscopic abdominal tumor resection, and his surgery was without complication, and he did not require a blood transfusion. Under the laparoscopic we found that there were about 30 tumors of various sizes from 0.5 × 0.8 cm to 0.3 × 0.6 cm distributing within the abdominal cavity which couldn't be removed because of their wide distribution besides two big tumors which were removed during the surgery, and the postoperative pathology proved to be GIST. Thyrotropin baseline obtaining by blood routine test that was performed immediately before the initiation of sunitinib therapy revealed hypothyroidism, with a thyrotropin (TSH) level of 28 mU/liter (normal range, 0.5 to 5.0 mU/liter), a total thyroxine level of 3.1 µg per deciliter (normal range, 5.0 to 11.0 µg/deciliter), and a triiodothyronine uptake of 40% (normal range, 25 to 35%). Testing for serum thyroperoxidase antibodies was negative. Three week after surgery, despite the administration of levothyroxine doses as high as 300 µg (3 µg/kg) daily and excellent adherence, the patient's subsequent serum TSH levels remained elevated (range, 11 to 36 mU/ liter), with subnormal levels of serum thyroxine (range, 1.2 to 3.1 µg/deciliter) and triiodothyronine (12 to 34 ng/deciliter; normal range, 70 to 170 ng/deciliter). During hypothyroxinemia, the level of serum reverse triiodothyronine was elevated, at 1545 pg/milliliter (normal range, 30 to 250 pg/milliliter (2.37 nmol/liter; normal range, 0.05 to 0.38 nmol/liter). Sunitinib was continued until the patient's death from tumor progression 23 months later.

# Materials and methods

# Pathology

For hematoxylin and eosin (HE) staining tumor tissues were fixed in 4% formaldehyde, dehydrated with gradient ethanol, and embedded in paraffin wax. Tissue sections were dewaxed and rehydrated according to a standard protocol. Sections were stained with HE. For immunohistochemistry [2], using the D3-18 primary D3 antibody and 5-µm sections cut from formalin-fixed, paraffin-embedded tissue. Sections were incubated with D3-18 at 1:1500 dilution and then processed with the immunoperoxidase kit (Vector Laboratories, Inc., Burlingame, CA). Preimmune rabbit IgG (Santa Cruz Biotechnology, Santa Cruz, CA) was used for iso-tope controls.

# Semiquantitative reverse-transcriptase polymerase chain reaction

Total RNA was extracted from tumor and tissues using TRIzol Reagent (Invitrogen). All gene segments were amplified and verified by quantitative reverse-transcriptase polymerase chain reaction (gRT-PCR). cDNAs were reverse-transcribed from 2 µg total RNA. The PCR primer sequences (KIT exon 9 primers were sense: 5'-TCC TAG AGT AAG CCA GGG CTT-3'and antisense: 5'-TGG TAG ACA GAG CCT AAA CAT CC-3'. KIT exon 11 primers were sense: 5'-GAT CTA TTT TTC CCT TTC TC-3'and antisense: 5'AGC CCC TGT TTC ATA CTG AC-3'. KIT exon 13 primers were sense: 5'-GCT TGA CAT CAG TTT GCC AG-3' and antisense: 5'-AAA GGC AGC TTG GAC ACG GCT TTA-3'. KIT exon17 primers were sense: 5'-CTC CTC CAA CCT AAT AGT GT-3' and antisense: 5'-GTC AAG CAG AGA ATG GGT AC-3'. PDGFRA primers were sense: 5'-TTG GAT ATT CAC CAG TTA CCT GTC-3' and antisense: 5'-CAA GGG AAA AGC TCT TGG-3'. GAPDH primers were sense: 5'-ACC ACA GTC CAT GCC ATC AC-3' and antisense: 5'-TCA CCA CCC TGT TGC TGT A-3'). The products of PCR were checked by agarose gel electrophoresis, and the abundance of each mRNA was detected and normalized to that of GAPDH mRNA.

# Western blotting

Cell lysates were prepared in a buffer containing 100 mmol/L NaCl, 10 mmol/L Tris-HCl (pH 7.6), 1 mmol/L EDTA (pH 8.0), 1 µg/mL aprotinin, 100 µg/mL phenylmethylsulfonyl fluoride, and 1% (v/v) NP-40. After protein quantitation using the Lowery protein assay, equal amounts of proteins were separated by SDS-PAGE and blotted onto nitrocellulose membranes by the semi-dry blotting method using a three-buffer system. The membranes were incubated with a dilution of primary antibody (anti-KIT exon 9:1:2000, anti-KIT exon 11:1:2000, anti-KIT exon 13:1:1000, anti-KIT exon 17:1:1500, anti-PDGFRA: 1:2000, anti-GAPDH:1:1000), overnight at 4°C. The membrane was washed with TBST and incubated with a peroxidase-conjugated secondary antibody (1:1000) (Santa Cruz Biotechnology) for 1 h. Specific antibody binding was detected using a chemiluminescence detection system (Pierce, Rockford, IL, United States), according to the manufacturer's recommendations. Western blot film was scanned,

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**Figure 1.** Endoscopic findings of the stomach. A: Esophagogastroduodenoscopy (EGD) image demonstrates a 5.0 cm × 3.5 cm subepithelial protruding mass located in the fundus of the stomach, covered by normal mucosa. B: Endoscopic ultrasound (EUS) revealed some ill-defined heterogenous hypoechoic lesion with multiple hyperechoic spots, arising from the muscularis propria layer. C: Resection of GIST under laparoscopic surgery system.

and the net intensities of the bands were quantified using Image-QuanT software (Molecular Dynamics, Sunnyvale, CA, United States). After development, the membrane was stripped and reprobed with antibody against GAPDH (1: 1000) to confirm equal sample loading.



**Figure 2.** The expression of Type 3 lodothyronine Deiodinase in the tumor tissue. A: Hematoxylin and eosin staining of the tumor and peri-tumor tissues. B: D3 immunostaining is specific for the tumor and peri-tumor tissues. C: D3 protein was determined by western blotting.

#### Statistical analysis

We used analysis of variance to perform all comparisons in molecular biology experiments.

We applied the principle of closed testing to hold the familywise type I error rate < 5% for each of the group comparisons of protein and mRNA expression.

#### Results

Type 3 iodothyronine deiodinase expression

In this patient, the two large abdominal tumors (endoscopy and biopsy examination led to the diagnosis of a GIST), one in stomach and the other in the transverse colon (Figure 1A and 1B), and supernormal requirements for exogenous thyroid hormone raised the possibility of consumptive hypothyroidism, a rare endocrinopathy caused by the inactivation of circulating thyroid hormones by tumoral D3, This phenomenon has been found in a 21-Year-Old woman with consumptive hypothyroidism due to a vascular tumor expressing type 3 iodothyronine deiodinase [3]. In this case, Type 3 lodothyronine Deiodinase was overexpressed in vascular tumor, we suspected the situation also could be occurred in gastrointestinal stromal tumor, to test this hypothesis, we immunostained biopsy samples obtained before the initiation of sunitinib therapy, which showed strong D3 expression in the GIST cells. To confirm the expression of D3 protein, we assayed D3 protein expression in frozen GIST tissue that was stored at the time of the patient's

first surgery by WB. This assay showed robust D3 activity approximating that of term placenta, the tumor tissue with the highest D3 activity (**Figure 2**).

# Consumptive hypothyroidism influenced expression of CD34, CD117, and DOG1

To investigate the mechanism by which the highest D3 activity induces consumptive hypothyroidism in GIST, as shown in Figure 3, we detected expression levels of some well-known GIST diagnose protein (CD34, CD117, and DOG1) by Western blotting, and some GIST relative genes (KIT exon 9, KIT exon 11, KIT exon 13, KIT exon17, and PDGFRA) by qRT-PCR. The protein expression level of CD34, CD117, and DOG1 in peri-tumor tissue samples was lower than that in tumor tissue samples. The mRNA expression level of KIT exon17, in peri-tumor tissue samples was higher than that in tumor tissue samples. However, no significant difference in the expression level of KIT exon 9, KIT exon 11, KIT exon 13, and PDGFRA was found.

# Discussion

This is the first case report of consumptive hypothyroidism in an adult after surgical partial resection of the gastrointestinal stromal tumor. This case demonstrates that hyperthyrotropinemia may worsen after surgical resection of the gastrointestinal stromal tumor.

The definition of consumptive hypothyroidism was first put forward in children with infantile hemangiomas. And it is different from other forms of hypothyroidism, which result from impaired secretion. Consumptive hypothyroidism is caused by the accelerated degradation of circulating thyroid hormone at rates that exceed the synthetic capacity of the normal stimulated thyroid gland. The definition of pathophysiological mechanism has been made in infants, and the complete resolution of hypothyroidism after involution or resection of the hemangioma has shown its tumor dependence. Evidence of increased thyroid hormone inactivation either elevated levels of serum reverse triiodothyronine (the product of thyroxine inactivation) or supernormal requirements for exogenous thyroid hormone helps to diagnose consumptive hypothyroidism. 13 Serum thyroglobulin levels and thyroid radioactive iodine uptake are increased, reflecting stimulation of the normal thyroid gland [3, 4]. An increased TSH alone certainly does not contribute to accurate diagnosis of consumptive hypothyroidism, while an increasing TSH can be a result of noncompliance to the specific thyroid replacement therapy. It's impossible in this case due to many of our patient's LT4 doses were given while hospitalized with directly observed therapy and were documented as normal over 2 yr before hospitalization. Meanwhile, her FT4 was rising with increased LT4 doses, showing that she was both ingesting and absorbing the drug. On the contrary, a process has been showed that primarily decreased the T3 by her increasing TSH in conjunction with an increasing FT4 and dropping TT3, implicating robust D3 activity [5]. Perhaps the persistence of altered TFT after resection of the D3 over-expressing GIST is the most interesting aspect of this case. This patient's TSH was increased on an otherwise stable dose of LT4 before surgery, showing increased D3 activity preoperatively. This shows the presence of other D3-stimulating factors besides overproduction by the tumors.

One possible explanation stems from the effect of partial resection of GIST itself. It has been shown that euthyroidism is restored after complete resection of GIST in a GIST patient with consumptive hypothyroidism [6]. However, our patient had only a partial resection. It deserves attention that the patient mentioned in this paper didn't received any tyrosine kinase inhibitor when he developed hypothyroidism and when he began to take tyrosine kinase inhibitor there was strongly active three iodine thyroid original glycine iodine enzyme in the tumor tissue removed 2 years before. All of these suggest that consumptive hypothyroidism cause systemic hypothyroidism to patients with GIST. We conclude that in certain subtypes of GIST side effects of tyrosine kinase inhibitor will be strengthened, which are more likely to happen compare to other subtype GIST. And partial resection may enhance these side effects, so we tested the parting related gene and protein expression. CD117 (KIT protein) widely expressed in GIST cell surface and plasma while don't expressed in all non-GIST cells, which made it the GIST of diagnosis index. CD34 is a transmembrane glycoprotein, existing in endothelial cells and bone marrow hematopoietic stem cells, which is of some significance to the expression of mesenchymal tumors. CD34 was positive in 60%~70% of gastrointestinal stromal tumor [7]. We tested the expression of CD117 and CD34 in neoplasm with the WB to further study the relation among expression of CD117 and CD34 and consumptive hypothyroidism, and we found that the expression of CD117 and CD34 was positive. DOG1 (the

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Discovered on GIST-1) whose function is unknown is a kind of newly found protein encoded by DOG1 gene on the cell membrane surface. The latest findings show that there are 136 out of 139 cases with GIST tumor tissue where there's expression (sensitivity 97.8%) and CD117 expression is negative in 5-7% of the GIST, and its diagnosis depends on the type of gene mutation 80% of which are mutation of KIT and PDGFRA and these mutations can be detected in the early stage. There four types of KIT mutation, including KIT exon (10.3%) KIT exon 11 (87.2%), KIT exon 13 (2.1%), and KIT exon 17 (0.4%) and three types of PDGFRA mutation found in tumor without KIT mutation, including KIT exon 12 (3%), KIT exon 14 (< 1%), KIT exon 18 (97%) [8]. In this case the expression of CD34, CD117, and DOG1 is positive while mutation only happened to KIT exon17 among KIT exon 9, KIT exon 11, KIT exon 13, KIT exon17, and PDGFRA. Although our hypothesis is as yet untested, we posit that some special types of GIST [CD34 (+), CD117 (+), DOG1 (+), KIT exon17 mutation] may contribute to side effect of tyrosine kinase inhibitor.

# Conclusions

An awareness of consumptive hypothyroidism is of important significance to clinical treatment. Thyroid Function should be carefully monitored in patients with GIST and the clinician should prepare supernormal doses of LT4, when necessary. Because the tumor growth is likely to increase LT4 requirement, LT4 requirements may be far more than the general treatment dose. LT4 dose for patients with GIST should be increased rapidly to correct severe hypothyroidism if consumptive hypothyroidism can be diagnosed in the early stage and meanwhile the primary tumor should be completely handled as soon as possible. In addition, monitoring of the gene mutation is imperative during the process of diagnosis and treatment of GIST, and to patients with GIST and hypothyroidism TSH and gene mutation type should be monitored. Hypothyroidism can't be completely avoided even if the patient hasn't used tyrosine kinase inhibitor.

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#### Disclosure of conflict of interest

None.

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