



Comparison of Basal Versus Stimulated Serum Thyroglobulin Levels to Assess Treatment Response in Papillary Thyroid Carcinoma

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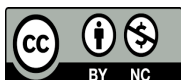
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Article Type	ABSTRACT
Research Paper	<p>Background and Objective: Papillary Thyroid Carcinoma (PTC) is the most common differentiated thyroid carcinoma. Persistent or recurrent disease occurs in approximately 20% of patients after treatment. Measurement of serum thyroglobulin (Tg) levels is one of the most important methods used for residual or recurrent disease management. The present study was conducted to investigate the role and significance of comparing two methods of measuring basal thyroglobulin (BTg) and stimulated thyroglobulin (STg) to assess treatment response and patient follow-up.</p> <p>Methods: In this cross-sectional study, patients with papillary thyroid carcinoma undergoing treatment were classified into three categories: excellent response (ER), indeterminate response (IR), and biochemical incomplete response (BIR) based on imaging tests and Tg levels. The primary outcome measure was the change in treatment response one year after initial treatment, which was achieved by comparing STg with BTg assessments.</p> <p>Findings: A total of 126 patients, including 96 women and 30 men, were assessed. One year after initial treatment, 28 patients (22.2%) showed a change in their classification when the treatment response based on BTg was compared with STg. Sixteen patients (12.7%) had a worse response, and their classification changed from the ER group to other groups. After measuring STg, structural recurrence was identified in 5 patients during the follow-up period. Based on STg, the highest recurrence was in the BIR group, followed by the IR group, which was statistically significant compared to the ER group ($p=0.001$).</p> <p>Conclusion: The results of this study demonstrated that compared to BTg, STg measurement better defines treatment response in low- or intermediate-risk patients when classified as indeterminate (IR and BIR). However, when BTg provides excellent response, STg measurement is not required.</p> <p>Keywords: <i>Differentiated Thyroid Carcinoma, Radioactive Iodine Therapy, Basal Thyroglobulin, Stimulated Thyroglobulin, Total Thyroidectomy.</i></p>
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Introduction

Patients with differentiated thyroid carcinoma (DTC) generally have a very good prognosis, with an overall 10-year mortality rate of less than 4% (1). The most common tumor of this type is papillary thyroid carcinoma (PTC), which generally exhibits a less aggressive behavior and a lower mortality rate. The most common treatment for PTC is total thyroidectomy (TT) followed by radioactive iodine (RAI) (2). However, persistent or recurrent disease, which is predominantly localized, occurs in approximately 20% of patients. Since DTC can recur at any time for years after initial treatment, long-term follow-up is essential. Follow-up should be guided by a method with a high negative predictive value (NPV) to exclude patients at low risk of recurrence from unnecessary investigations and to identify those at higher risk who deserve closer follow-up. Thyroglobulin (Tg) is a protein produced by thyroid cells and is used as an important biomarker in the follow-up of patients after treatment for PTC. After TT and RAI treatment, Tg levels should be very low or undetectable. An increase in Tg levels after treatment may indicate disease recurrence (3). Measurement of serum Tg levels is one of the most important methods used to monitor patients for residual or recurrent disease. Thyroglobulin has a high sensitivity and specificity in the diagnosis of thyroid cancer, especially after TT and radioiodine remnant ablation (RRA), which can be assessed with low/normal thyroid-stimulating hormone (TSH) as basal thyroglobulin (BTg) or with high TSH as stimulated thyroglobulin (STg), which is obtained endogenously or using recombinant human TSH. Along with Tg measurement, the level of Antithyroglobulin Antibody (TgAb) should always be checked, as this may interfere with the measurement method (3-6).

While most cases of PTC have positive outcomes, some patients experience disease progression. Traditional classification systems such as the American Thyroid Association (ATA) and American Joint Committee on Cancer (AJCC) provide an initial risk assessment but are unable to adapt to changes over time. To address this problem, a new dynamic classification system, based on initial treatment response, classifies patients based on factors such as imaging findings and thyroglobulin levels (7). While Tg is important for assessing response to treatment, current guidelines do not provide a specific preference for measuring Tg according to BTg or STg, probably because both methods are assumed to provide comparable results (8).

Several studies have shown that STg is more sensitive in detecting residual or recurrent disease than BTg (9). However, STg measurement may be associated with some problems, such as the need for thyroid hormone withdrawal and the side effects of hypothyroidism. On the other hand, BTg is usually easy to perform without additional steps and may be sufficient for some patients (10, 11). The aim of this study was to investigate the role and importance of Tg measurement in assessing treatment response and follow-up of patients with PTC and to compare the two methods of measuring BTg and STg.

Methods

This single-center cross-sectional study was conducted after approval by the Ethics Committee of Babol University of Medical Sciences with the code IR.MUBABOL.HRI.REC.1403.274. The medical records of all patients with DTC who underwent TT surgery with or without cervical lymph node dissection and were treated with RAI in the Nuclear Medicine Department of Shahid Beheshti Hospital were reviewed. Demographic information, thyroid and neck ultrasound history, serum TSH, Tg, TgAb levels, and details of levothyroxine treatment, surgical reports, and histopathological results were collected and recorded, along with information about RAI treatment, radiographic findings, and treatment outcomes. Patients aged 17 years and older with a history of TT with or without cervical lymph node dissection, performed up to 12

weeks before RAI treatment for residual thyroid tissue, were included in the study. PTC histology, availability of information regarding serum Tg, TSH, and TgAb levels were followed for at least one year. Patients classified as high risk based on the ATA guidelines or positive for TgAb at any time during the follow-up period were excluded from the study. Patients with other malignant tumors (cancer) or Hashimoto's thyroiditis (due to high TgAb levels), or patients who took iodine-containing drugs, drugs or foods that affect iodine absorption within 4 weeks before RAI treatment, received iodinated contrast media within 3 months before RAI treatment, pregnant or breastfeeding patients, as well as patients with structural disease detected by ultrasound or other imaging methods during the 1-year follow-up were excluded from the study.

Postoperative neck ultrasound using multifrequency doppler probes was performed to detect lymph node metastasis after thyroidectomy. Patients were initially stratified based on risk of recurrence according to ATA and based on risk of mortality according to AJCC (TNM). Although these staging systems are useful for predicting recurrence and mortality, they only assess the patient at the initial time point and do not consider changes that occur during follow-up or response to interventions. In this staging system, patients are classified into four response categories: excellent response (ER), indeterminate response (IR), biochemical incomplete response (BIR), and structural incomplete response (SIR), based on the results of imaging tests and serum TgAb concentrations, but mainly on Tg concentrations. Tg, TgAb, and TSH levels were retrospectively analyzed at different times (3, 6, 12, 18, and 24 months) after RAI. STg levels were measured 12 months after RAI in accordance with current ATA guidelines. To achieve this goal, patients underwent a 4-week levothyroxine withdrawal period to induce endogenous TSH levels above 30 mU/L, a standard criterion for radioiodine diagnostic methods. The primary outcome measure was the therapeutic response achieved one year after initial treatment, which was classified as excellent response (ER), biochemical incomplete response (BIR), or indeterminate response (IR) (Table 1) using BTg and STg. Additional tests routinely performed to assess response to treatment included ultrasound and 131-iodine whole-body scan.

Table 1. Patient outcomes based on American Thyroid Association response criteria

Definition	Category
Negative imaging or suppressed Tg less than 0.2 ng/mL or stimulated Tg less than 1 ng/mL	Excellent response (ER)
Negative imaging and suppressed Tg greater than or equal to 1 ng/mL or stimulated Tg greater than or equal to 10 ng/mL or increased anti-Tg antibody levels	Biochemical Incomplete Response (BIR)
Structural or functional evidence of disease with any Tg level with or without anti-Tg antibodies	Structural Incomplete Response (SIR)
Nonspecific findings on imaging studies, poor uptake in the thyroid gland on iodine-131 scan, detectable suppressed Tg but less than 1 ng/mL or detectable stimulated Tg but less than 10 ng/mL, or persistent or decreased anti-Tg antibodies in the absence of structural or functional disease	Indeterminate response (IR)

The primary outcome measure was the change in treatment response one year after initial treatment, as measured by comparison of stimulated thyroglobulin (STg) with baseline thyroglobulin (BTg). STg was measured one month after BTg and by endogenous TSH stimulation after a 30-day levothyroxine withdrawal period. Patient clinical outcomes were assessed based on their final status (excellent response,

biochemical incomplete, structural incomplete, or indeterminate response) and disease-free survival. Data analysis was performed using SPSS version 24. Descriptive statistics were used to summarize the data, mean and standard deviation were used for continuous variables and frequency was used for qualitative variables. Due to the retrospective nature of the study, calculations were performed based on the available data and not the entire sample size. First, the normality of the data was examined using the Kolmogorov-Smirnov test, and because the data did not follow a normal distribution, the Kruskal-Wallis test was used. The chi-square test was used to examine the relationship between qualitative variables, and $p < 0.05$ was considered significant.

Results

A total of 126 patients with PTC who underwent total thyroidectomy and received RAI were included in the study. No residual disease was detected on neck ultrasound in these patients and Tg-Ab remained negative between 9 and 12 months after initial treatment. Initial risk stratification of these patients was performed according to the 2015 ATA guidelines. 92 patients (73%) were classified as low-risk and 34 patients (27%) as intermediate-risk. Patients classified as high-risk were excluded from the study.

The mean age of the patients was 42.6 ± 15 years (ranging from 17 to 79). A total of 96 patients were female (76.2%) and 30 were male (23.8%). Residual thyroid was absent in post therapy I-131 WB scan (PTWBS) in only 6 (4.8%) patients and was present in 120 (95.2%) patients. The dose of radioactive iodine administered varied from 30, 100, 125, and 150 mCi, with most patients (69%) receiving a dose of 30 mCi. The mean time interval between surgery and the administration of the first dose was 4.5 months (Table 2).

Table 2. Characteristics of study patients

Patient characteristics	Number(%)
Gender	
Male	30(23.8)
Female	96(76.2)
Age (years)	
Total	42.5 ± 15
Age (years)	
Below 45	73(57.9)
Above 45	53(42.1)
Initial risk level based on ATA	
Low	92(73)
Intermediate	34(27)
Iodine dose received (millicuries)	
Less than 100	87(69)
Greater than or equal to 100	39(31)

One year after initial treatment (TT and RAI), 101 (80.2%) patients were in the ER group, 17 (13.5%) patients were in the IR group, and 8 (6.3%) patients were in the BIR group based on serum BTg levels. 91 (72.2%) patients were in the ER group, 19 (15.1%) patients were in the IR group, and 16 (12.7%) patients were in the BIR group based on serum STg levels. 28 (22.2%) patients showed a change in classification when treatment response based on STg was compared with BTg. Furthermore, 16 (12.7%) patients showed a worse treatment response, so that their classification changed from the ER group to other groups. Of these,

9 patients were transferred to the IR group and 7 patients to the BIR group. Also, of the 17 patients who were in the IR group, 9 patients showed a change in classification; Thus, 5 patients were transferred to the ER group and 4 patients to the BIR group. Of the 8 patients in the BIR group, 3 patients were classified into other groups (2 patients to the IR group and 1 patient to the ER group). As a result, 6 patients (4.7%) showed an improvement in therapeutic response using STg, of which 5 patients from the IR group and 1 patient from the BIR group were transferred to the ER group and were classified as excellent response.

The mean serum levels of Tg, TSH, and TgAb in baseline and stimulated conditions and in all patients are shown in Table 3. The frequency of patients in the treatment response group after measuring serum levels of BTg and STg is also shown in Table 4.

After STg measurement, patients were followed for 12-24 months. Structural disease was identified in 5 patients, all of whom were presented with neck metastases detected by ultrasound (Table 5). No distant metastases were seen. After reoperation, 4 patients were disease-free, with BTg < 0.1 ng/mL and normal ultrasound. One patient still had detectable BTg < 1 ng/mL but no abnormalities were seen on ultrasound.

One recurrence was observed among patients in the ER group based on BTg whose response changed to IR after STg, while no recurrences were observed in patients who remained in the ER group, but this difference was not significant. In patients in the IR and BIR groups based on BTg, no recurrences were observed among those whose response changed to ER after STg, while 11.8% and 25% of those who remained in their original group or their response changed to IR and BIR groups, respectively, developed structural disease. The highest recurrences were in the BIR group (18.8%), followed by the IR group (10.5%) based on STg, which was statistically significant compared to the ER group (no recurrence) according to the result of the chi-square test ($p=0.001$).

Table 3. Mean serum levels of BTg and STg and other diagnostic tests in patients

Variable	ER Mean±SD	IR Mean±SD	BIR Mean±SD	Total Mean±SD	p-value*
Basal thyroglobulin (BTg)	0.1±0.05	0.56±0.22	3.7±2.5	0.39±1.07	<0.001
Basal thyroid stimulating hormone (BTSH)	0.98±0.96	1.08±1.01	1.1±0.7	1±0.95	0.745
Basal thyroglobulin antibody (BTgAb)	21.56±18.1	22.56±19.3	14.2±18.6	21.2±18.3	0.287
Stimulated thyroglobulin (STg)	1.6±4.3	5.16±5.84	11.3±6.7	2.73±5.33	<0.001
Stimulated thyroid-stimulating hormone (STSH)	62.3±22.8	59.9±21.21	61.71±22.34	61.9±22.4	0.073
Stimulated thyroglobulin antibody (STgAb)	29.5±17	30±20.9	15.5±12.9	28.7±17.55	0.038

*Kruskal-Wallis test

Table 4. Comparison between patients in treatment response classification and serum BTg and STg levels

Classification	S-ER Number(%)	S-IR Number(%)	S-BIR Number(%)	p-value*	Total
B-ER	85(84.2)	9(8.9)	7(6.9)	<0.001	101
B-IR	5(29.4)	8(47.1)	4(23.5)	<0.001	17
B-BIR	1(12.5)	2(25)	5(62.5)	<0.001	8
Total	91	19	16	<0.001	126

*Chi-square test

Table 5. Frequency of disease recurrence considering treatment response based on serum BTg and STg levels

BTg-based recurrence	Recurrence based on STg			Total
	S-ER	S-IR	S-BIR	
B-ER (1 patient out of 101)	patients out of 85 0	1 patient out of 9	0 patient out of 7	101
B-IR (2 patients out of 17)	0 patients out of 5	1 patient out of 8	1 patient out of 4	17
B-BIR (2 patients out of 8)	0 patients out of 1	0 patient out of 2	2 patients out of 5	8
Total (5 patients out of 126)	0 patients out of 91	2 patients out of 19	3 patients out of 16	126

Discussion

In this study, about 22% of PTC patients had a change in their treatment response one year after initial treatment based on STg compared to BTg, which suggests that it is a better predictor of excellent treatment response. Although one of the main aspects assessed for the treatment response of patients with PTC is the measurement of serum Tg levels, whether the measurement should be done simultaneously with levothyroxine tablets (BTg) or after its discontinuation and after the increase in TSH levels (STg) is still controversial. Although in recent years, the emergence of highly sensitive Tg measurement methods has called into question the use of STg, this issue is still not fully clarified.

In our study, 28 patients (22.2%) showed a change in treatment response after TT and RAI treatment based on STg, which was associated with a worse response in 71.4% of patients. This finding is consistent with the results of Barreto et al., who observed that STg was associated with a change in treatment response in about 20% of cases and that the response worsened in about 60% of patients (5).

Although our study is relatively consistent with the results of Rosario et al., as STg was associated with a change in treatment response in about 24% of cases in their study, but unlike our study, the majority of their patients showed a better treatment response and only about 30% of those patients had a worse treatment response (12). It is important to emphasize that based on STg, most cases had worse responses than the ER group, most of whom were transferred to the IR group. Therefore, even a small increase in Tg levels (from less than 0.2 ng/mL to 1 ng/mL) can have debatable clinical significance. Furthermore, in our study, the biggest change in treatment response was in the IR group, with about 53% of patients in this group showing a change in treatment response, such that 55.5% of patients had a better treatment response and were transferred to the ER group; the reasons for this finding remain to be clarified. In agreement with this finding, Rosario et al. showed that among 108 patients with an indeterminate response (IR) based on BTg, a change to the excellent response (ER) category was observed in 44% of patients after STg (12).

In the present study, about 28.6% of patients had an improvement in treatment response with STg. In similar studies, Brassard et al. showed that 41% of 117 patients in the IR group had STg < 1.4 ng/mL (13). Moreover, in the study of Malandrino et al. and Trimboli et al., 36% and 39% of patients in the IR group had STg ≤ 1 ng/mL, respectively (14, 15).

Using BTg to define treatment response, the recurrence rate was significantly lower for the ER group (0.9%) compared with the IR and BIR groups (16%). This difference was also significant using STg (0 vs. 14.5%). There was no significant difference in recurrence frequency between the ER groups (0.9% vs. 0%) or the IR and BIR groups (16% vs. 14.5%) using BTg or STg. A similar result was reported in the study by Rosario et al. In their study, a significant difference in recurrence rate was observed between the ER and IR groups based on BTg and STg, whereas this difference was not observed between the ER groups with each other and between the IR groups with each other. As in their study, there was no risk of recurrence in patients whose response changed from IR and BIR to ER based on STg (12).

Similar results were reported by Brassard et al., who found recurrence in only 2% of patients with $STg < 1.4$ ng/mL (13). Malandrino et al. and Trimboli et al. also reported no short-term recurrence in patients with $BTg > 0.1$ ng/mL and $STg < 1$ ng/mL (14, 15). The frequency of recurrence in patients in the IR and BIR groups based on BTg alone was 16% and based on both BTg and STg was 20%. In agreement with our study, two recent studies by Rosario et al. and Malandrino et al. reported biochemical or structural recurrence in 11% and 9%, respectively, of patients initially classified as indeterminate responders (IR) based on BTg and STg (12, 16).

In agreement with other studies in this field, it seems reasonable to obtain STg in patients with an uncertain initial response (IR and BIR groups) based on BTg . With this change in classification, these patients may no longer need to repeat ultrasound, and the intervals between laboratory markers can be increased, and there is no need to suppress TSH in this group of patients (12, 17). On the other hand, some patients in the IR group switched to the BIR group after STg , a finding that favors a worse therapeutic response and requires more diagnostic follow-up and more intense TSH suppression (17, 18).

This study showed that STg better defines the response to treatment than BTg in patients with low or intermediate initial risk (based on ATA) when classified as indeterminate (IR and BIR groups). However, when BTg provides an excellent response (ER group), STg measurement is not required.

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