ORIGINAL ARTICLE

Prevalence of Thyroglobulin Antibody (TgAb) Positivity in Patients Post Treatment for Differentiated Thyroid Carcinoma and Verification of Serum Thyroglobulin Measurements by Thyroglobulin Recovery Test and TgAb assay

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ABSTRACT

Introduction: Thyroglobulin (Tg) measurement is important for detection of disease recurrence in patients with differentiated thyroid carcinoma (DTC). However, its measurement is affected by Tg autoantibodies (TgAb). Calculation of Tg recovery may complement quantitative TgAb measurement in detection of interference. This study aimed to determine the prevalence of TgAb positivity in Tg samples received from post-thyroidectomy DTC patients in Hospital Pulau Pinang (HPP). Additionally, we assessed the use of the Tg II Confirmatory Test (Roche Diagnostics) assay to calculate Tg recovery in detecting Tg assay interference. **Method:** Samples received for Tg measurements from post-thyroidectomy DTC patients with TgAb positivity were tested with Tg II Confirmatory Test. The Tg levels [categorised as biochemically detectable (≥1.0 µg/l) vs biochemically undetectable (<1.0 µg/l)], TgAb and Tg recovery [categorised as correct (70%-130%) vs compromised (<70% and >130%) recovery] were interpreted with the diagnostic radioiodine uptake (RAI) results. **Results:** In this study, 58/73 (79.5%) samples with TgAb positivity had undetectable Tg. A compromised Tg recovery was observed in three (4.1%) samples. Only 51 out of 73 subjects had an RAI performed, out of which 27 (52.9%) had increased RAI uptake (radiological evidence of persistent/recurrent disease). Of those with increased RAI uptake, 17 (63%) had biochemically undetectable Tg, out of which none had compromised Tg recovery. **Conclusion:** The presence of TgAb prevents reliable measurement of Tg. The Tg II Confirmatory assay for calculation of Tg recovery did not provide additional complementary value to quantitative TgAb measurement in the detection of interference in Tg measurements.
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Keywords: Differentiated thyroid carcinoma, Thyroglobulin, Thyroglobulin autoantibodies, Thyroglobulin recovery, interference

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INTRODUCTION

Carcinoma of the thyroid is the most common endocrine malignancy (1). In 2021, the National Cancer Institute in the United States estimates the number of new thyroid cancer cases to be 44,280, representing 2.3% of all new cancer cases (1). In Malaysia, the National Cancer Registry (NCR) for 2012-2016, reported thyroid carcinoma to be the eighth and 14th most common malignancy in females and males, respectively with the highest incidence seen in the Malays (2). Thyroid carcinoma is classified into differentiated and undifferentiated carcinomas, with the former being more common. Differentiated thyroid carcinoma (DTC), develops from thyrocytes and includes papillary, follicular and mixed varieties. Most thyroid malignancies have an excellent prognosis with complete cure or long-term remission possible, provided appropriate and adequate treatment is administered (3).

Near- or total thyroidectomy, and/or high dose radioactive remnant ablation (RRA) therapy, and hormone substitution, form the basis of the treatment (3,4). Serum thyroglobulin (Tg) is measured during follow-up to detect disease recurrence and is not to be performed sooner than six weeks post-surgery or RRA therapy (5). The level decreases to very low or undetectable levels following successful total thyroidectomy and RRA (6). For patients who have undergone partial thyroidectomy, Tg levels remain measurable depending on how much thyroid tissue is left after surgery. Significantly increasing Tg levels during follow-up are interpreted as a sign of disease recurrence (6).

Tg measurements are however affected by the presence of Tg antibodies (TgAb), leading to recommendations for simultaneous measurement of TgAb for detection of Tg assay interference (5,6). In the commonly used automated immunometric Tg assays, the interfering TgAb causes underestimation of Tg levels, hence masking the disease and risking failure to detect disease recurrence (5-8). TgAb is found in up to 25% of patients with thyroid cancer but the prevalence varies depending on the specific Tg assay used and the study population (7, 9). TgAb is defined as positive when the result is above the stated cut-off level for a particular assay (5). TgAb measurement should be repeated in such cases, usually at six-monthly intervals. The absence of TgAb positivity, however, does not exclude the possibility of Tg assay interference, as it may also be affected by interferents such as heterophilic antibodies (7).

In contrast to the TgAb assay which directly measures TgAb, the Tg recovery test indirectly measures the presence of interfering TgAb. It typically involves measurement of Tg before and after a known amount of Tg is added to an aliquot of the test serum (7). A recovery value of >80% often indicates the absence of interference, hence validating the Tg result (7). However, care must be taken to ensure that the exogenous Tg preparation mimics the interactions between endogenous Tg and TgAb. The concentration of Tg added should also not exceed the endogenous Tg concentration and sufficient time be allowed for the added Tg to equilibrate with the endogenous Tg and TgAb serum constituents. Unfortunately, these criteria are not usually met.

In Hospital Pulau Pinang (HPP), Tg is measured using the Elecsys Tg II (Roche Diagnostics, Germany) assay. Each Tg sample received by the laboratory is routinely analysed for TgAb using the Anti-Tg (Roche Diagnostics, Germany) assay. In cases with TgAb positivity, a comment "positive for TgAb, Tg value may be unreliable" will be added to the laboratory report. Apart from measurement of TgAb, the manufacturer also states the use of Tg II Confirmatory Test for calculation of Tg recovery to confirm Tg measurements (10). This study thus aimed to determine the prevalence of TgAb positivity in Tg samples received from post-thyroidectomy DTC patients. Additionally, the aim was to assess the use of the Tg II Confirmatory Test (Roche Diagnostics, Germany) assay for the calculation of Tg recovery for the detection of Tg assay interference in samples with TgAb positivity.

MATERIALS AND METHODS

Study design

A cross-sectional study involving subjects aged ≥18 years old who had undergone total thyroidectomy and/or RRA for DTC, under the follow-up of the Nuclear Medicine Department of HPP and had their blood samples for Tg measurement either with or without rhTSH stimulation or L-T4 withdrawal from 1st March 2019 till 31st December 2019. Samples were excluded if it was a repeat sample, or if the samples were from patients with high suspicion of metastatic disease. In HPP, each Tg sample received by the laboratory is routinely analysed for TgAb. A sample is defined as TgAb positive when the value is >115 IU/mL (11). For this study, all samples with TgAb positive were further tested with the Tg II Confirmatory test.

Sample size calculation

Sample size calculation was made based on the prevalence of thyroid cancer patients with detectable TgAb i.e., 25% with the following formula used to calculate the sample size (7):

$n = z^2 1-\alpha/2\ p(1-p) / d$

n = number of sample size; z 1-α/2 = 1.96 ; p=prevalence, d=0.09

The sample size was hence determined as 72.

Laboratory analysis

Tg assay

Tg measurements were performed using the Elecsys Tg II (Roche Diagnostics, Germany) assay, based on a sandwich electrochemiluminescence immunoassay (ECLIA) on Roche Cobas e411. The assay has a functional sensitivity of 0.1 ng/ml. Values above the measuring range (after 10-fold dilution) will be reported as >5000 ng/ml. The assay is calibrated against the Certified Reference Material (CRM) 457.

TgAb

TgAb measurements were performed using the Elecsys anti-Tg (Roche Diagnostics, Germany) assay, based on a competitive electrochemiluminescence immunoassay (ECLIA) on Roche Cobas e411. Values below the lower detection limit are reported as <10 IU/ml whilst values above the detection limit are reported as 4000 IU/mL.

Tg II Confirmatory test

The test is based on the pretreatment of samples with a confirmatory reagent followed by the assay procedure using the Elecsys Tg II assay.

Tg levels obtained before the addition of Tg II confirmatory reagents, and the levels obtained after the sample was added with Tg II confirmatory reagents were entered into a formula to obtain the percentage recovery of Tg (12):

$$\frac{\text{Concentration Tg (sample + confirmatory reagent) - 0.8 x concentration Tg (sample)}}{\text{0.2 x concentration of Tg in the Tg II confirmatory reagent}} \times 100$$

A Tg recovery between 70 and 130% is considered a correct recovery whilst values above or below these limits indicate that the Tg result should be interpreted with caution (12).

Statistical analysis

Statistical calculations were performed using the standard statistical software package, IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp. Numerical variables are presented as mean ± standard deviation and median [inter-quartile range (IQR)] values. whereas categorical variables will be presented as counts and percentages.

Ethics

Ethical approval was granted by the Medical Research Ethical Committee (MREC) Ministry of Health, Malaysia (NMMR-18-3327-43047 (IIR).

RESULTS

Out of 744 samples received, 91 (11%) had positive TgAb, out of which 18 were excluded (eight for distant metastases and ten were repeated samples), hence only 73 samples had a Tg II confirmatory test performed.

The demographic and clinical profiles of the 73 patients are shown in Table I. The median age was 47 years old with the majority (n=26, 36%) being ≥ 50 years old. The majority were also female (n=61, 84%) and Malays (n=45, 62%). In terms of clinical history, the majority (n=61, 84%) had received a combination of total thyroidectomy and RRA and 47 (64%) had their post-treatment Tg samples taken after 18 months of treatment.

The results for Tg, TgAb and Tg II Confirmatory Test are shown in Table II. The median for Tg and TgAb was 0.1 ng/ml and 845 IU/mL, respectively. There were 50 (69%) subjects with Tg levels below the assay's functional sensitivity of 0.1 ng/ml. In HPP, a Tg level of <1.0 ng/ml is considered biochemically undetectable, and if the RAI is also negative, then the patient is considered to have an excellent response to therapy. In this study, 58 (79.5%) samples with TgAb positivity had a Tg value <1.0 ng/ml.

Table I: Demographic and clinical profiles of the study subjects (N=73)

Parameters	N	%
Gender		
- Male	12	16
- Female	61	84
Age (years)		
– <21	2	3
– 21-30	9	12
– 31-40	12	16
– 41-50	24	33
– ≥50	26	36
Ethnicity		
– Malay	45	62
– Chinese	20	27
– Indian	5	7
– Others	3	4
Clinical history		
– Total thyroidectomy +RRA	61	84
– Total thyroidectomy only	12	16
Post-treatment Tg samples		
– <6 months	6	8
– 6-12 months	10	14
– 12-18 months	10	14
– >18 months	47	64

Table II: Tg, TgAb and Tg II Confirmatory Test in all subjects (N=73)

Investigation	Median (IQR)	Range
TgAb (IU/mL)	845 (2008)	120 - 4000
Tg (ng/ml)	0.1 (0.4)	< 0.1 - 72.9
	n	%
Tg (ng/ml)		
– < 1.0	58	79.5
– ≥ 1.0	15	20.5
Tg II confirmatory test		
– Tg recovery <70% or >130%	3	4.1
– Tg recovery 70%-130%	70	95.9

Fig. 1 shows the results of individual Tg recovery with TgAb. Only three (4.1%) samples were classified as having compromised Tg recovery. There was an over-recovery (180%) in one sample with a serum Tg of 53.8 ug/L. In this patient, the RAI was also positive. Two samples had Tg recovery below 70% and in both samples, the serum Tg values were <1.0 ng/ml. In general, there is a wide range of TgAb titres (120 – 4000 IU/mL) and the levels did not demonstrate any specific pattern with Tg recovery.

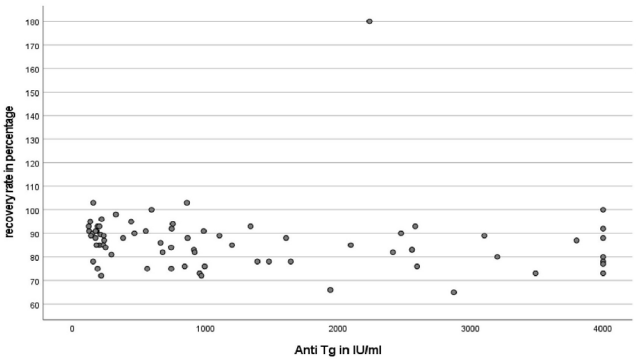

Figure 1: Tg recovery in clinical samples containing TgAb

Table III shows the results of Tg recovery following the Tg II Confirmatory Test in samples with detectable Tg (Tg ≥ 1.0 ng/ml) and undetectable Tg (Tg <1.0 ng/ml). In those with undetectable Tg (n=58), 56 (96.5%) were considered to have correct Tg recovery. In contrast, only two (3.4%) had compromised recovery, i.e., indicating the presence of interference in the Tg measurement.

Out of 73 patients, 51 (69.9%) subjects underwent post-treatment RAI. Increased RAI uptake was noted in 27 (52.9 %) subjects, while the remainder (n=24, 47.1%) had negative RAI. The Tg results (detectable vs undetectable) in those with and without increased RAI uptake are shown in Table IV. Increased RAI uptake indicates radiological evidence of disease recurrence

Table III: Results of Tg II Confirmatory Test in samples with detectable and undetectable Tg (N=73)

Tg II Confirmatory Test	Tg	
	Detectable (Tg ≥ 1.0 ng/ml) n (%)	**Undetectable (Tg <1.0 ng/ml) n (%)**
Compromised (Tg Recovery <70% or >130%)	1 (7.1)	2 (3.4)
Normal (Tg Recovery 70%-130%)	14 (92.9)	56 (96.6)

Table IV Tg (detectable vs undetectable) and Tg II Confirmatory Test results in those with and without increased RAI uptake (n=51)

Parameters		RAI	
		No increase in uptake (No radiological evidence of recurrence) (n=24)	Increase uptake (Radiological evidence of recurrence) (n=27)
Tg			
–	Detectable (Tg ≥1.0 ng/ml)	4 (17%)	10 (37%)
–	Undetectable (Tg <1.0 ng/ml)	20 (83%)	17 (63%)
Tg II Confirmatory Test			
–	Tg recovery <70% or >130%	1 (4.2%)	1 (3.7%)
–	Tg recovery 70%-130%	23 (95.8%)	26 (96.3%)

while no increase in RAI uptake supports a disease-free state. Four out of 24 (17%) subjects without any detectable lesion seen radiologically were noted to have biochemically detectable Tg (Tg ≥1.0 ug/L), whereas 17 out of 27 (63%) with increased RAI uptake had undetectable Tg (Tg <1.0 ug/L), out of which none had compromised Tg recovery.

DISCUSSION

There was a female and older age group preponderance. Although, the development of TgAb increases with age and autoantibodies are generally more commonly seen in females (9, 13), the findings were also most likely contributed by the higher prevalence of DTC among females (2.9-times higher rate compared to males) (1,14). Similarly, the median age of 47 also corresponds to the 45 -51 years old age group commonly seen for thyroid cancer (1).

In this study, 11% of all serum Tg samples received from DTC post-treatment patients were positive for TgAb, similar to a recently reported study in Spain, which reported a prevalence of 9.5% (9). Most of the samples were taken after 18 months post-treatment. A previous study showed that nearly two-thirds of patients demonstrated TgAb positivity 15–18 months after the initial therapy (15). The presence of TgAb, in these cases, was thought to be due to the persistence of minimal remnants of thyroid tissue or incomplete cure of DTC.

Sixty-nine percent of the samples had levels below the functional sensitivity of Tg assay (0.1 ng/ml) while 79.5% of samples had biochemically undetectable Tg (<1.0 ng/ml). This is expected as Tg immunometric methods tend to underestimate serum Tg in the presence of autoantibodies as the endogenous Tg forms a complex with TgAb preventing its interaction with the reagent antibodies used in the assay. A study evaluated the accuracy and behaviour of four Tg immunoassays, including the Roche Elecsys Tg II assay as well as two Tg radioimmunoassays and two Tg mass spectrometry (Tg-MS) assays (8). The presence of TgAb causes false-low bias in all Tg immunoassays with the underestimation and the rate of undetectable Tg concentrations was about 2-fold lower for the Beckman Access Tg (Beckman Coulter), Thermo-Brahms Tg (Thermo Scientific), and Roche Tg Roche Elecsys Tg II assays compared with the Siemens-Immulite Tg (Siemens) assay.

There is a wide range of TgAb concentrations that can cause falsely low/undetectable serum Tg values, in our case the TgAb concentrations ranged between 120 to 4000 IU/mL. The cut-off used to define TgAb positivity depends on the specific TgAb assay used, and the reference range stated by the assay's manufacturers. Nevertheless, TgAb interference with the Tg assay has been reported to be variable among patients and only loosely correlates with TgAb concentrations (8). Therefore, the degree of interference by TgAb cannot be predicted (9), as demonstrated in this study.

In the 27 subjects that had increased uptake on RAI, 63% had no "biochemical response" Tg (Tg <1.0 ug/L), hence a false negative Tg in the presence of TgAb positivity. Conversely, four (17%) subjects with no radiological evidence of recurrence had "biochemical response" Tg. Thus, the importance of continuing to monitor all these patients.

There were only three samples with TgAb positivity that showed compromised Tg recovery, out of which one was from a patient with increased RAI uptake and increased Tg, one with no increase in RAI uptake and undetectable Tg while the other had no RAI performed. The remainder were categorised as having a normal Tg recovery. In those with increased RAI but with biochemically undetectable Tg, none showed compromised Tg recovery in sera with TgAb positivity. This study thus shows poor performance of Tg recovery in recognising Tg-TgAb interferences. The findings concurred with previous studies that reported normal Tg recoveries in samples with interfering TgAb (7, 16,). Possible contributing factors for this include inadequate equilibrium between the added exogenous Tg with endogenous Tg and TgAb constituents (7). Other reasons include the difference between the epitopes of the exogenous and the endogenous Tg (7).

CONCLUSION

The presence of circulating TgAb prevents reliable measurement of Tg. The Tg II Confirmatory assay for calculation of Tg recovery did not provide additional complementary value to quantitative TgAb measurement in the detection of interference in Tg measurements.

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