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ORIGINAL ARTICLE

Does ^{131}I Radioactivity Interfere with Thyroglobulin Measurement in Patients Undergoing Radioactive Iodine Therapy with Recombinant Human TSH?

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Abstract
Objectives Recombinant human thyroid-stimulating hormone (rhTSH) is widely used in radioactive iodine therapy (RIT) to avoid side effects caused by hypothyroidism during the therapy. Owing to RIT with rhTSH, serum thyroglobulin (Tg) is measured with high ^{131}I concentrations. It is of concern that the relatively high energy of ^{131}I could interfere with Tg measurement using the immunoradiometric assay (IRMA). We investigated the effect of ^{131}I administration on Tg measurement with IRMA after RIT.
Methods A total of 67 patients with thyroid cancer were analysed retrospectively. All patients had undergone rhTSH stimulation for RIT. The patients' sera were sampled 2 days after ^{131}I administration and divided into two portions: for Tg measurements on days 2 and 32 after ^{131}I administration. The count per minute (CPM) of whole serum (200 μl) was also measured at each time point. Student's paired *t*-test and Pearson's correlation analyses were performed for statistical analysis.
Results Serum Tg levels were significantly concordant between days 2 and 32, irrespective of the serum CPM. Subgroup analysis was performed by classification based on the ^{131}I dose. No difference was noted between the results of the two groups.
Conclusions IRMA using ^{125}I did not show interference from ^{131}I in the serum of patients stimulated by rhTSH.

Keywords Immunoradiometric assay · Thyroglobulin · Radioactive iodine therapy · Recombinant human TSH

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Introduction

Immunoradiometric assay (IRMA) is widely used for the measurement of thyroglobulin (Tg) levels [1]. Despite debates on the discordance between commercial assay kits [2–6], high-sensitivity human Tg IRMA can detect serum Tg concentrations of 0.2 ng/ml, and serum Tg concentrations <0.2 ng/ml are used to indicate complete remission [7]. Because the serum Tg level is used for surveillance after total thyroidectomy to evaluate the residual and recurrent differentiated thyroid cancer (DTC), accurate estimation of the serum Tg level is important [8].

Radioactive iodine therapy (RIT) is commonly used for the ablation of remnant tissue, treatment of functioning metastasis, and diagnostic purposes [9, 10]. RIT was conventionally performed with thyroid hormone withdrawal. However, recently, recombinant human thyroid-stimulating hormone (rhTSH) was approved for RIT for thyroid hormone stimulation, thereby substituting thyroid hormone withdrawal. Compared to the use of conventional thyroid hormone withdrawal, the use of rhTSH for TSH stimulation significantly improved the quality of life (QOL) during RIT, avoided iatrogenic hypothyroidism symptoms, and sustained the liver and kidney functions [11, 12].

^{131}I emits beta and gamma rays simultaneously. Therefore, it can be used for simultaneous RIT and diagnostic imaging.

^{131}I predominantly emits 363 and 637 keV of energy in a two-step decay process. On the other hand, ^{125}I, which is used for IRMA, emits four kinds of gamma rays with a maximum energy of 35 keV. Because Tg measurement is performed a day after ^{131}I administration, the relatively high energy of ^{131}I can interfere with the measurement of Tg using IRMA. Serum Tg elevation is highest at 48 h after ^{131}I administration, and optimal Tg measurement is obtained after ^{131}I administration [13]. Therefore, measurement of Tg levels after ^{131}I administration may be influenced by the high radioactivity of the serum ^{131}I. However, to the best of our knowledge, no study has investigated the influence of ^{131}I on the detection of ^{125}I anti-human-Tg antibody. The present study aimed to evaluate the interference of ^{131}I in the measurement of Tg using ^{125}I anti-human-Tg antibody.

Materials and Methods

Patients

A total of 67 patients with pathologically confirmed papillary thyroid cancer (PTC), who underwent RIT between January and February 2014 were included in this study (Table 1). In all patients, TSH stimulation was performed with rhTSH.

Study Protocol

TSH stimulation was performed with rhTSH, as per the instructions of the manufacturer (Genzyme Corp., Cambridge, MA, USA), and patient sera were collected after ^{131}I administration. Two intra-muscular injections of rhTSH, each of 0.9 mg, were given at 24-h intervals and ^{131}I was administered 48 h after the first rhTSH injection. Then, patients' sera were collected after 48 h of ^{131}I administration and divided in two portions for Tg concentration measurements at two different time points: at day 2 after administration and at day 32 (the serum was stored at −20 °C) (Fig. 1).

Table 1 Patient characteristics

		Patient number
Sex	M	17
	F	50
Age	<35 years	13
	>35 years	54
RAI dose	148 MBq	20
	1,110 MBq	47
PTC stage	I	29
	II	12
	III	26
	IV	0

PTC papillary thyroid carcinoma

Tg Measurement Protocol Using IRMA

Tg was measured using the IRMA commercial kit (Tg-plus; Brahms Diagnostica, Berlin, Germany), having an analytical sensitivity of 0.08–250 ng/ml, as per the manufacturer's instructions. Briefly, all kit components and patient samples were stored at room temperature. All liquid reagents including patients' sera were agitated gently before use. Standard solution, controls, Tg-free serum, and patients' sera (100 μl) were pipetted into test tubes coated with anti-h-Tg (polyclonal, rabbit). The tubes were briefly agitated on a sample mixer and incubated overnight (18±4 h) at room temperature. Next, 2 ml of the washing solution was added and the tube was washed twice. The tubes were placed upside down on adsorbent paper for a minimum of 10 min. Finally, 200 μl tracer of ^{125}I-labeled monoclonal Tg antibody was pipetted into each tube. The tubes were incubated for 2–3 h at room temperature with shaking (170–300 rpm) and washed again with 2 ml of the washing solution. All tubes were placed upside down on adsorbent paper for at least 10 min twice. Radioactivity of each tube was measured using a gamma counter (Gamma-10; Shinjin Medics, Goyang, Korea) with a countable isotope energy of 15–2,000 keV.

Statistical Analysis

The relationship between the Tg levels of days 2 and 32 were analysed by Student's paired *t*-test and Pearson's correlation analysis. Statistical analyses were performed using SPSS (version 18.0; IBM Software, Chicago, IL, USA).

Results

Patients' Demographic Information

Patients' information is summarised in Table 1. A total of 67 patients (age range: 27–69 years) pathologically diagnosed with PTC after surgery were included. The patients received either 148 MBq or 1,110 MBq of ^{131}I for remnant thyroid

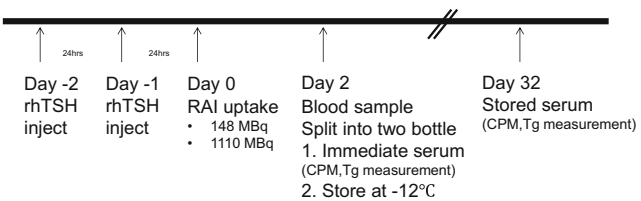

Fig. 1 The study protocol

ablation. The stages of PTC were distributed from I to III, with no patient having distant metastasis.

The Relationship Between Serum Tg Levels at Days 2 and 32

The serum Tg level of each sample was measured on days 2 and 32; levels <0.2 ng/ml were regarded as "undetected". The mean Tg levels of days 2 and 32 were 0.90±1.88 ng/ml and 0.95±2.11 ng/ml, respectively. No significant difference was noted between the two samples (p=0.201; Fig. 2). Furthermore, a significant correlation was found between the two samples (r=0.992, p<0.001; Fig. 3).

The serum count per minute (CPM) determined at the two different time points showed a significant decline from day 2 to day 32 (mean CPM on days 2 and 32 were 1,004±834 and 116±63, respectively; p<0.001). In addition, decay correction performed between day-2 serum CPM and day-32 serum CPM was significantly correlated (p<0.001).

Subgroup Analysis Classified by Radioactive Iodine Dose

From among 67 patients, 20 patients underwent RIT with ^{131}I 148 MBq, and 47 patients underwent RIT with ^{131}I 1,100 MBq. In the ^{131}I 148 MBq group, the day-2 and day-32 Tg levels were equal for all samples (mean value: 0.27±0.03 ng/ml). In the ^{131}I 1,100 MBq group, the mean Tg values of day 2 and day 32 were 1.27±0.31 ng/ml and 1.34±0.35 ng/ml, respectively. Conclusively, the mean Tg level in each group showed no significant difference (p=1.000 in the ^{131}I 148 MBq group and p=0.202 in the ^{131}I 1,100 MBq group; Fig. 4).

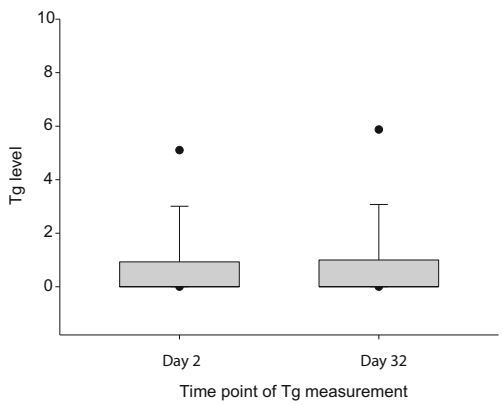

Fig. 2 Box plot of Tg measurements on days 2 and 32. The mean Tg value was 0.90±1.88.ng/ml and 0.95±2.11 ng/ml in each group, with no statistically significant difference, as determined by the paired t-test (p=0.201)

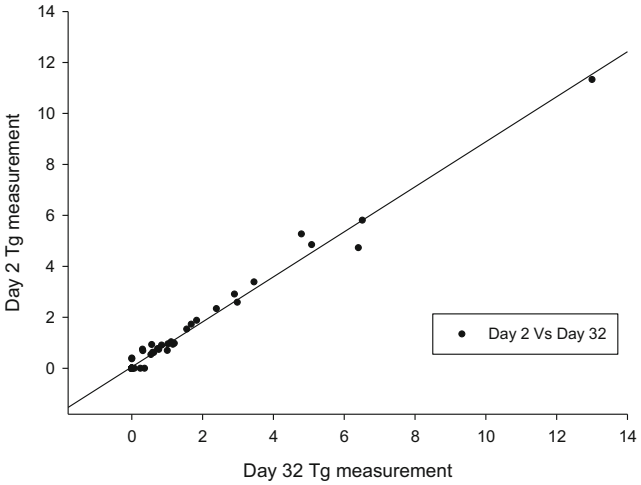

Fig. 3 Significant correlation was noted between the Tg values on days 2 and 32 (r=0.992, p=0.000)

Discussion

IRMA is a reliable method for the measurement of Tg in the follow-up of DTC after total thyroidectomy [1]. Our study evaluated whether ^{131}I administration interferes with Tg

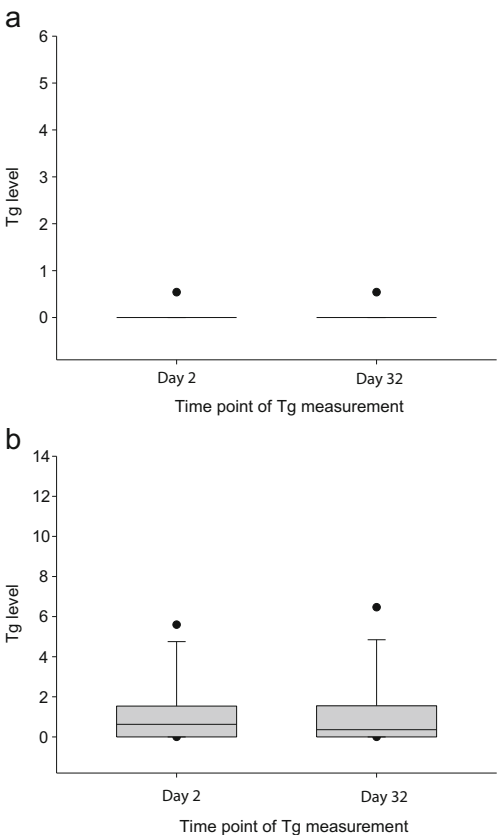

Fig. 4 Subgroup analysis of the Tg level measurement. a The 148 MBq ^{131}I group, which shows no significant change in the Tg value between days 2 and 32. b The 1,110 MBq ^{131}I group, which shows no significant change in the Tg value between days 2 and 32

measurement using IRMA because of the relatively high energy of ^{131}I compared to ^{125}I. Usage of rhTSH for stimulation of TSH is becoming easy because of insurance coverage in Korea. On using rhTSH for TSH stimulation, the serum Tg elevation was highest at 48 h after rhTSH administration, which is a day after ^{131}I administration [13]. As a result, the optimal Tg measurement was performed after ^{131}I administration. So when we use rhTSH for stimulation of TSH, optimal Tg measurement is done with high serum background radioactivity due to the administration of ^{131}I. If the administered ^{131}I interferes with the serum Tg measurement, the Tg levels determined by IRMA can be inaccurate. However, in our study, the Tg levels of serum samples with high ^{131}I and decayed ^{131}I showed no significant difference. Therefore, we conclude that the ^{131}I radioactivity of serum does not interfere with the measurement of Tg by IRMA.

The Tg level was correlated with the remnant tumour burden and prognosis. A high pre-ablation Tg level is known to be the most significant predictor of therapeutic failure [14]. The stimulated Tg level is related to the prediction with stimulation using rhTSH. The first rhTSH stimulation Tg level showed excellent prediction of remission [15]. Furthermore, Tg level measurement is a sensitive method for monitoring response and recurrence during the treatment process. As mentioned earlier, knowledge of the Tg level is important to make precise clinical decisions; therefore, the Tg value should be accurately measured.

The half-life of ^{131}I is 8.01 days and that of ^{125}I is 59.4 days. Therefore, we believe that, after a month, the radioactivity of ^{131}I significantly decreases to <7 % of the initial activity, although the radioactivity of ^{125}I decreases to approximately 30 %. The biological half-life of ^{131}I may be 18 h because of its excretion from the body [16]. Although considering the biological half-life of ^{131}I, the sera on day 2 still contain high radioactivity of ^{131}I. Therefore, our study confirmed the reliability of immediate Tg measurement in sera with high radioactivity of ^{131}I.

TSH stimulation for RIT is conventionally performed with thyroid hormone withdrawal. Hypothyroidism is inevitable before RIT, and hypothyroidism decreases patient QOL. rhTSH improves a patient's QOL by avoiding long-term hypothyroidism [11]. rhTSH is expensive; hence, there is considerable controversy surrounding the cost-effectiveness of using rhTSH [17]. However, the ablation success rate by the proposed method is comparable to that obtained using thyroid hormone withdrawal, even in high-risk patients with metastatic thyroid cancer; the long-term outcome is also similar between both protocols [18–20]. Therefore, it is recommended that rhTSH be applied routinely for RIT to improve patient QOL. Furthermore, diagnostic monitoring is feasible using rhTSH as compared with that using thyroid hormone withdrawal because of not only the efficacy of rhTSH but also its effect on improving the QOL [21, 22].

The radioactive iodine dose was different in each patient. For diagnostic purposes, the remnant thyroid tissue was examined and very low dose of ^{131}I was administered (148 MBq). However, for treatment purposes, a low dose of ^{131}I was administered for remnant ablation (1,110 MBq) [9]. As the administered dose could influence the serum CPM at the time of blood collection, subgroup analysis classified by the radioactive iodine dose may affect our data interpretation. However, no significant difference was noted between the Tg values of days 2 and 32 in each group. The limitation of our study is that it did not include high-dose RAI patients. However, no difference was noted between the results of doses of 148 MBq and 1,110 MBq, suggesting that the binding force between the antibody and Tg was strong enough to neglect the serum CPM.

A serum Tg level <0.2 was regarded as "undetectable". The cutoff level of Tg for surveillance remains controversial; however, the cutoff level of 0.2 could be rational for the surveillance of DTC after total thyroidectomy [23–26]. In our study, the Tg level of most patients was undetectable. There was a concern that an increase in Tg level may occur between days 2 and 32. However, a significant correlation was observed between the dual time points for detectable Tg levels, and the results were significant when analysed by the paired *t*-test. Thus, IRMA has a high reproducibility irrespective of the administered dose of ^{131}I and the serum Tg level.

Conclusion

The ^{131}I in the serum of the patients stimulated by rhTSH did not interfere with the IRMA using ^{125}I. In the follow-up of DTC, IRMA can be used for disease surveillance, irrespective of the RIT used.

Declaration of Interest The authors fully declare any financial or other potential conflict of interest.

Conflict of Interest Sohyun Park, Ji-In Bang, Ho-Young Lee, and Sang-Eun Kim declare that they have no conflict of interest.

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