

The Effect of Radioiodine Treatment on TRAb, Anti-TPO, and Anti-TG in Graves' Disease

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Keywords

Graves' disease · TRAb · Anti-TPO · Anti-TG · Radioiodine

Abstract

Background: In Graves' disease (GD), immunocompetent cells infiltrate thyroid tissue with release of TSH-receptor antibodies (TRAb), and radioiodine treatment is known to elicit an immune response with an increase in TRAb. **Objectives:** The aim was to study if all patients treated with radioiodine respond with a release of TRAb, anti-thyroperoxidase (anti-TPO), and anti-thyroglobulin (anti-TG). **Methods:** This is a prospective observational study. GD patients ($n = 131$) were admitted for treatment with radioiodine. Thyroid antibodies were measured before and 3 months after iodine-131 treatment. **Results:** After 3 months, a fold change >1.1 was found in 66% of the GD patients, while the remaining 34% did not have a change or decrease in TRAb. Anti-TPO and anti-TG also increased; the former showed an increase in 73% and the latter of 52%, while 27 and 48% decreased/were unchanged. A significant positive correlation was found between TRAb and anti-TPO, but not between TRAb and anti-

TG. In the group with an increase in TRAb, the median fold change was 5.1, but there were no additional effects of tobacco smoking. The proportion of females below the median age (51.5 years) was significantly higher in the group that increased in TRAb compared to the one that decreased/was unchanged (66 vs. 34%). **Conclusions:** Treatment with radioiodine elicits an increase in thyroid antibodies, but not in all GD patients. The proportion of responders varied and was affected by age, resulting in a stronger immune response at younger age. However, there were no additional effects of smoking.

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Introduction

In Graves' disease (GD), immunocompetent cells infiltrate thyroid tissue with release of TSH-receptor antibodies (TRAb), resulting in hyperthyroidism. The triggering of the autoimmune response depends on the interplay of genetic [1, 2] and environmental factors [3]. One strong risk factor is tobacco smoking, which results in

higher TRAb at diagnosis of GD and during treatment with thiamazole than in nonsmokers [4]. It has also been shown that smokers treated with carbimazole had a much slower reduction of stimulating TRAb compared to nonsmokers with GD [5]. Tobacco smoking is a risk factor for the development of both GD and Graves' ophthalmopathy (GO), and in a recent review it is stated that current smoking increases the risk of GD approximately twofold and GO approximately threefold [6]. Independently of smoking, it has also been shown that higher TRAb increase the risk of GO both at and after diagnosis of GD [7, 8]. Treatment of GD with radioiodine is also a risk factor for the development of GO, and the pathophysiological process of GD and GO may be mediated by activating TRAb [9]. Treatment with radioiodine results in an increase of TRAb with a maximum after 3 months. This is in contrast with the other two treatment modalities, anti-thyroid drugs (ATD) or thyroidectomy, where TRAb slowly declines without a prior increase [10]. It has been suggested that the increase in TRAb after radioiodine treatment was mediated by a transient release of thyroid antigens [11, 12]. However, in some patients, the increase in TRAb persists for several years, indicating the existence of other mechanisms involving the activation of specific immunocompetent cells. Indeed, irradiated Hashimoto lymphocytes have been studied *in vitro*, and it was suggested that irradiated lymphocytes of the thyroid are important in the synthesis of autoantibodies in response to iodine-131 [13]. The aim of the present study was to prospectively investigate if all patients respond in a similar manner and if anti-thyroperoxidase (anti-TPO) and anti-thyroglobulin (anti-TG) also showed the same pattern, and to study factors that may modulate these responses.

Subjects and Methods

Patients

All treatments with iodine-131 in Skåne County are centralized to the Department of Oncology at Lund University where all patients with hyperthyroidism intended for iodine-131 are referred. The choice of the treatment modality is made by the patients' ordinary clinician.

This is a prospective observational study that included all GD patients admitted to the Department of Oncology, Skåne University Hospital, for treatment with radioiodine during August 2016 until May 2017. The patients were classified as having GD on the basis of clinical signs, the presence of TSH receptor antibodies (TRAb), and/or a diffuse uptake on thyroid technetium scintigraphy. There were 3 patients included without measurable TRAb in the group that did not respond to radioiodine and out of these, 1 patient was negative for both anti-TPO and anti-TG before and

after treatment. In the group that responded to radioiodine, 5 patients were negative for TRAb before treatment, but all responded with an increase in TRAb.

In total, 131 patients not previously treated with radioiodine were included, and the following parameters were registered: age (median = 55 years), female/male ($n = 100/31$), born in Sweden ($n = 97$), born outside Sweden ($n = 31$), tobacco smoking ($n = 28$), duration of GD (median = 9 months), ophthalmopathy ($n = 11$), and treatment with corticosteroids ($n = 27$). As a clinical routine, 120 Gy were used, but in some patients, up to 300 Gy were administered if the aim was to decrease the risk of relapse. Methimazole or propylthiouracil with or without L-thyroxine was used in some patients before the decision of radioiodine treatment and was stopped 2 weeks before the administration of iodine-131. The ALARA (as low as reasonably achievable) principle was used when defining the activity needed to achieve the described doses, and the activity (MBq) was estimated by use of the following formula: Dose (D) \times Mass (m) / 0.043 \times uptake day zero (U0) \times effective half-life (Teff). U0 and Teff were calculated from the iodine uptake at 24 h and at 7 days, as measured after the administration of a pretreatment dose of 0.4 MBq for individualized treatment planning. The thyroid mass was calculated from ^{99m}Tc -pertechnetate scintigraphy.

The absorbed doses used were distributed as follows: 120 Gy ($n = 102$), >120–150 Gy ($n = 2$), >150–200 Gy ($n = 12$), and >200–300 Gy ($n = 15$).

TRAb, anti-TPO, and anti-TG were measured 7 days before and 3 months (+/–14 days) after iodine-131 treatment, and the fold changes were registered.

Assays

TRAb was measured with a competitive electrochemiluminescence immunoassay (ECLIA) according to the manufacturer's instructions (Roche). The detection limit was 0.3 IU/L, and the variation coefficient 5% at 16 IU/L. The cutoff for a positive value of TRAb was >1 IU/L.

The anti-TPO titer was measured with a competitive sandwich ELISA (Roche) according to the manufacturer's instructions (detection limit 5 IU/L, variation coefficient 11% at 34 IU/L). The cutoff for a positive value of anti-TPO was >34 IU/L.

Anti-TG was measured with a competitive sandwich ELISA (Roche) according to the manufacturer's instructions (detection limit 10 IU/L, variation coefficient 10% at 73 IU/L). The cutoff for a positive value of anti-TG was >115 IU/L.

The samples were analyzed in clinical routine laboratories at the Department of Clinical Chemistry in Malmö and Lund.

Statistical Analysis

The fold changes of thyroid antibodies were determined, and a change of 1.1 or more was judged as an increase. If lower than 1.1, the change was judged as unchanged or decreased. The *t* test (continuous variables) and the χ^2 test (categorical variables) were used to assess statistical significance of differences between the groups. Linear regression analysis was used in the study of correlations between the parameter fold changes of TRAb, anti-TPO, anti-TG, and age in years. All statistical analyses were carried out using the SPSS 22.0 statistical software (SPSS, Chicago, IL, USA).

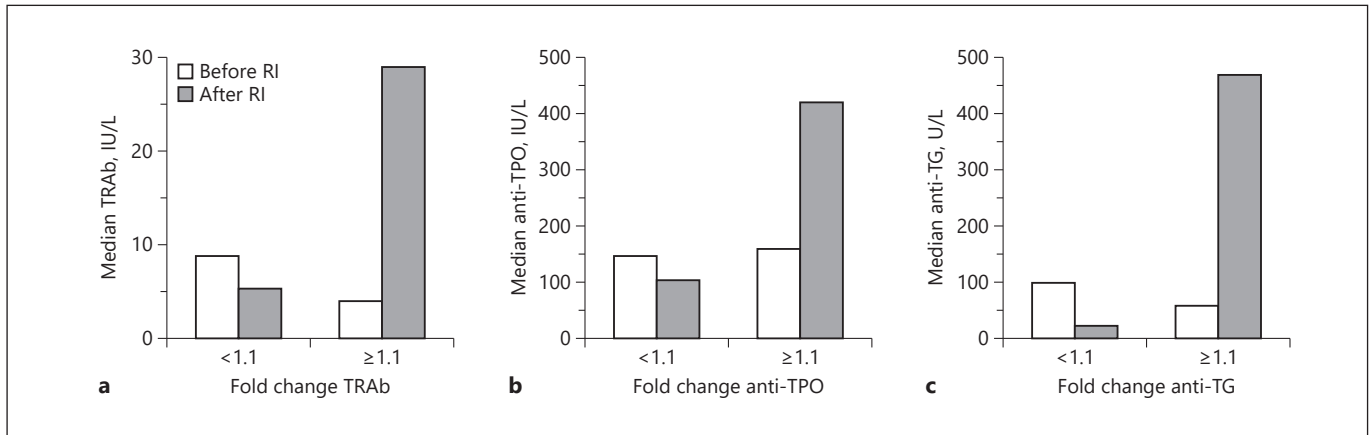


Fig. 1. Changes of TRAb, anti-TPO, and anti-TG 3 months after treatment of GD with radioiodine. The median values of TRAb, anti-TPO, and anti TG before and after radioiodine (RI) in the group with a fold change <1.1 and in the group with a fold change ≥ 1.1 were all significant with p values <0.01 (t test).

Results

Thyroid antibodies were measured at baseline and 3 months after administration of iodine-131. The TRAb fold change was above 1.1 in 66% of the GD patients, and the remaining 34% did not change or decrease in TRAb. The median values of TRAb showed the same kinetics as in the group with an increased value, and the median TRAb raised from 4 (range 1–32) to 29 (range 2.9–39) IU/L. In the group without change or decrease, the median TRAb decreased from 8.8 (range 1–39) to 5.3 (range 1–39) IU/L (Fig. 1a).

The proportion of patients in whom anti-TPO increased was 73%, and in 27%, it did not change or decrease. In the group in which anti-TPO increased, the median value at baseline raised from 158 (range 8–3,962) to 419 (range 10–9,999) IU/L after 3 months (Fig. 1b), and in the group without change or decrease in anti-TPO, the value changed from 144 (range 9–2,243) to 103 (range 7–1,936) IU/L after 3 months (Fig. 1b).

The fold change in anti-TG showed a different distribution. The proportion of patients in whom the value increased was 52%, and in the group without change or decrease, 48% had showed decrease or unchanged values (Fig. 1c). However, when analyzing the median values in the group where anti-TG increased, it raised from 58 (range 20–1,286) to 468 (range 26–4,000) IU/L. In the other group without change or decrease in anti-TG, the value changed from 98 (range 20–7,910) to 21 (range 20–4,000) IU/L (Fig. 1c).

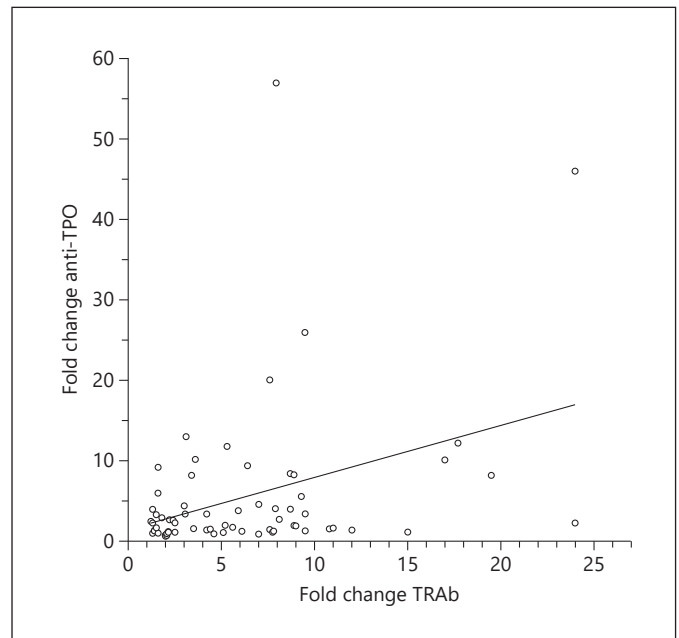


Fig. 2. Correlation of the fold change in TRAb with the fold change in anti-TPO 3 months after treatment with radioiodine ($R = 0.362$, $p = 0.003$).

A positive correlation was seen between TRAb and anti-TPO (Fig. 2), which contrasted with anti-TG where no significant correlation with TRAb ($R = 0.082$, $p = 0.574$) was demonstrated. In the linear regression analysis, it was also shown that a lower age was associated with a higher fold increase in TRAb (Fig. 3).

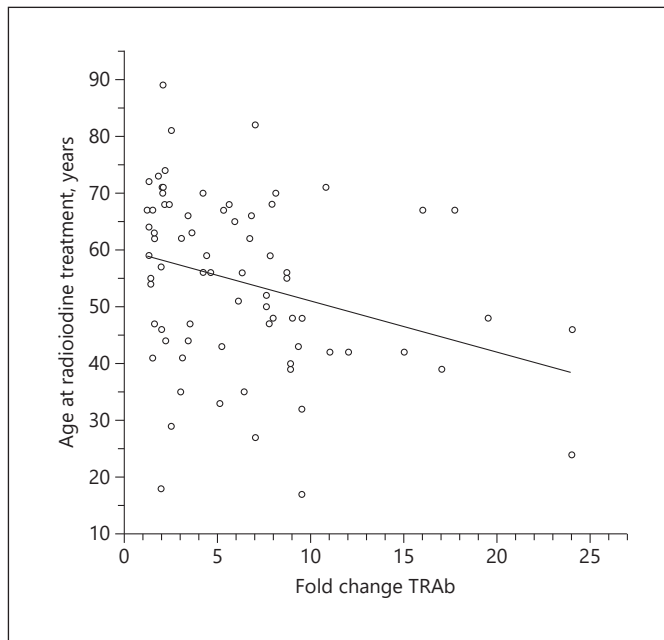


Fig. 3. Correlation of the fold change in TRAb 3 months after treatment with radioiodine with age at treatment with radioiodine ($R = 0.305$ $p = 0.008$).

When comparing the distribution of factors that may affect the groups with or without increase in TRAb (fold change <1.1 and ≥ 1.1), we found no significant change in the distribution of smoking habits, sex, or age (Table 1). The same analysis was performed for anti-TPO and anti-TG, with no significant difference except for median age. The median age for the group with an increase in anti-TPO was 51 years, and in the group where the value did not change or decrease, it was 63 years ($p = 0.003$). For anti-TG, the median age in the group with increased values, the median age was 50 years, and in the group with unchanged or decreased values, it was 61 years ($p = 0.018$). The distribution of the patients treated with corticosteroids or higher doses of radioiodine (>120 Gy) was not significantly different. The duration of disease was not significantly different for TRAb and anti-TPO, but there was a significant difference between the groups in the duration of disease for anti-TG ($p = 0.03$). The median duration was 6 months (range 2–120) in the group with decreased or unchanged values versus 22 months (range 1–96) in the group with increased values. There was a significantly higher proportion of women below the median age of 51.5 years (Table 2). A lower age was seen in patients with a fold change of TRAb <5.1 ; the median age was 48 years as compared to patients with a fold change

<5.1 who had a median age of 59 years. The same analysis was not significant for anti-TPO and anti-TG (data not shown).

Discussion

The triggering of an autoimmune response in GD depends on the genetic background and environmental factors resulting in the production of stimulating TRAb and the activation of TSH receptors in thyroid follicular cells followed by an increased release of thyroid hormones, resulting in thyrotoxicosis. Previously defined environmental triggers are a changed iodine status, stress, the postpartum period, the menopausal period, and tobacco smoking [3, 6]. In a randomized study, Laurberg et al. [10] compared the 3 treatment modalities ATD, thyroidectomy, and radioiodine and found that the two former methods resulted in a continuous decrease in TRAb, whereas the latter one resulted in a distinct increase in TRAb with a maximum after 3 months followed by a slow decrease, in some cases for several years. This study is the first that investigated the effect of radioiodine treatment on TRAb, anti-TPO, and anti-TG and found that all three antibodies increased after 3 months, but not in all patients. A subgroup was identified where the antibody concentrations were decreased or unaltered, and the proportion of the total differed depending on the antibody analyzed. The proportion of patients that had increased values was similar in TRAb and anti-TPO, where approximately 2/3 were increased. However, in anti-TG, only half of the patients showed an increase. Tobacco smoking has previously been shown to result in higher concentrations of TRAb [4, 5], but this was not the case because the proportion of smokers was similar in the group of patients with increased values compared to the group with unchanged or decreased values. Neither were there any differences depending on sex or age nor was there an overrepresentation of patients born outside Sweden (not significant). This observation has also been made in a previous study in Malmö where the incidence of GD was higher in patients born outside Sweden, with a maximum around the menopausal age [14]. In contrast with this finding, the present study shows that the proportion of women <51.5 years old was increased in the group with the highest fold change of TRAb, and the mean age in this group was 48 years. In the group with a lower increase, the median age was 59 years. This is in line with the knowledge that younger GD patients are more prone to produce higher TRAb levels and to have an increased relapse rate [15]. The role of anti-TG

Table 1. Changes in TRAb after treatment with radioiodine and relation to clinical parameters

Fold change TRAb	<1.1	≥1.1	<i>p</i> value*
Females	82 (31)	78 (58)	0.69
Males	18 (7)	22 (16)	0.69
Median age females and males, years	52	56	0.63
Median age females, years	51	52	0.46
Smokers	11 (4)	23 (17)	0.11
Nonsmokers	89 (34)	77 (57)	0.11
Born in Sweden	82 (31)	70 (52)	0.18
Born outside Sweden	18 (7)	30 (22)	0.18
Treatment with corticosteroids	26 (10)	16 (12)	0.20
Treatment with >120 Gy	21 (8)	20 (15)	0.92
Median duration of disease (range), months	10 (1–132)	9 (1–120)	0.56
Primary treatment with ATD	66	55	0.29

Values are presented as % (*n*), unless otherwise stated. * χ^2 test.

in the pathogenesis of GD is unclear, but in a previous study examining discontinuation of ATD, it was shown that patients with low levels of anti-TG at the diagnosis of GD had a lower chance to stop the treatment with ATD, indicating a higher activity of GD in these patients [16]. A low level of anti-TPO at the diagnosis of GD has been coupled with an increased risk to develop GO both at diagnosis and during follow-up [8].

In a recent study, a previously TRAb-negative patient with toxic adenoma developed TRAb, anti-TPO, and anti-TG within 3–4 months after treatment with radioiodine [17]. It may seem paradoxical to use a treatment modality for an autoimmune disease that triggers an antibody response, so what is the mechanism? It has been suggested that this response depends on a transient release of autoantigens due to the destruction of thyroid tissue. If this is the explanation, you would expect all patients to be antibody negative within a relatively short period of time. However, it is well known that many patients are TRAb positive for several years. Another explanation could be that local irradiation not only destroys the follicular cells but also affects the present immunocompetent cells, resulting in an imbalance of the regulatory T-cells and the antibody-producing B cells. This could be the case if the regulatory T cells need longer for restitution than the antibody-producing B cells, which are known to have a higher proliferation rate. In an in vitro study on immunocompetent cells, patients with Hashimoto thyroiditis were irradiated with different doses, and it was shown that the antibody production was higher in cells irradiated with an intermediate high dose compared to a high dose [13].

Table 2. Increase in TRAb after radioiodine treatment and relation to clinical parameters

Fold change TRAb in group >1.1	Median <5.1	Median >5.1	<i>p</i> value*
Proportion of females with a median age <51.5 years	34 (13)	66 (24)	0.02
Smokers	24 (9)	22 (8)	0.78
Nonsmokers	76 (28)	78 (29)	0.78

Values are presented as % (*n*). * χ^2 test.

In patients with GD and/or GO, specific antibodies are directed against the TSH receptor, and the antigen is suggested to be shared between the thyroid and orbital tissue. It has previously been shown that the use of radioiodine increased the risk of developing GO [18]. This finding was not explained by hypothyroidism [9], and the risk could be diminished by pretreatment with corticosteroids that decrease the TRAb titers [19]. One hypothesis is that the triggering of high titers of TRAb could be the link between the thyroid and the orbital tissue that explains the increased risk to develop GO after treatment with radioiodine. If so, patients who respond with decreased or unaltered TRAb should have a lower risk to develop GO.

GD during pregnancy is in most cases limited by the pregnancy-related immunosuppression, which reduces the levels of TRAb. However, some patients will still have detectable TRAb in the late third trimester [20]. In addi-

tion, patients treated with radioiodine several years before pregnancy may also have detectable TRAb during the whole pregnancy period. Our observation that not all patients respond with an increase in TRAb could also be of importance when treating women in fertile age with radioiodine because if these women become pregnant, there would be a lower risk for the development of fetal thyrotoxicosis mediated by TRAb passing the placenta barrier.

To conclude, treatment with radioiodine does not elicit an increase in thyroid antibodies in all GD patients, and whether these patients are less prone to develop GO or pregnancy-related complications might be evaluated in a follow-up study. The proportion of responders varied and was affected by age but not tobacco smoking.

Acknowledgement

We are grateful to the nurses at the Department of Oncology, Skåne University Hospital, for the help with the blood tests.

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Statement of Ethics

The study was approved by the Regional Research Ethics Committee at Lund University (project number 160610). Informed consent has been obtained from each patient or subject after full explanation of the purpose and nature of all procedures used was given.

Disclosure Statement

There are no conflicts of interest to disclose.

Funding Sources

This work was supported by grants from the Faculty of Medicine at Lund University, the Skåne Research Foundation, and the Research Funds of Skåne University Hospital.