Efficacy and safety of radioiodine therapy for mild Graves' ophthalmopathy in dependence on cigarette consumption - a half year of follow-up

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**Authors:** Agata Czarnywojtek, Krzesiwa Komar-Rychlicka, Małgorzata Zgorzalewicz-Stachowiak, Nadia Sawicka-Gutaj, Kosma Woliński, Paweł Gut, Maria Teresa Plazinska, Barbara Torlińska, Ewa Florek, Joanna Waligórska-Stachura, Marek Ruchała

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Efficacy and safety of radioiodine therapy for mild Graves' ophthalmopathy in dependence on cigarette consumption - a half year of follow-up

Short title: Radioiodine therapy for mild Graves' ophthalmopathy

Agata Czarnywojtek¹,²*, Krzesisława Komar-Rychlicka³*, Małgorzata Zgorzalewicz-Stachowiak⁴, Nadia Sawicka-Gutaj¹, Kosma Woliński¹, Paweł Gut¹, Maria Teresa Plazinska⁵*, Barbara Torlińska⁶, Ewa Florek⁷, Joanna Waligórska-Stachura¹, Marek Ruchala¹

* Both authors contributed equally to this work

1 Department of Endocrinology, Metabolism and Internal Medicine, Poznan University of Medical Sciences, Poznań, Poland
2 Department of Pharmacology, Poznan University of Medical Sciences, Poznań, Poland
3 Department of Ophthalmology, Poznan University of Medical Sciences, Poznań, Poland
4 Laboratory of Medical Electrodiagnostics, Department of Health Prophylaxis, Poznan University of Medical Sciences, Poznań, Poland
5 Nuclear Medicine Department, Medical University of Warsaw, Warsaw, Poland
6 Centre for Endocrinology, Diabetes & Metabolism Institute of Biomedical Research, School of Clinical and Experimental Medicine, University of Birmingham, United Kingdom
7 Laboratory of Environmental Research, Department of Toxicology, Poznan University of Medical Sciences, Poznań, Poland
Correspondence to: Maria Teresa Plazinska, PhD, Nuclear Medicine Department, Medical University of Warsaw, Warsaw, Poland, e-mail: plazinska@poczta.onet.pl

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Abstract

Introduction  Graves' Orbitopathy (GO) is an autoimmune disease associated with Graves' disease. Therapy is largely dependent on the severity and activity of ocular changes. Particular attention should be given to radioiodine therapy (RIT). Although its application is a valuable method of the treatment of hyperthyroidism, it may be followed by worsening of GO.

Objectives  The aim of the present article is to indicate how the severity of nicotine addiction influences the response for RAI treatment in GO patients.

Patients and methods  106 patients with mild GO treated with 800 MBq of radioiodine (RAI) were included. Serum levels of TSH, thyroid hormones, autoantibodies against thyroperoxidase (TPO-Abs), thyroglobuline (Tg-Abs) and TSH receptor (TSHR-Abs), urine cotinine levels and severity of ophthalmopathy were assessed; analyses were conducted at baseline (before RAI treatment), 2 and 6 months after therapy.

Results  Statistically significant differences in serum levels of TSHR-Abs were found between non-smokers and smokers 2 and 6 months after RIT, whereas there was no difference at baseline. In smokers, there were statistically significant differences in the severity of ophthalmopathy and the concentration of serum TSHR-Abs assessed at baseline and after 6 months of follow-up. Six months after RIT, 46.2% of smokers and 4.3% of non-smokers ($P <0.001$) were subsequently upstaged from mild to moderate GO.

Conclusions  High level of cotinine in urine in smokers was associated with the deterioration of ocular changes after RAI. High dose of RAI did not induce exacerbation of GO in non-smokers administered with oral steroid prophylaxis.

Key words  cotinine, Graves’ disease, Graves’ ophthalmopathy, radioiodine, smoking
Introduction  Graves' Orbitopathy (GO) is an ophthalmological manifestation of Graves' disease. The therapy of GO is largely dependent on the severity and activity of ocular changes [1]. Additionally, the severity of GO is influenced by both endogenous (gender, genetic factors, pregnancy) and exogenous factors, such as thyroid dysfunction, cigarette smoking or radioiodine therapy (RIT) [2-6]. Particular attention should be given to RIT. Although its application is a valuable method of treatment of hyperthyroidism, it may be followed by a worsening of GO [3,7,8]. RIT can induce increased production of TSHR-Abs and subsequently the eye injury. To avoid these effects glucocorticoid prophylaxis should be considered [9]. However, question about a dose of radioiodine (RAI) to avoid a relapse or deterioration of GO remains not answered.

The aim of the study was to analyze how the severity of cigarette smoking assessed with the cotinine concentration in urine influences the course of GO in patients treated with RAI.

Patients and methods

Patients  Patients with GO treated in the Outpatient Clinic of the Department of Endocrinology, Metabolism and Internal Medicine in Poznan between March 2014 and October 2014 were included. Serum levels of TSH, thyroid hormones, thyroid autoantibodies and urine cotinine level were assessed before RIT, and two and six months after. All patients were examined by the same experienced ophthalmologist (K.K.-R) before RIT and after six months. Inclusion criteria were: mild GO or inactive and moderate-to-severe GO, treatment with 800 MBq (22 mCi) of radioiodine. Exclusion criteria were: active and moderate-to-severe GO, sight-threatening GO, lack of compliance (patients who did not come for the control examination after 2 or 6 months), refusal for radioiodine treatment.

The study was approved by The Ethical Committee of Poznan University of Medical Sciences. Written informed consents were given by all participants.
Antithyroid drugs (ATDs), RAI and corticosteroid therapy

Ninety five patients were treated with methimazole and 11 with propylthiouracil before RIT. Treatment with ATDs was discontinued at least 24 hours prior to RIT. In 24% patients, ATDs were reintroduced at least 7 days after RAI. All patients were prescribed a prophylactic therapy with oral glucocorticoids (oGCS), i.e. prednisone (0.3–0.5 mg/kg body weight daily) initiated after RAI and gradually tapered down and withdrawn after 6 weeks [11,12].

**Ophthalmological evaluation**

Ophthalmological examination was performed according to the EUGOGO recommendations and its Polish adaptation [17, 18]. Activity of GO was assessed using the Clinical Activity Scale (CAS) [13,14] (spontaneous retrobulbar pain, pain on attempted up or down gaze, redness of the eyelids, redness of the conjunctiva, swelling of the eyelids, inflammation of the caruncle and/or pica, and conjunctival oedema). GO was classified as active when CAS was ≥3/7. NOSPECS was used to assess the severity of GO [15,16] (N, no signs or symptoms; O, only signs, no symptoms; S, soft tissue involvement; P, proptosis; E, extraocular muscle involvement; C, corneal involvement; S, sight loss).

**Progression of ophthalmopathy**

**Worsening of GO in ophthalmologic examination indicated the disease progression**

**Urine cotinine level**

Urine cotinine [(5S)-1-methyl-5-(3-pyridyl)pyrrolidin-2-one)] is a nicotine metabolite which reflects the tobacco-smoke exposure. Patients were divided into two groups based on these results. Non-smokers and those not exposed to the environmental tobacco smoke (ETS) had urine cotinine levels of 5 ng/ml in urine/mg creatinine; individuals exposed to ETS had 5 to 50 ng cotinine in urine/mg creatinine (passive exposure); and active smokers > 50 ng cotinine in urine/mg creatinine (from 50 to 500 ng/ml in light smokers; 500-2500 ng/ml in moderate smokers, and above 2500 ng/ml in heavy smokers) [19].
Cotinine was dissolved in methanol to create a standardized solution (Wao Pure Chemicals; Osaka, Japan and Sigma Chemical St Louis, MO, USA), which further was added to urine (non-smokers) and distilled water to prepare a range of concentrations: 2.1, 27.6, 55.2, 82.8, 110, 138, 276, 552, 828, 1100 and 1380 ng/ml for cotinine.

A gas chromatograph (GC-14B, Kyoto, Japan) equipped with a capillary column and flame thermionic sensor was utilized for quantifying cotinine. The injection port and detector temperature was 260˚C. The column temperature was constant at 150˚C for 2 min., then raised to 260˚C at the rate of 10C/min., and held constant for 2 min. as the final temperature. Nitrogen at 15 kPa was the carrier gas.

One milliliter of urine was added to 0.1 ml of 12 µg/ml of carbinoxamine maleate in methanol and 1ml of 1M carbonate buffer (pH 9.7). The upper aqueous phase was aspirated and disposed of, while the remaining organic phase was transferred to a new single use tube. The organic phase was re-extracted with 1ml of 0.1N HCl and vigorously vortexed for 30 s. The resulting aqueous phase was transferred to a new disposable tube and 1ml carbonate buffer, added, and re-extracted using 4 ml dichloromethane vortexed for 30 s. Organic phase was mixed with 20 µl of isoamyl alcohol and dichloromethane, along with succeeding evaporation under a mild flow of nitrogen in a heated block at 35˚C. 1 µl of the remaining alcohol solution was then injected into the gas chromatograph.

Assays TSH, FT4 and FT3 (free triiodothyronine) were measured using the electrochemiluminescence technique in Cobas E 601 (norm ranges: TSH 0.27–4.2 mU/l; FT4 11.5–21.5 pmol/l; FT3 3.9- 6.8 pmol/l). Estimation of TSHR-Abs was performed using second-generation antibodies (RIA-2 Dynotest TRAK human, BRAHMS Diagnostica GmbH, Berlin, Germany) (norm range<2 IU/ml). Tg-Abs, and TPO-Abs were measured with radioimmunoassay (norm ranges <115 IU/ml and <35 IU/ml, respectively).
Sonography, radioiodine uptake and scintigraphy  The thyroid ultrasonography (The Aloka IPC-1530, Tokyo, Japan) performed by a 7.5-MHz with linear transducer was used to perform thyroid volume and the ellipsoid model (width x length x thickness x 0.52 for each lobe) was used for calculation [19]. A RAI uptake was measured in every patient before RIT 5-h and 24-h after administration of 2 MBq (54 µCi) of 131I. All patients received the same therapeutic activity of 800 MBq of RAI [10] (TABLE 1).

Statistical analysis  Statistical analyses were performed using Statistica 10 software by StatSoft. P value of less than 0.05 was considered as statistically significant. Categorical dichotomous data was analyzed using McNemar’s test; continuous data using either paired samples T-test or repeated measures ANOVA test. Data was presented with means and standard error (SE) or percentages.

Results  Of 126 preliminary assessed subjects, 106 were eligible for the inclusion in the study. Reasons for ineligibility (n = 20, 15.8%) included: (i) inadequate information (n = 11, 8.7%), and (ii) the incomplete laboratory data (n = 9, 7.1%). Finally, 106 patients (93 females, 13 males) were included.

Baseline characteristics of the patients are shown in TABLE 1. The results concerning the thyroidal status two and six months after RAI were presented in Table 2.

Thyroid function  At the end of follow-up, 46 out of smoking patients (79.3%) were successfully treated (persistent hypothyroidism or euthyroidism), 5 (8.6%) developed subclinical hyperthyroidism, and 7 (12.1%) remained symptomatically hyperthyroid. Among the non-smokers, 8 (16.7%) had persistent hypothyroidism, 34 (70.8%) were euthyroid, and 6 (12.5%) had recurrent thyrotoxicosis.

Serum level of autoantibodies TSHR-Abs, TPO-Abs, Tg-Abs  Levels of autoantibodies, TSH and thyroid hormones during follow-up are shown in TABLES 1 (baseline evaluation) and 2. Among smoking patients, a statistically significant increase of TRAbs following RAI
therapy was observed (P <0.001). FIGURE 1 shown regression standardized predicted value of TSHR-Abs in tobacco smoking patients. In the case of non-smokers, there were no statistically significant differences in the serum level of TSHR-Abs at baseline and during follow-up (P >0.05).

**Serum level of TSHR-Abs, urine level of cotinine and CAS**  
Non-smokers and smokers differed significantly according to the levels of the urine level of cotinine before RAI therapy as well as after 6 months of follow-up (P <0.001). Statistical analyses of the urinary level of cotinine and the serum level of TSHR-Abs in tobacco smoking patients indicated significant differences between baseline and evaluation after 6 months of follow-up (P <0.001). In addition, statistically significant differences in the assessment of the activity of ophthalmopathy (CAS), concentration of serum TSHR-Abs at baseline and after follow-up (P <0.001, ANOVA) were observed in smokers. In the latter group, the association between the urine level of cotinine and CAS was statistically significant at baseline as well as after 6-months of follow-up (P <0.001), whereas no significant associations were observed in the non-smoking subgroup.

**Ophthalmological changes (activity and severity measures)**  
CAS was significantly higher in smokers before (P = 0.003, TABLE 1) as well as 6 months after RAI therapy (P = 0.0001, Fig. 2) than in non-smokers. In none of the cases of non-smokers conjunctival oedema occurred. Spontaneous retrobulbar pain was common in smokers and deteriorated after 6 months, whereas in the non-smoking group in most of the patients spontaneous retrobulbar pain did not occur.

There were statistically significant differences between smokers and non-smokers in proptosis at baseline and after 6 month of follow-up (NOSPECS 3, P <0.001). Moreover, statistical analysis showed significant changes after 6 months of follow-up according to the appearance of worsen of diplopia (NOSPECS 4, P <0.05) (FIGURE 3). Similar changes were observed
in lid aperture (NOSPECS 1) and visual acuity that were increased in smokers as compared to non-smokers (P <0.01). Decrease of visual acuity resulting from corneal disorder or neuropathy of n. II were not observed. Additionally, in our study retraction and exophthalmos were separately analyzed (TABLE 1). In none of the cases, corneal involvement, punctuate keratopathy, ulcer (NOSPEC 5) or optic nerve involvement (NOSPECS 6) were observed. Twenty seven of tobacco smokers (46.2%) and two of non-smokers (4.3%) patients had deterioration of ophthalmopathy 6 months after RAI treatment and were subsequently upstaged to moderate-to-severe GO (TABLE 3).

Discussion In the present study, the influence of nicotine consumption on the efficacy and safety of radioiodine therapy for mild Graves' ophthalmopathy was assessed. Smoking and non-smoking patients were examined by ophthalmologist before RAI therapy and 6 months later. The dose of 800 MBq of RAI was applied in both groups. Smoking habits declared by the patients were confronted with urine cotinine concentrations being a “gold standard” of assessment of the total nicotine exposure regardless of direct cigarette smoking. The association between RAI therapy and worsening of GO in patients has been addressed and investigated in numerous studies [17,21-24]. The radioiodine therapy has been proved to have an actual impact on the deterioration and development of ophthalmopathy in 15 to 39% of patients [16,20,21]. Randomized case-control trials confirmed a significantly higher risk of deterioration of ophthalmopathy after radioiodine treatment in comparison with antithyroid drugs [21-23].

The results of our study clearly demonstrate that RAI treatment is associated with ocular changes and worsening of GO particularly in the group of smokers. According to our results as well as study previously published by Kobe et al. [10] RAI therapy is not always followed by increased ophthalmic abnormalities when a high dose of RAI (800 MBq) is applied. In our study the worsening of ophthalmopathy was present in less than 5% of non-smoking patients
In order to reduce the risk of worsening of GO, all patients received oral glucocorticoid prophylaxis (30mg/d). Such treatment might provide protective influence [6,8,11].

It is difficult to explain such good effects of the high dose of RAI combined with oGCS. One might only speculate that a small dose of RAI leads to a prolonged worsening of autoimmunity against the TSHR-Abs, as compared to high doses of RAI (800 MBq). Our findings may have practical implications. Considering the fact that more advantageous effects can be obtained by using the high dose of RAI, we can ignore its unnecessary calculation, abandoning in this way the RAI uptake.

According to our results as well as many other published observations [25-28], RAI therapy is very often followed by persistent hypothyroidism, which is additionally detrimental as it can cause worsening of ophthalmopathy Therefore, to prevent the exacerbation of ocular changes it is very important to prevent development of hypothyroidism by thyroid function monitoring after RAI and, if necessary, early administration of L-thyroxine [25,26].

A major problem in the management of smoking patients is how to break the addiction to smoking. High values of cotinine (a metabolite of nicotine) as a biomarker for the exposure to tobacco smoke directly show how smoking influences the radioiodine therapy in terms of ocular changes. Our investigation has proved the relationship between the urine level of cotinine and increased CAS in smokers (P <0.001), whereas no significant differences of the kind were reported in the non-smoking group. This marker is used to determine the incidence of smoking, even after several days (up to one week) after cigarette consumption [19]. Furthermore, cotinine is a valuable marker in those cases when patients are not always completely honest with their doctors while referring to their smoking habits.

Our study, for the first time show connection between RAI treatment and consumption of cigarettes and exacerbation of GO apart from the preliminary study, performed by the same
authors using similar methodology on the distinct, over two times smaller group of patients [29].

The effect of cigarette smoking on TPO-Abs and Tg-Abs still remains controversial. As other studies suggest, cigarette smoking is associated with a lower prevalence of TPO-Abs and Tg-Abs [30,31]. Belin et al. [30] established a negative correlation between the cotinine concentration in smokers and the serum level of TPO-Abs and Tg-Abs.

**Conclusions** 1. Ocular changes occur with much higher intensity in the group of smokers with high level of urine cotinine 2. High dose of RAI does not induce exacerbation of ophthalmopathy in non-smokers.

**References**


Belin RM, Astor BC, Powe NR, et al. Smoke exposure is associated with a lower prevalence of serum thyroid autoantibodies and thyrotropin concentration elevation and a higher prevalence of mild thyrotropin concentration suppression in the Third
Table 1 Basic demographic, biochemical, thyroid and eye specific differences at baseline between smoking and non-smoking patients. Continuous values expressed as a mean (SE) ± standard error of mean (SEM), categorical in numbers (N) with proportion in brackets

<table>
<thead>
<tr>
<th></th>
<th>non-smokers (n = 48)</th>
<th>smokers (n = 58)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>age, years</td>
<td>39.2 (±1.14)</td>
<td>37.2 (±0.97)</td>
<td>NS</td>
</tr>
<tr>
<td>females</td>
<td>43 (89.6%)</td>
<td>50 (86.2%)</td>
<td>NS</td>
</tr>
<tr>
<td>TSH, µIU/ml</td>
<td>0.06 (±0.01)</td>
<td>0.05 (±0.009)</td>
<td>NS</td>
</tr>
<tr>
<td>fT4, pmol/l</td>
<td>25.3 (±0.3)</td>
<td>27.0 (0.35)</td>
<td>NS</td>
</tr>
<tr>
<td>fT3, pmol/l</td>
<td>8.8 (±0.36)</td>
<td>10.3 (±0.44)</td>
<td>NS (0.07)</td>
</tr>
<tr>
<td>Tg-Abs, IU/ml</td>
<td>218 (±85.6)</td>
<td>246.1 (±171.8)</td>
<td>NS</td>
</tr>
<tr>
<td>TPO-Abs, IU/ml</td>
<td>232.8 (±140.2)</td>
<td>246 (±145.3)</td>
<td>NS</td>
</tr>
<tr>
<td>TSHR-Abs, IU/l</td>
<td>11.7 (±6.3)</td>
<td>15.9 (±6.2)</td>
<td>NS</td>
</tr>
<tr>
<td>urine cotinine, ng/ml</td>
<td>3.8 (±1.6)</td>
<td>371.9 (±48.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>thyroid ultrasound</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>measurement volume, ml</td>
<td>26.7 (±0.9)</td>
<td>31.3 (±1.1)</td>
<td>0.02</td>
</tr>
<tr>
<td>RAIU, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>after 5</td>
<td>26.7 (±7.4)</td>
<td>27.5 (±8.4)</td>
<td>NS</td>
</tr>
<tr>
<td>and 24h</td>
<td>55.0 (±17.1)</td>
<td>53.5 (±7.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Correct vision acuity</td>
<td>44 (91.7%)</td>
<td>45 (77.6%)</td>
<td>NS (0.06)</td>
</tr>
<tr>
<td>-----------------------</td>
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</tr>
<tr>
<td><strong>NOSPECS scale</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>35 (72.9%)</td>
<td>28 (48.3%)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3 (6.3%)</td>
<td>19 (32.8%)</td>
<td>0.001</td>
</tr>
<tr>
<td>3</td>
<td>10 (20.8%)</td>
<td>6 (10.3%)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>5 (8.6%)</td>
<td></td>
</tr>
<tr>
<td><strong>Exophthalmos, mm</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right eye</td>
<td>17.3 (±0.4)</td>
<td>19.9 (±0.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Left eye</td>
<td>17.0 (±0.3)</td>
<td>20.2 (±0.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Max. exophthalmos</td>
<td>18.1 (±0.3)</td>
<td>20.7 (±0.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Max. Retraction, mm</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>14 (29.2%)</td>
<td>6 (10.3%)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>31 (64.6%)</td>
<td>36 (62.1%)</td>
<td>0.003</td>
</tr>
<tr>
<td>2</td>
<td>3 (6.3%)</td>
<td>16 (27.6%)</td>
<td></td>
</tr>
<tr>
<td><strong>CAS, points</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>25 (52.1%)</td>
<td>14 (24.1%)</td>
<td>0.003</td>
</tr>
<tr>
<td>3</td>
<td>23 (47.9%)</td>
<td>44 (75.9%)</td>
<td></td>
</tr>
<tr>
<td><strong>Incorrect eye mobility, mm</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right eye</td>
<td>3 (6.3%)</td>
<td>17 (29.3%)</td>
<td>0.003</td>
</tr>
<tr>
<td>Left eye</td>
<td>3 (6.3%)</td>
<td>17 (29.3%)</td>
<td>0.003</td>
</tr>
<tr>
<td>Any eye</td>
<td>3 (6.3%)</td>
<td>17 (29.3%)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Abbreviations: CAS, clinical activity score; fT3, free triiodothyronine; fT4, free thyroxine; Tg-Abs, autoantibodies against thyroglobulin; TPO-Abs, autoantibodies thyroperoxidase; TSH,
thyroid stimulating hormone; TSHR-Abs, autoantibodies against TSH receptor
Table 2 Serum levels of thyroid parameters, urine level of cotinine and thyroid volume in non-smoking and smoking patients with mild Graves’ ophthalmopathy

<table>
<thead>
<tr>
<th>Group</th>
<th>TSH, U/ml</th>
<th>fT4, pmol/l</th>
<th>fT3, pmol/L</th>
<th>TPO-Abs, IU/ml</th>
<th>Tg-Abs, IU/ml</th>
<th>TSHR-Abs, IU/l</th>
<th>Cotinine, ng/ml</th>
<th>Thyroid volume, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>After 2 months</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smokers</td>
<td>0.56 ± 1.49</td>
<td>17.2 ± 3.7</td>
<td>6.5 ± 1.2</td>
<td>336.2 ± 223.4</td>
<td>276.5 ± 154.2</td>
<td>14.0 ± 7.6</td>
<td>2.6 ± 7.9</td>
<td>25.6 ± 4.6</td>
</tr>
<tr>
<td>Smokers</td>
<td>0.2 ± 0.9</td>
<td>20.0 ± 5.3</td>
<td>7.9 ± 3.7</td>
<td>399.0 ± 236.6</td>
<td>345.8 ± 193.4</td>
<td>23.5 ± 8.5</td>
<td>473.4 ± 362.6</td>
<td>27.9 ± 8.1</td>
</tr>
<tr>
<td><em>P value</em></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>&lt; 0.001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td><strong>After 6 months</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smokers</td>
<td>1.98 ± 2.58</td>
<td>22.3 ± 2.9</td>
<td>4.5 ± 0.8</td>
<td>312.9 ± 223.7</td>
<td>278.5 ± 154.0</td>
<td>12.0 ± 5.2</td>
<td>4.1 ± 13.3</td>
<td>23.2 ± 5.0</td>
</tr>
<tr>
<td>Smokers</td>
<td>2.1 ± 2.3</td>
<td>17.2 ± 3.0</td>
<td>5.3 ± 1.9</td>
<td>315.4 ± 193.0</td>
<td>271.5 ± 125.4</td>
<td>26.6 ± 8.9</td>
<td>599.5 ± 387.5</td>
<td>26.6 ± 7.4</td>
</tr>
<tr>
<td><em>P value</em></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>&lt; 0.001</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD.

Normal values in the laboratory are as follows: free T₄: 11.5 - 21.5 pmol/L; free T₃: 3.9 – 6.8 pmol/L; TSH: 0.27 - 4.2 µU/ml, TSHR-Abs, < 2 IU/L; Tg-Abs, < 115 IU/ml; and TPO-Abs, < 35 IU/ml. The concentration of cotinine in urine: non-smokers (< 5 ng/ml), passive smokers (from 5 to 50 ng/ml), and smokers (> 50 ng/ml). Thyroid volume was measured by ultrasonography (normal values range
from 18 to 25 ml).

Abbreviations: fT3, free triiodothyronine; fT4, free thyroxine; NS - not significant; Tg-Abs, autoantibodies against thyroglobuline; TPO-Abs - autoantibodies thyroperoxidase; TSH, thyroid stimulating hormone; TSHR-Abs - autoantibodies against TSH receptor

Figure 1  Regression standardized predicted value of TSHR-Abs in tobacco smokers patients. TSHR-Abs – autoantibodies to the thyrotropin receptor.
Figure 2  Distribution of CAS grade in non-smokers and smokers at baseline and at 6 months of follow-up ($P<0.001$).
Figure 3  Distribution of NOSPECS grade in non-smokers and smokers at baseline and at 6 months of follow-up ($P<0.001$).