

CLINICAL STUDY

Smoking is negatively associated with the presence of thyroglobulin autoantibody and to a lesser degree with thyroid peroxidase autoantibody in serum: a population study

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Abstract

Background: Autoimmune thyroid diseases are common and the prevalence of circulating thyroid antibodies (thyroid peroxidase antibody, TPO-Ab and thyroglobulin antibody, Tg-Ab) is high in the population. The knowledge of a possible association between lifestyle factors and circulating thyroid antibodies is limited.

Aim: To evaluate the correlation between smoking habits and the presence of circulating TPO-Ab and Tg-Ab.

Material and methods: In a cross-sectional comparative population study performed in two areas of Denmark with moderate and mild iodine deficiency, 4649 randomly selected subjects from the population in some predefined age groups between 18 and 65 years were examined. Blood tests were analysed for TPO-Ab and Tg-Ab using assays based on the RIA technique. The participants answered questionnaires, were clinically examined and blood and urine samples collected.

Results: Data were analysed in multivariate logistic regression models. There was a negative association between smoking and the presence of thyroid autoantibodies in serum. This association was observed for the presence of TPO-Ab and/or Tg-Ab, TPO-Ab (without respect to Tg-Ab status), Tg-Ab (without respect to TPO-Ab status) and both antibodies together. The association between smoking and thyroid autoantibodies was stronger for Tg-Ab than for TPO-Ab. There was no association between smoking and TPO-Ab measured alone or between smoking and TPO-Ab when Tg-Ab was included in the model as an explanatory variable.

Conclusion: Smoking was negatively associated with the presence of thyroid autoantibodies with the strongest association between smoking and Tg-Ab. The study design precludes any conclusions as to the cause of the negative association between smoking thyroid autoantibodies.

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Introduction

Like many other autoimmune diseases, autoimmune thyroiditis is thought to be multifactorial in origin with both genetic and environmental factors playing a role in disease development (1, 2).

There is a good correlation between the thyroid autoimmunity evaluated from lymphocytic infiltration in the thyroid and the presence of thyroid autoantibodies in serum (3, 4).

Several mechanisms have been suggested for the development of circulating thyroid peroxidase antibody (TPO-Ab) and thyroglobulin antibody (Tg-Ab) (5). Some mechanisms involve more general aberrations of the immune system, while others focus on abnormalities

in presentation or structure of individual antigens. We previously described a similar prevalence rate of TPO-Ab and Tg-Ab in the population (6) and suggested a common mechanism behind the generation of the two antibodies.

During the last few years, there has been a focus on a more general association between smoking and the immune system, and the incidence and severity of some autoimmune diseases seem to be influenced by tobacco smoking (7). A negative association between smoking and celiac disease and ulcerative colitis has been observed (8, 9), whereas an increased incidence of psoriasis and Crohn's disease has been found in smokers when compared with non-smokers (10, 11).

Thyroid abnormalities may be associated with smoking (12–16). In iodine-deficient populations, a positive

association between smoking and multinodular goitre and between smoking and the level of thyroglobulin has been reported (13, 17). The positive association between smoking and Graves' hyperthyroidism and Graves' orbitopathy has been described unambiguously (18, 19). Subclinical hypothyroidism seems to occur less commonly in smokers (13, 20), whereas results from the studies of overt autoimmune hypothyroidism are inconsistent (19). Smoking has also been reported to be a risk factor for the development of *post-partum* thyroid dysfunction (21). Knowledge of a possible association between lifestyle factors including smoking and circulating thyroid antibodies (TPO-Ab and Tg-Ab) in the population is limited (22).

The aim of the present study was to evaluate the correlation between smoking habits and the presence of circulating TPO-Ab and Tg-Ab in the population.

Subjects and methods

Study population

This study is part of The Danish Investigation of Iodine Intake and Thyroid Diseases (DanThyr), which is the official clinical monitoring of the Danish iodine fortification programme. The present study took place from March 1997 to June 1998 before iodine fortification of salt was implemented in Denmark.

A sample of females within the following age groups, young adults (18–22 years), mid-gestational (25–30 years), premenopausal (40–45 years) and post-menopausal (60–65 years) and males aged 60–65 years living in either Aalborg (Northern Jutland) or Copenhagen, was drawn from the Civil Registration System. The sample contained 40 233 subjects who were given random numbers within each group using computer software and invited to the study examination in the order of the given random numbers. The number of subjects invited in each group was adjusted throughout the study period to obtain uniform numbers of participants in each group. Out of 9274 subjects invited, 4649 (50.1%) participated in the examination.

The two sub-cohorts had moderate and mild iodine deficiency respectively reflected by median iodine concentrations in spot urine of 45 µg/l (Aalborg) and 61 µg/l (Copenhagen) when the subjects taking iodine supplements were excluded (53 and 68 µg/l in all the subjects) (23). The cohort has been described in detail previously (24).

Participants previously treated for thyroid disease ($n=224$), and women who had been pregnant within the past 12 months ($n=141$) were excluded from this study. The participants with missing measurements (thyroid antibodies, $n=98$; serum thyroid-stimulating hormone (TSH), $n=4$; urinary iodine, $n=45$ or information on smoking habits, $n=6$ or alcohol consumption, $n=6$) were also excluded leaving 4125

for the analysis (females aged 18–22 years, $n=884$; 25–30 years, $n=788$; 40–45 years, $n=847$; 60–65 years, $n=717$ and males aged 60–65 years, $n=889$).

Data collection

The participants answered questionnaires concerning previous treatment for thyroid disease and lifestyle factors. They were asked about present or previous smoking, daily or occasional smoking, type of smoking (cigarettes, cheroots, cigars or pipe tobacco), amount of tobacco consumed, years of smoking and years since cessation of smoking. A clinical examination and an interview were conducted by a medical doctor. Blood and urine samples were collected.

Laboratory procedures

Serum TPO-Ab and Tg-Ab were both measured by RIA (DYNO test anti-TPO and DYNO test anti-Tg; BRAHMS Diagnostica, Berlin, Germany). The assays and evaluation of cut-off values have been described in detail previously (11). The following detection limits were used: TPO-Ab >30 U/l and Tg-Ab >20 U/l, both corresponding to the functional sensitivity given by the manufacturer.

The iodine content in spot urine was measured in duplicate by Ce/As method after alkaline ashing (25), as described previously (26). The analytical sensitivity of the assay was 2 µg/l.

Definition of data and statistical analysis

Most of the smokers smoked only cigarettes (33.1% of participants), 1% were smokers of cheroots, 0.2% cigars and 2.6% smoked pipe tobacco. The different types of preferred smoking were combined in the analyses; thus, cigarettes were regarded as containing 1 g tobacco each, cheroots 3 g and cigars 5 g. The smoking habit of each participant was classified in two separate ways: 1) daily smoker, occasional smoker or non-smoker and 2) never smoker (never daily use tobacco), ex-smoker (previous daily use of tobacco), moderate smoker (1–19 g/day) or heavy smoker (≥ 20 g/day). The classification of smoking habits was based on questionnaires only. Alcohol consumption was classified in two classes according to the recommendations of the National Board of Health in Denmark (low and moderate alcohol intake (0–14 drinks/week for females and 0–21 drinks/week for males) and high alcohol intake (>14 drinks/week for females and >21 drinks/day for males)).

The data analyses were performed with SPSS software (version 13.0 SPSS software, Inc., Chicago, Illinois, USA). The χ^2 -test was used to compare the number of subjects in different groups. In some of the analyses, the group of occasional smokers ($n=194$) was excluded.

The association between smoking and thyroid autoantibodies (TPO-Ab and Tg-Ab) was analysed in logistic regression models for dichotomous variables. Data were generally analysed in multivariate models allowing adjustment for age and sex group, region of inhabitancy, iodine excretion, alcohol consumption and familial occurrence of thyroid disease.

There was a difference in the frequency of smoking between regions and between the five age and sex groups. Interactions between smoking and region and between smoking and age and sex group were tested in the models to describe a possible difference in the impact of smoking on thyroid autoantibodies between regions and age and sex groups. No significant interaction was found.

The study was approved by the regional Ethics Committees in Northern Jutland and Copenhagen. All the participants gave written informed consent.

Results

Overall, thyroid autoantibodies (TPO-Ab and/or Tg-Ab) were more common in non-smokers when compared with daily smokers (21.4% vs 15.4%, $P < 0.001$; Table 1). The prevalence rate of TPO-Ab and/or Tg-Ab was equal in occasional smokers compared with daily smokers (15.4% vs 15.5%, $P = n.s.$). The distribution of different combinations of TPO-Ab and Tg-Ab in subgroups of the participants and the prevalence rate of daily smoking are shown in Table 2.

As a first step, the association between thyroid autoantibodies and smoking was analysed in multivariate logistic regression models with smoking divided into three subclasses: daily, occasional and non-smokers. There was a negative association between thyroid autoantibodies (TPO-Ab and/or Tg-Ab) and daily smoking (daily smoking versus non-smoking: odds ratio (OR), 0.66 and 95% confidence intervals (CI), 0.55–0.79). A small and non-significant tendency of a lower risk for TPO-Ab and/or Tg-Ab in occasional

smokers when compared with non-smokers was present (OR, 0.82 and CI, 0.54–1.24). As the small group of occasional smokers ($n = 194$) was difficult to classify precisely, it was excluded from the following analyses.

To evaluate the impact of the amount of tobacco smoked, the data were analysed with smoking divided into four subclasses: heavy, moderate, ex- and never smokers (Fig. 1). In both heavy and moderate smokers, the risk for having TPO-Ab and/or Tg-Ab, Tg-Ab (without respect to TPO-Ab) or both antibodies measured together was lower when compared with never smokers. There was no statistical difference in the risk between moderate and heavy smokers (moderate versus heavy, TPO-Ab and/or Tg-Ab, OR (CI), 1.09 (0.81–1.47); TPO-Ab, 1.04 (0.74–1.44); Tg-Ab, 1.26 (0.86–1.86) and both antibodies, 1.23 (0.75–2.02)). Neither were there statistical differences in the risks between ex- and never smokers for having different combinations of thyroid autoantibodies (Fig. 1).

For the next analyses, the definition of smoking was simplified and divided into smoking (heavy and moderate smokers) and non-smoking (ex- and never smokers). The associations between smoking in two classes and different combinations of thyroid antibodies are shown in Fig. 2. There was no association between smoking and TPO-Ab measured alone, but a weak negative association between smoking and TPO-Ab (without respect to Tg-Ab status) was present. The most pronounced association was found between smoking and Tg-Ab (without respect to TPO-Ab status) and Tg-Ab measured alone.

As expected, there was a clear association between the presence of TPO-Ab and Tg-Ab in serum ($P < 0.001$). The inclusion of TPO-Ab as an explanatory variable in the logistic regression model with Tg-Ab as dependent variable did not influence the association between smoking and Tg-Ab. When Tg-Ab as explanatory variable was included in the model for TPO-Ab, the weak association between TPO-Ab and smoking disappeared.

Table 1 Prevalence rate (%) of thyroid peroxidase antibody (TPO-Ab) and thyroglobulin antibody (Tg-Ab) in participants with different smoking habits.

Smoking subclasses	TPO-Ab and/or Tg-Ab	TPO-Ab ^a	Tg-Ab ^b	TPO-Ab and Tg-Ab	TPO-Ab only	Tg-Ab only
1) Daily smoker, occasional smoker or non-smoker						
Daily ($n = 1542$)	15.4	11.7	8.6	5.0	6.7	3.6
Occasional ($n = 194$)	15.5	8.2	11.9	4.6	3.6	7.2
Non-smoker ($n = 2389$)	21.4	14.4	15.9	9.0	5.5	6.9
2) Never smoker, ex-smoker, moderate smoker or heavy smoker						
Heavy ($n = 562$)	14.6	11.6	7.5	4.4	7.1	3.0
Moderate ($n = 980$)	15.8	11.8	9.3	5.3	6.5	4.0
Ex-smoker ($n = 793$)	20.8	15.1	14.4	8.7	6.4	5.7
Never smoker ($n = 1596$)	21.7	14.1	16.7	9.1	5.0	7.6

Prevalence rate in percentage of different combinations of detectable thyroid antibodies according to smoking habits in participants with no previously treated thyroid disease or pregnancy within 12 months. Classification of smoking habits: 1) daily smoker, occasional smoker or non-smoker; 2) never smoker, ex-smoker, moderate smoker or heavy smoker. In 2, occasional smokers have been excluded.

^aWithout respect to Tg-Ab status.

^bWithout respect to TPO-Ab status.

Table 2 Prevalence rate (%) of smoking and thyroid peroxidase antibody (TPO-Ab) and thyroglobulin antibody (Tg-Ab) in the cohort stratified according to age and sex.

Gender and age (years)	Number	Smoking	TPO-Ab and/or Tg-Ab	TPO-Ab ^a	Tg-Ab ^b	TPO-Ab alone	Tg-Ab alone	TPO-Ab and Tg-Ab
Female 18–22	806	33.7	12.0	7.3	8.8	3.2	4.7	4.1
Female 25–30	728	38.3	19.4	12.9	14.8	4.5	6.5	8.4
Female 40–45	823	47.5	24.9	19.3	15.6	9.4	5.6	10.0
Female 60–65	709	33.7	29.5	21.6	19.9	9.6	7.9	12.0
Male 60–65	865	41.8	11.1	7.1	7.5	3.6	4.0	3.5
Total	3931	39.3	19.0	13.4	13.1	6.0	5.6	7.4

Prevalence rate in percentage of smoking and detectable thyroid antibodies in the participants with no previously treated thyroid disease or pregnancy within 12 months. Occasional smokers and cases with missing blood or urine test results have been excluded. Prevalence rate in percentage of different combinations of detectable thyroid antibodies according to smoking habits in participants with no previously treated thyroid disease or pregnancy within 12 months. Classification of smoking habits: 1) daily smoker, occasional smoker or non-smoker and 2) never smoker, ex-smoker, moderate smoker or heavy smoker. In 2, occasional smokers have been excluded.

^aWithout respect to Tg-Ab status.

^bWithout respect to TPO-Ab status.

Finally, we studied whether the negative association between smoking and the presence of thyroid autoantibodies depended on the level of serum TSH. As illustrated in Fig. 3, thyroid antibodies were much more common when TSH was elevated, but no difference in the effect of smoking was apparent. This was confirmed in logistic regression models.

Discussion

In this random sample of 4125 subjects from the population, we found a significant negative association between smoking and the presence of thyroid autoantibodies. The association was most pronounced for Tg-Ab and was independent of TPO-Ab. There was only a weak association between smoking and TPO-Ab, and one might question whether that association was due to the

higher prevalence of Tg-Ab in TPO-Ab-positive than TPO-Ab-negative subjects.

It is difficult to compare results from different epidemiological studies on thyroid abnormalities as different biochemical and epidemiological methods have been applied. This includes differences in the sensitivity and cut-off values of the assays and whether the study has been population-based or patient-based. The Third National Health and Nutrition Examination Survey (NHANES III) study is the only study prior to the present looking at the possible association between smoking and the prevalence of circulating autoantibodies in the population (20). In the NHANES III study, TPO-Ab and Tg-Ab were measured in 15 592 subjects representative of the US population. In accordance with the present study, there was a lower proportion of smokers with TPO-Ab and/or Tg-Ab compared with non-smokers when adjusted for possible confounders. The association persisted between smoking and TPO-Ab (independent of

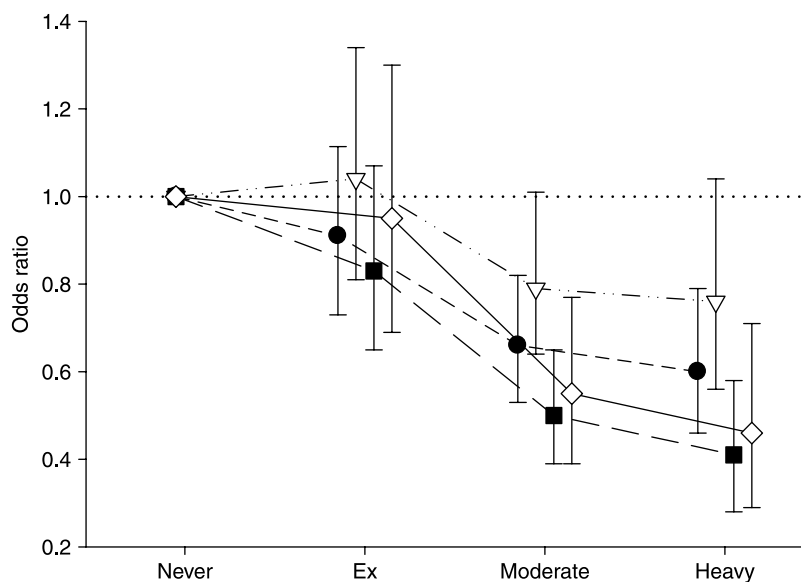


Figure 1 The association between tobacco smoking and the presence of thyroid autoantibodies (TPO-Ab and/or Tg-Ab (●), TPO-Ab (▽), Tg-Ab (■) and TPO-Ab and Tg-Ab (◇)) in 3931 randomly selected subjects from the Danish population with no previous thyroid disease and pregnancy within 1 year. Occasional smokers have been excluded. Data were analysed in multivariate logistic regression models adjusting for age and sex group, region of inhabitancy, iodine excretion, alcohol consumption and familial occurrence of thyroid disease. Vertical bars represent 95% confidence intervals (CI). Odds ratio values are significantly different from the reference (never smoking) when the CI does not include 1.

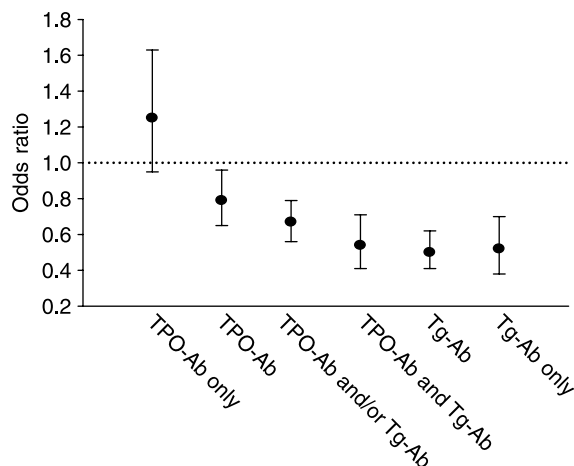


Figure 2 The association between tobacco smoking and presence of different combinations of thyroid antibodies in 3931 randomly selected Danes with no previous treated thyroid disease or pregnancy within 1 year prior to the study. Occasional smokers have been excluded. Data were analysed in multivariate regression models. Vertical bars represent 95% CI for the OR. There was a negative association between thyroid antibodies and smoking with the strongest association when Tg-Ab was present.

Tg-Ab status) and Tg-Ab (independent of TPO-Ab status). The association between smoking and TPO-Ab or Tg-Ab present alone was not described. In a study by Strieder *et al.* (27) including 803 first- or second-degree female relatives of patients with documented autoimmune thyroid disease, there was a higher proportion of non-smokers than smokers in the group of participants with positive TPO-Ab. No information on Tg-Ab was given. Goh *et al.* found significantly lower levels of TPO-Ab in smokers compared with non-smokers with eye complications due to debuting GD (28). No information on Tg-Ab was given.

The major cause for spontaneous overt hypothyroidism is an autoimmune destruction of the thyroid gland (29). Nearly all patients with newly diagnosed overt hypothyroidism are TPO-Ab- and/or Tg-Ab-positive

with TPO-Ab being more common than Tg-Ab (30–32). Only a modest correlation between the two antibodies has been found and it has been suggested that it is more or less random which antibody is produced (30). Seronegative individuals with spontaneous overt hypothyroidism do exist, but in some of these patients intrathyroidal production of TPO-Ab and Tg-Ab can be demonstrated *in vitro* (10).

A number of studies have shown a lower incidence of subclinical hypothyroidism in smokers compared with non-smokers (13, 20), whereas a meta-analysis reported a small and non-significant negative association between smoking and overt hypothyroidism (19).

The mechanisms behind the association of smoking and different thyroid abnormalities are not known. It has been speculated that the effect of smoking on the development of goitre and increased thyroglobulin in serum might be mediated via generation in smokers of thiocyanate from detoxification of cyanide in tobacco smoke (13). Thiocyanate is a competitive inhibitor of the sodium-iodide symporter responsible for the accumulation in the thyroid gland of iodine and thereby smoking might aggravate the goitrogenic effect of iodine deficiency (33). It might be speculated that the same mechanism is behind the lower prevalence of subclinical hypothyroidism in smokers. This is supported by several epidemiological studies finding a relatively low prevalence of subclinical hypothyroidism in areas with moderate and mild iodine deficiency (34–36). Another possibility could be that the lower prevalence of subclinical hypothyroidism in smokers is secondary to the lower prevalence of thyroid autoantibodies. The present study does not directly support this hypothesis as one would expect a stronger association between smoking and TPO-Ab, which has been shown in several studies to correlate better to impaired thyroid function than Tg-Ab (31, 37).

We have no ready explanations for the reduced risk of thyroid autoimmunity in smokers or for the stronger negative association between smoking and Tg-Ab when

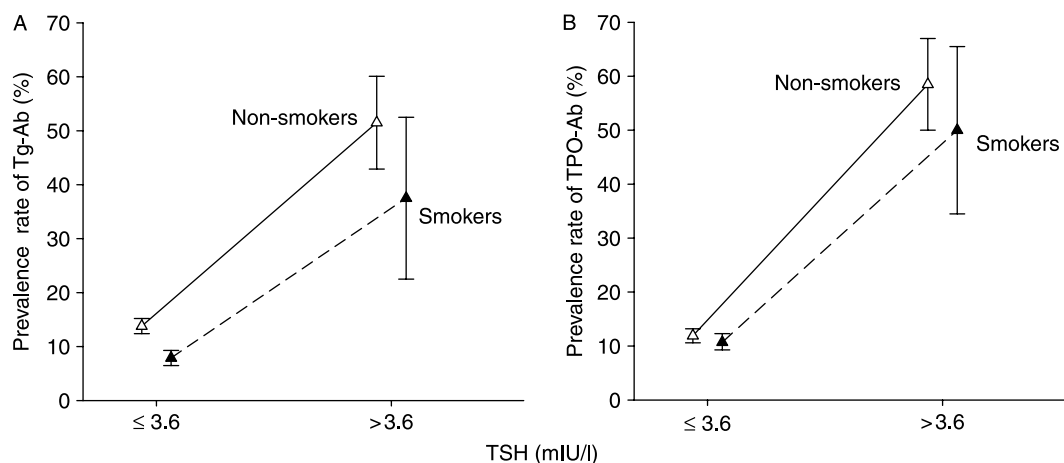


Figure 3 Prevalence rate in percentage of Tg-Ab (A) and TPO-Ab (B) in smokers (▲) and non-smokers (Δ) with different levels of TSH.

compared with TPO-Ab. It could be speculated that the lower prevalence rate of subclinical hypothyroidism and thereby a lower stimulation of the thyroid gland might reduce thyroid autoimmunity. This phenomenon has also been suggested as a possible explanation for the reduced risk of thyroid cancer in smokers (38). However, we found no evidence that the effect of smoking depended on serum TSH. Other possibilities are direct effects of smoke on the immune system via among others the nicotinic anti-inflammatory pathway (7), via increased cortisol release (39) or a direct interference with iodide transport and organification. Finally, it cannot be excluded that the reduced risk of thyroid autoantibodies in smokers could be caused by an unknown lifestyle factor associated with smoking rather than smoking itself.

In conclusion, we found that smoking was negatively associated with the presence of thyroid autoantibodies with the strongest association between smoking and Tg-Ab. The study design precludes any conclusions as to the cause of the negative association between smoking and development of thyroid autoantibodies.

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