

Thyroid autoantibodies in women with and without thyroid disorders in an iodine-replete area

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الأضداد الذاتية في المصابات وغير المصابات باضطرابات الدرقية في منطقة يعوّض فيها اليود
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الخلاصة: كان الهدف من هذه الدراسة مقارنة معدل انتشار الأضداد الذاتية الإيجابية في المصابات وغير المصابات باضطرابات الدرقية، في إحدى المناطق التي يعوّض فيها اليود في جمهورية إيران الإسلامية. وقد شملت الدراسة 930 مريضة (منهن 286 مصابة بقصور الدرقية و140 مصابة بفرط نشاط الدرقية و272 مصابة بدُراق goiter بسيط)، إلى جانب 232 من الأصحاء. وأجريت قياسات للثيروكسين T4 وثلاثي يودوثيرونين T3 والهرمون المنبه للدرقية وكذلك لأضداد الدرقية، واكتشفت إيجابية الأضداد الذاتية لدى 75.5% من المصابات بقصور الدرقية ولدى 73.6% من المصابات بفرط نشاط الدرقية، ولدى 48.9% من المصابات بدُراق بسيط ولدى 35.8% من مجموعة الشاهدات المعافيات ($P < 0.001$). مما يعني أنه قد يكون للمناعة الذاتية دور في إحداث الاضطرابات الدرقية الشائعة.

ABSTRACT To compare the prevalence of positive autoantibodies in patients with thyroid disorders and healthy subjects in an iodine-replete area of the Islamic Republic of Iran, we studied 930 women in a clinic-based study: 698 patients (286 hypothyroid, 140 hyperthyroid, 272 with simple goitre) and 232 healthy women. Serum thyroxine (T₄), triiodothyronine (T₃), thyroid stimulating hormone, and anti-thyroid antibodies were measured. Positive autoantibodies were detected in 75.5% of patients with hypothyroidism, 73.6% of those with hyperthyroidism, 48.9% of those with simple goitre and 35.8% of the control group ($P < 0.001$). Autoimmunity may have a role in the genesis of common thyroid disorders.

Autoanticorps thyroïdiens chez les femmes avec et sans troubles thyroïdiens dans une région riche en iode

RÉSUMÉ Afin de comparer la prévalence des autoanticorps positifs chez les patients atteints de troubles thyroïdiens et chez des sujets en bonne santé dans une région riche en iode de la République islamique d'Iran, nous avons effectué une étude clinique sur 930 femmes : 698 patientes (286 hypothyroïdiennes, 140 hyperthyroïdiennes et 272 présentant un goitre simple) et 232 femmes en bonne santé. La thyroxine (T₄), la triiodothyronine (T₃), la thyroïdostimuline, ou TSH (pour *thyroid stimulating hormone*) sériques et les anticorps antithyroïdiens ont été mesurés. Des autoanticorps positifs ont été détectés chez 75,5 % des patientes atteintes d'hypothyroïdie, 73,6 % de celles atteintes d'hyperthyroïdie, 48,9 % de celles présentant un goitre simple et 35,8 % dans le groupe témoin ($p < 0,001$). L'auto-immunité peut avoir un rôle dans l'apparition des troubles thyroïdiens courants.

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Introduction

Iodine deficiency disorders were prevalent in the Islamic Republic of Iran prior to 1989 when the national salt iodization programme was initiated [1]. A law for the mandatory production of iodized salt for households was passed in 1994. Since then, the country has achieved a sustainable control programme for iodine deficiency [1,2].

Isfahan is a centrally located city in the Islamic Republic of Iran with a population of about 1 000 000. In a study conducted on 3000 Isfahan students after 8 years of iodine repletion, 94% had sufficient iodine consumption [2]. Despite this, however, the prevalence of goitre was still high (63%) [2]. So the question of iodine-induced autoimmune thyroid disease was raised [2,3]. A randomized, double-blind, placebo-controlled trial reported the transient occurrence of high levels of thyroid antibodies in 6 of 31 patients with endemic goitre treated daily for 6 months with a supraphysiologic dose of 500 µg/day iodine. Therefore, it was concluded that iodine may induce thyroid autoimmunity in patients with endemic goitre [3].

The clinical implications of thyroid autoimmunity following correction of iodine deficiency are, however, still unclear [4]. In a survey done in Shahriar, Islamic Republic of Iran, positive antithyroid antibodies were observed in 3.1% of the population before iodine supplementation [5]. In a clinic-based study in Shiraz in 1981, antithyroid antibodies were found in 2% of healthy volunteers, 3% of patients with simple goitre, 41% of those with hyperthyroidism and 67% of those with hypothyroidism [6].

As high titres of antithyroid antibodies are strongly suggestive of thyroid autoimmunity [7], we investigated the frequency of thyroid autoantibodies 12–14 years after iodine repletion in Isfahan by measuring

these antibodies in patients with thyroid disorders and in a healthy control group.

Methods

Participants

Women were chosen for this study as it is known they have a higher prevalence of thyroid autoimmune markers and goitre compared with men [7]. Isfahan Endocrine and Metabolism Research Centre is a referral centre for endocrine disorders. All 990 women in Isfahan who met the inclusion criteria and who were referred to the outpatient endocrine clinic during the period April 2001–March 2003 were invited to participate in the study; 930 agreed and gave voluntary informed consent. Those who were pregnant or on medications which would affect thyroid function tests (e.g. oral contraceptive pills, levothyroxine or thionamides) were excluded.

Participants were classified into 4 groups, hypothyroidism, hyperthyroidism, simple goitre, and a healthy control group (no goitre and normal thyroid function). The women in the control group were those with certain nonspecific symptoms who had been referred with suspected thyroid disorder, but after history-taking and physical examination, were diagnosed as completely healthy and their thyroid function tests were normal.

Initial clinical evaluation included taking demographic data, noting symptoms of hypo- and hyperthyroidism, physical examination for signs of thyroid dysfunction and presence or absence of goitre. A thyroid gland whose lateral lobes had a volume greater than the terminal phalanges of the thumbs of the person examined was considered goitrous [4].

The protocol was approved by the Institutional Review Board and Medical Ethics

Committee of Isfahan University of Medical Sciences.

Laboratory measurements

Commercially-available kits were used to measure serum concentrations of thyroxine (T4) and triiodothyronine (T3) by radioimmunoassay using a gamma counter (Berthold Technologies GmbH, Germany), and thyroid-stimulating hormone (TSH) by immunoradiometric assay (Kavoshyar, Tehran). The normal (reference) ranges were: T4: 5.50–11.03 µg/dL; T3: 80–200 ng/dL; TSH: 0.36–3.68 mIU/L. Those with TSH values > 3.68 mIU/L were diagnosed as having hypothyroidism. Those with T4 values > 11.03 µg/dL and/or T3 > 200 ng/dL and TSH < 0.36 mIU/L were diagnosed as having hyperthyroidism. Simple goitre was defined as presence of goitre with normal thyroid function (T4, T3 and TSH within normal range).

Serum antithyroid peroxidase (anti-TPO/TPOAb) and antithyroglobulin (anti-Tg/TgAb) were measured by enzyme-linked immunosorbent assay standardized against the WHO reference preparation 66/387 for anti-TPO and 65/93 for anti-Tg antibodies (DRG Instruments GmbH, Germany). The intra-assay variation was 5.3% for TPOAb and 3.3% for TgAb. The inter-assay vari-

ation for was 2.0% TPOAb and 4.0% for TgAb.

Serum TPOAb < 50 IU/mL was defined as normal (negative) and > 75IU/mL as elevated (positive). Serum TgAb < 100 IU/mL was defined as normal (negative) and > 150 IU/mL as elevated (positive).

Statistical analysis

Data with normal distribution were shown as mean and standard deviation (SD), unless stated otherwise. We compared variables using analysis of variance, chi-squared and *t*-tests as appropriate using *SPSS*, version 12, and *Epi-Info*, version 6.04. *P* < 0.05 was considered statistically significant.

Results of variables not distributed normally are shown as box plots with 25th, 50th and 75th percentiles.

Results

Mean age in hypothyroid, hyperthyroid, simple goitre and control groups was 27.0 (SD 12.1), 30.1 (SD 13.5), 28.2 (SD 13.9) and 27.3 (SD 13.1) years, respectively. There was no difference in age between groups.

Mean levels for serum T4, T3 and TSH are shown in Table 1. As expected, mean T4

Table 1 Mean thyroid hormone levels in women with thyroid disorders and in a control group in Isfahan

Variable	Patients						Controls	
	Hypothyroidism (n = 286)		Hyperthyroidism (n = 140)		Simple goitre (n = 272)		(n = 232)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
T4 (µg/dL)	6.9*	4.2	16.5*	6.9	7.9	1.4	8.1	1.4
T3 (ng/dL)	112.7*	47.7	229.8*	151.9	131.1	24.9	128.4	26.1
TSH (mIU/mL)	23.5*	32.3	0.1*	0.1	1.5	0.8	1.6	0.8

**P* value < 0.05.

SD = standard deviation.

T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid stimulating hormone.

and T3 levels were statistically significantly lower in hypothyroid women and statistically significantly higher in the hyperthyroid group than in the control group ($P < 0.05$). Mean TSH level was higher in hypothyroid patients than in those who were euthyroid, and was suppressed in those with hyperthyroidism.

Medians for TPOAb and TgAb were higher in the hypo- and hyperthyroidism and simple goitre groups than in the control group. The distribution of autoantibody concentration is shown as box plots (Figures 1 and 2). Median (range) for TPOAb in these groups was 200 (< 1–8361), 200 (5–5200), 30 (< 1–2975) and 20 (< 1–2336) IU/mL, respectively and for TgAb was 197

(1–9317), 165 (4–5500), 67 (< 1–4219) and 27 (< 1–4219) IU/ mL, respectively.

Frequency of positive antithyroid antibodies was statistically significantly higher in the hypo- and hyperthyroidism groups than in the control group (Table 2). This was also higher in the simple goitre group, but not statistically significantly. Frequency of positive TPOAb and/or TgAb was similar in the hypo- and hyperthyroidism groups. Frequency of positive autoantibodies did not increase with age in patients with thyroid disorders or in the control group.

Negative thyroid antibody levels were lower in the hypothyroidism ($P < 0.001$), hyperthyroidism ($P < 0.001$) and simple goitre ($P < 0.05$) groups than in the control

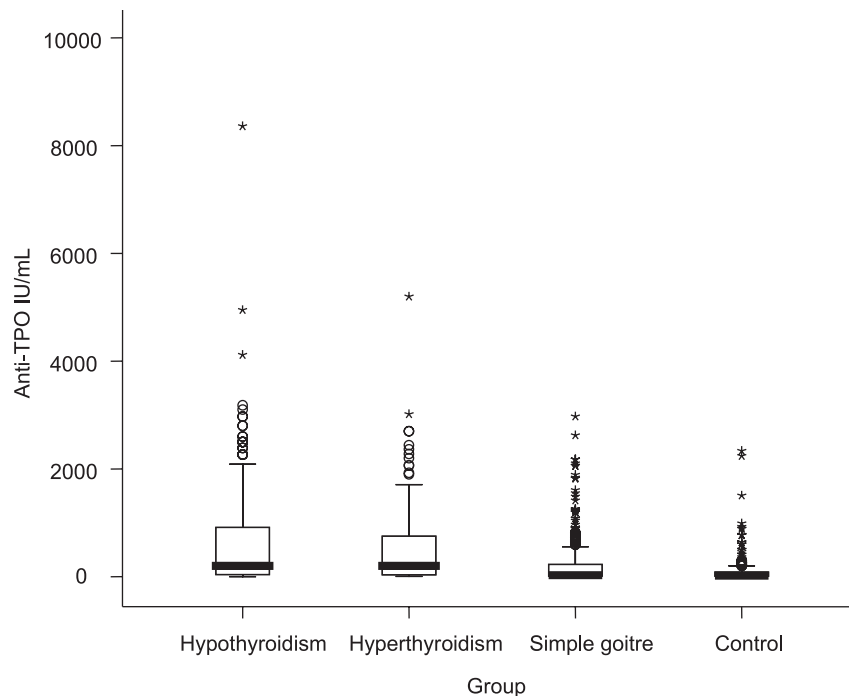


Figure 1 Serum antithyroid peroxidase (TPO) antibody levels in women with thyroid disorders and in a control group in Isfahan (box plot, SPSS 12)

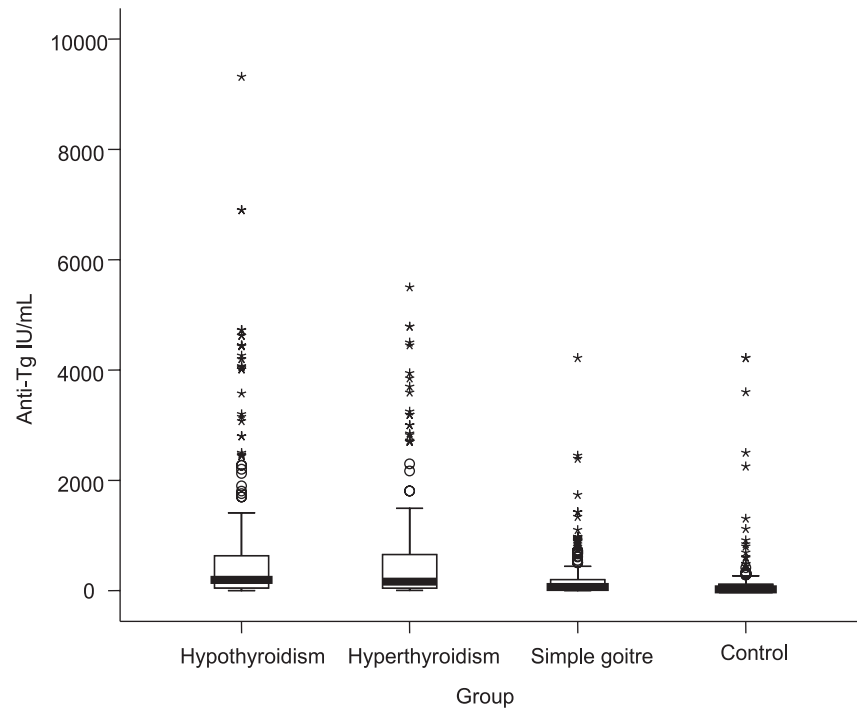


Figure 2 Antithyroglobulin (Tg) antibody levels in women with thyroid disorders and in a control group in Isfahan (box plot, SPSS 12)

group (Table 2). The difference was statistically significant except for TPOAb in the simple goitre group.

Only-positive TPOAb was detected in 22.0% of those with hypothyroidism, 20.7% of those with hyperthyroidism, 16.5% of those with simple goitre and 14.7% of the control group. For only-positive TgAb, corresponding values were 9.4%, 11.4%, 11.8% and 9.1%, respectively. Only-positive TPOAb was more frequent than only-positive TgAb in the hypo- ($P < 0.001$) and hyperthyroidism ($P < 0.05$) groups.

Of those women with positive TPOAb, 66.7% in the hypothyroidism group, 65.9% in the hyperthyroidism group, 55.4% in the simple goitre group and 42.4% of the control group also had positive TgAb; 82.4%,

77.8%, 63.6% and 54.3% of those with positive TgAb in these groups also had positive TPOAb. The difference was not statistically significant except in the hypothyroid group ($P < 0.001$).

Discussion

In this study, thyroid autoantibodies were measured in women referred to an endocrine clinic in Isfahan, an iodine-replete area. Serum concentrations of both TPOAb and TgAb were much higher in all 3 groups with thyroid disorders in comparison with the healthy control group.

Prevalence of positive antithyroid antibodies (TPOAb and/or TgAb) was 75.5%,

Table 2 Antithyroid antibodies in patients with thyroid disorders and in a control group in Isfahan

Antithyroid antibody	Patients						Controls (n = 232)	
	Hypothyroidism (n = 286)		Hyperthyroidism (n = 140)		Simple goitre (n = 272)		No.	%
	No.	%	No.	%	No.	%		
<i>Positive^a</i>								
TPOAb	189*	66.1	87*	62.1	101	37.1	62	26.7
TgAb	153*	53.5	72*	51.4	88	32.4	46	19.8
TPOAb + TgAb	126*	44.1	56*	40.0	56	20.6	25	10.8
TPOAb or TgAb	216*	75.5	103*	73.6	133*	48.9	83	35.6
<i>Negative^b</i>								
TPOAb	76*	26.6	47*	33.6	152	55.9	154	66.4
TgAb	107*	37.4	55*	39.3	166*	61.0	164	70.7
TPOAb + TgAb	45*	15.7	29*	20.7	111*	40.8	128	55.2

TPOAb = serum antithyroid peroxidase; TgAb = serum antithyroglobulin.

*P value < 0.05.

^aPositive TPOAb > 75 IU/mL; positive TgAb > 150 IU/mL.

^bNegative TPOAb < 50 IU/mL; negative TgAb < 100 IU/mL.

73.6%, 48.9% in patients with hypothyroidism, hyperthyroidism and simple goitre. It was much higher than in the control group (35.8%), although this was also high. In a clinic-based study in Shiraz, Islamic Republic of Iran, during the iodine deficient period (1981), corresponding values were 67%, 41%, 3% and 2% (control group) [6]. In our study in Isfahan, after iodine supplementation, the prevalence of positive antithyroid antibodies was much higher than in Shiraz in all groups. The concentration of these antibodies had not been measured in Isfahan before iodine supplementation so we could not compare our data with previous findings for the same population. This is one of the limitations of this study.

In a Greek study, carried out 7 years after iodine repletion, a 3-fold increase in the prevalence of autoimmune thyroiditis was observed among schoolchildren evidenced by TPOAb and/or TgAb levels [8]. In Iran, frequency of positive thyroid autoantibodies was 2% in Shiraz in a disease-free popula-

tion before iodine supplementation [6]. In our sample in Isfahan, after elimination of iodine deficiency, it was 35.6% in healthy subjects, 18 times higher after iodine repletion.

In Zimbabwe, an iodine-replete country, 39% of hyperthyroid patients had positive TPOAb or TgAb. None of these antibodies was positive in patients with simple goitre [9]. In Isfahan, it was 75.5% and 48.9% in hypothyroid and simple goitre patients. It seems that iodine prophylaxis-induced autoimmune thyroid disorders are more prevalent in Isfahan than Zimbabwe, even in goitre without thyroid dysfunction. These simple goitres may progress to hypo- and hyperthyroidism in future.

In this study from Isfahan, TPOAb was positive in 66.3%, 62.1% and 26.8% of women with hypo- and hyperthyroidism, simple goitre and control groups, respectively. In a recent study in Tehran on women with postpartum thyroiditis and a control group, positive anti-TPO was observed

in 61.5% and 19.0% respectively [10]. Prevalence of positive autoantibodies was similar in women with thyroid dysfunction in Tehran and Isfahan, but was higher in the Isfahan control group (Table 2).

In Pakistan, positive TPOAb was observed in 78.9% of patients with Graves' disease and in 13.3% of the control group; positive TgAb was observed in 57.9% in patients and 6.7% of the control group [11]. Prevalence of positive antibodies in those with thyroid disorders was similar to the findings in our study, but lower in the control group. While the prevalence of thyroid autoantibodies is generally higher in women, and all participants in our study were female [12–14], higher prevalence of antibodies in healthy Isfahani subjects compared with Pakistani people cannot be explained by patient selection as higher prevalence of positive antibodies has been observed in disease-free women in Tehran, too [10]. In comparison to reports from Zimbabwe, Pakistan and Tehran, prevalence of positive antibodies in the control group was higher in Isfahan [9–11]. It appears that Isfahani people are more susceptible to thyroid autoimmunity.

In Sri Lankan schoolgirls, the prevalence of TgAb has been markedly raised since iodine supplementation, ranging between 14.3% (11-year-old girls) and 69.7% (16-year-old girls), but the prevalence of TPOAb was $\leq 10\%$ in all age groups [14]. In our study, the prevalence of positive antibodies did not increase with age in any group. In Thailand, prevalence of anti-thyroid antibodies was higher in an elderly compared to a younger age group (14.69% versus 5.02%) [12]. Prevalence of thyroid antibodies in the population of the United States of America, National Health and Nutrition Examination Survey (NHANES

III) study, increased with age [13]. These 3 studies were population-based but ours was clinic-based. This might explain why we found no correlation between age and positive antithyroid autoantibodies.

In the NHANES III study and also in a study from Bangladesh, TgAb alone in the absence of TPOAb was not significantly associated with thyroid disease [13,15]. In Budapest, 57.9% of hyperthyroid patients who had positive anti-TPOAb were negative for anti-TgAb, whereas 9.4% of those with positive anti-TgAb were anti-TPOAb negative [16]. Corresponding values were 22.0% and 9.4% respectively in our study.

In recent study, only-positive TPOAb was more prevalent than only-positive TgAb in hypo- and hyperthyroidism, but the difference was not as great as in other studies [13,15,16]. However, the higher prevalence of TPOAb compared to TgAb and both combined, similar to the above studies, makes measurement of anti-Tg superfluous. Prevalence of negative anti-thyroid antibodies was much higher in the control group, and serum titres of these antibodies were significantly higher in those with thyroid disorders than in the control group in Isfahan, Islamic Republic of Iran.

Although the findings of the present study may suggest that iodine-induced autoimmune thyroid disease is increasing in the Islamic Republic of Iran since iodine repletion, a cohort population-based study is recommended to investigate this possibility. It appears that TPOAb measurement would be enough to survey thyroid autoimmunity.

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