

Increased risk of papillary thyroid cancer in Systemic Sclerosis associated with autoimmune thyroiditis.

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List of Abbreviations:

AbTg: anti-thyroglobulin antibody
AbTPO: anti-thyroperoxidase antibody
ERK: extracellular signal-regulated kinase
FNA: fine-needle aspiration
FT3: free triiodothyronine
FT4: free thyroxine
MEK: mitogen-activated protein kinase
PTC: papillary thyroid cancer
TC: Thyroid cancer
TSH: thyroid stimulating hormone

Abstract

Objectives: Patients with SSc have an increased risk of malignancy compared with the general population. Before now, no study has evaluated the risk of thyroid cancer in SSc patients.

Methods: We studied the prevalence of thyroid cancer in 327 unselected SSc patients in comparison with 2 population-based, gender- and age-matched control groups: 1) 654 subjects from an iodine deficient area; 2) 654 subjects from an iodine sufficient area. Thyroid status was assessed by measurement of circulating thyroid hormones and autoantibodies, thyroid ultrasonography and fine-needle aspiration cytology (when necessary).

Results: Circulating thyroid stimulating hormone, anti-thyroglobulin and anti-thyroperoxidase antibodies levels, and the prevalence of hypothyroidism were significantly higher in SSc patients ($P < 0.01$, for all). Six patients with papillary thyroid cancer (PTC) were detected among SSc patients, whereas only one case was observed both in control 1 and control 2 ($P = 0.007$, for both). In SSc all patients with thyroid cancer had evidence of thyroid autoimmunity vs. 40% of the other SSc patients ($P = 0.001$).

Conclusions: These data suggest a high prevalence of PTC in SSc patients, in particular in the presence of thyroid autoimmunity; a careful thyroid monitoring is opportune during the follow-up of these patients.

Introduction

Patients with SSc may have an increased risk of malignancy compared with the general population [1]. A wide array of cancers has been reported in scleroderma, although lung and breast cancers are thought to be the most common [2].

Papillary thyroid cancer (PTC) presents most commonly between 30-50 years of age, with a preponderance female/male=3:1. The radiation exposure is a risk factor of PTC [3].

The prevalence of PTC is higher where iodine intake is excessive, or in case of iodine prophylaxis [4].

A longitudinal follow-up study has shown a high incidence of new cases of hypothyroidism and thyroid dysfunction in female sclerodermic patients, suggesting that the ones who are at high risk (a borderline high [even if in the normal range] thyroid stimulating hormone (TSH) value, anti-thyroperoxidase antibody (AbTPO) positivity, and a hypoechoic and small thyroid) should have periodic thyroid function follow-up [5].

A few studies have reported sporadic cases of PTC in patients with SSc [6,7].

The present (cases/controls) study prospectively investigated prevalence and features of thyroid cancer (TC) in a large series of unselected patients with SSc in comparison with two matched sample from the general population with different iodine intake.

Methods

Subjects

SSc patients. Three hundred twenty-seven SSc patients consecutively referred to the Rheumatology Units of the University of Pisa and Modena (from 1995 to 2009) were recruited into the study. SSc was classified according to the American College of Rheumatology 1980 preliminary criteria [8]. Standardized criteria were followed for the evaluation of clinico-serological features, main visceral organ involvement and disease

activity [9]. In SSc patients disease duration ranged from 7 to 112 months; skin sclerosis was observed in all patients (diffuse 52% or limited 48%); visceral involvement included: peripheral vascular system, 94%; gastrointestinal system, 51%; lung, 64%; joint/tendons, 21%; heart, 35%; kidney, 9%. The prevalence of autoantibodies, evaluated according to standard methodologies [9], was: antinuclear 91% (with nucleolar pattern 24%), anticentromere 37%, anti-topoisomerase I (anti-Scl-70) 41%.

Controls. Since iodine intake differs within Tuscany, and reliable data on local iodine intake based on urinary iodine excretion are available, two different control groups were used.

1) *Controls from an iodine deficient area.* Among 3124 subjects randomly extracted from the general registry of North-West Tuscany and systematically screened for thyroid disorders, 2 individuals of same gender and similar age (± 5 years), for each SSc patient, were randomly selected and used as a population-based, gender- and age-matched control group (**Table 1**). The majority (87%) of these control subjects had resided in an iodine-deficient area for 20 years or more, which was considered a criterion of historical iodine deficiency.

2) *Controls from an iodine sufficient area.* Another control group was obtained choosing 2 individuals of same gender and similar age (± 5 years), for each SSc patient, who were randomly extracted from the background population of an area of iodine sufficiency (central Tuscany) who had been screened for thyroid disorders. Among these control subjects 19% had resided in an iodine-deficient area for 20 years or more (**Table 1**).

Control subjects with previous radiotherapy were excluded.

All patients and controls gave informed consent to the study, which was approved by the Institutional Ethics Committee.

All patients and controls underwent a complete clinical evaluation (**Table 1**) and were prospectively evaluated by TSH, free thyroxine (FT4), free triiodothyronine (FT3), anti-

thyroglobulin antibody (AbTg), AbTPO determination, and physical examination, thyroid ultrasonography, fine-needle aspiration (FNA) (if necessary).

Ultrasonography of the neck and FNA.

Neck ultrasonography and FNA were performed as previously reported [3]. FNA was performed in palpable nodules; nodules that were not palpable were examined by FNA only when clinical findings or the echographic pattern [10,11] suggested the opportunity of excluding malignancy (larger than 8 mm, solid hypoechoic appearance, and/or irregular or blurred margins, and/or microcalcifications) [12].

Laboratory evaluation.

Serum levels of TSH (DiaSorin, USA; reference range 0.3-3.6 mIU/L), FT3 and FT4 (AMERLEX-MAB FT3/FT4 Kit; Amersham, Little Chalfont, UK), AbTPO and AbTg (ICN Pharmaceuticals, USA; positivity was set at > 100 kIU/L) were evaluated.

Statistical analysis.

Data are expressed as mean \pm SD. For continuous variables, group differences were tested by analysis of variance; when this was significant at the $P \leq 0.05$ level, between-group comparisons were carried out by the Bonferroni-Dunn test. Group differences were tested for categorical variables, by Fisher's Exact test (two-tailed). Furthermore, Odds Ratio was used when at least 1 case of thyroid disorder was observed in controls. A P value < 0.05 was considered significant. We used StatView software, Version 5.0, SAS Institute Inc., SAS Campus Drive, Cary NC 27513, USA, for statistical analysis.

Results

The samples had similar distributions by gender, age (by selection criteria) and smoking habits (**Table 1**). A positive family history of thyroid disease was significantly more frequent in controls from the iodine-deficient area and in SSc patients than in controls from the iodine-sufficient area.

In SSc patients, serum TSH levels, AbTg and AbTPO titers were significantly higher, while FT3 and FT4 were significantly lower in comparison to the other groups (**Table 1**).

Hypothyroidism (defined as a TSH level > 4 mIU/L, in the presence of normal or low values of FT3 and/or FT4) was significantly more common in SSc patients than in the other groups.

The prevalence of subclinical and clinical hyperthyroidism (defined as a TSH level < 0.3 mIU/L, in the presence of normal or high values of FT3 and/or FT4) was not significantly higher in SSc patients (**Table 1**). Non-thyroidal illness syndrome (defined as low serum FT3, normal to low FT4, high reverse T3 [ranging from 1.08 – 2.46 nmol/L], and normal TSH) was observed in 3% of SSc patients.

The prevalence of thyroid nodules was higher in control subjects from the iodine-deficient area and in SSc patients than in the controls from the iodine-sufficient area (**Table 1**). FNA was performed in 14% of SSc patients (57 nodules in 46 patients were biopsied, 1.2 [mean] nodules biopsied [range 1-2] per patient receiving FNA; median nodule size was 19 mm), in 13% of iodine-deficient controls (108 nodules biopsied in 85 patients, 1.3 [mean] nodules biopsied [range 1-3] for each patient submitted to FNA; median nodule size, 21 mm) and 9% of iodine-sufficient controls (77 nodules biopsied in 59 patients, 1.3 [mean] nodules biopsied [range 1-2] per patient submitted to FNA; median nodule size, 18 mm).

Following standard criteria [13], the cytological samples were subdivided into classes: class 1, macrophages and colloid with no or rare follicular cells; class 2, benign nodule; class 3, indeterminate follicular lesion; class 4, suspect or frankly malignant. The distributions of cytological results in SSc, iodine-deficient and iodine-sufficient groups were: class 1 (7%,

8%, 8%, respectively); class 2 (82%, 85%, 86%, respectively); class 3 (4%, 7%, 8%, respectively). No significant differences in the distribution of class 1, 2 and 3 results among the three groups were observed (χ^2 , $P = 0.9$).

Four class 4 (7%), suspect or frankly malignant cytological results were observed in SSc patients. This class of results was only observed in the SSc group. Thyroidectomy was performed in all patients and controls with class 3 cytology; upon histological examination, a PTC was found in two SSc patients and one subject from both control groups. In the 4 SSc patients with class 4 cytology, post-thyroidectomy histological examination revealed PTC.

To sum up, six PTCs were detected in the SSc series, while only one case was observed in both controls (**Table 1**) ($P = 0.007$, by Fisher's Exact test (two-tailed) P -Value, for SSc vs. each control group). The size of nodules with cancer, at histological examination, varied from 8 to 43 mm (median 19 mm). The TNM Classification of Malignant Tumours (TNM) stage [14] was T1 in 3, T2 in 2, and T3 in 1 of the PTC patients; no case of lymph node, or distant metastasis was detected.

The ^{V600E}*BRAF* mutation was observed in 1 among 3 patients evaluated. Other endocrine neoplasias were not found in SSc patients.

The Odds Ratio for PTC was significantly higher in SSc patients than in iodine-sufficient controls (Odds Ratio = 12.2; c.i. 1.9-77.4).

SSc patients with PTC did not differ from the other SSc patients (**Table 2**) in terms of gender distribution (M/F: 1/5, 27/294; respectively), age (54 ± 12 , 55 ± 11 , years; respectively), or serum TSH (TSH in non-nodule-SSc, in benign-nodule-SSc and in PTC-SSc were, 3.5 (0.01-51.4), 2.9 (0.01-11.2), 3.1 (0.4-6.7), respectively; $P = ns$), FT3, FT4, AbTg, and AbTPO concentrations. Five SSc patients with PTC had positive circulating thyroid autoantibodies (AbTg titer: 11, 28, 42, 191, 641, 823 kIU/L, respectively; AbTPO titer: 11, 109, 122, 218, 292, 713 kIU/L, respectively) at the time of surgery, and three had PTC in the context of

chronic thyroiditis on histological examination. Altogether, all SSc patients with PTC had evidence of thyroid autoimmunity (6 vs. 0), while only 40% (128 vs. 193) (Fisher's Exact test P -Value = 0.004) of SSc patients without TC exhibited evidence of thyroid autoimmunity (**Table 2**). The presence of PTC was not associated with any specific SSc treatment, or duration of the disease.

Discussion

This study first shows that PTC is observed with higher prevalence in SSc patients, with respect to age- and gender-matched iodine-deficient controls, and iodine-sufficient controls. To eliminate bias in the observed prevalence of TC due to differences in iodine uptake, control groups from both high and low iodine intake regions were used [4]. Nonetheless, the results show a significantly higher prevalence of PTC in SSc patients than in both controls. The low prevalence of PTC in the control groups is in agreement with the low prevalence of TC (1/1411 subjects screened) recently reported from a population-based study in Southern Italy [10]. However, we cannot completely rule out that surveillance bias may be a possible explanation for finding more cancers in the cases than in controls undergoing routine screening.

The mechanisms connecting SSc and malignancies are unknown. The occurrence of different cancer types with SSc or SSc-like disorders suggests different underlying mechanisms, including altered immune response, common genetic and environmental links, disease-dependent factors, tumor-derived biologic substances and therapies [2]. The process of sclerosis itself may favour cancer in certain sites, such as lung and breast [2].

In our patients autoimmune thyroid involvement and hypothyroidism were more frequently observed in patients with SSc than in the comparison groups, in agreement with results from

other studies [15,16]. An association between TC and chronic thyroiditis has been observed in many studies [17,18].

In our study, features of thyroid autoimmunity were observed in all SSc patients with PTC, in comparison to 40% of SSc patients without TC, reinforcing the hypothesis that thyroid autoimmunity may be a predisposing condition for PTC. In our SSc patients, TSH was higher in the SSc group than in controls, and it is known that nodules are statistically more often malignant in patients with higher TSH [19]. Even if TSH levels in non-nodule-SSc, in benign-nodule-SSc and in PTC-SSc were not significantly different, we cannot exclude that a high TSH could be associated with PTC in SSc.

Another hypothesis proposed to explain the increase of cancers in SSc patients is the exposure to medications [2]. However, the presence of PTC was not associated with any specific treatment of SSc in our series. Furthermore, no specific treatment of SSc is known to be associated with TC.

If the BRAF-MEK-ERK pathway activation plays a common role in PTC and SSc remains to be investigated; however, we have found the ^{V600E}*BRAF* mutation was observed in 1 among 3 patients evaluated, in line with the frequency observed in PTC patients without SSc [20].

In conclusion, this study first shows an increased risk of PTC in SSc patients, and an association with thyroid autoimmunity. As such, we recommend that careful thyroid evaluation (by ultrasonography, FT4, TSH, AbTPO measurements) should be carried out during the follow-up of these patients.

Key messages

PTC is predominantly observed in SSc patients with respect to iodine-deficient and iodine-sufficient controls

All SSc patients with PTC have thyroid autoimmunity, vs. 40% of the other SSc patients

A careful thyroid evaluation should be carried out during the follow-up of SSc patients

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The authors have nothing to declare.

Conflict of Interest statement

The authors declare that they have no conflicts of interest.

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Table 1. Clinical characteristics and comparison of thyroid status between patients with SSc and Controls.

	<i>Controls (iodine deficient)</i>	<i>SSc</i>	<i>Controls (iodine sufficient)</i>	<i>P</i>
n	654	327	654	
Age (years)	55 ± 11	54 ± 14	54 ± 13	0.724
Men/women (%)	8/92	8/92	8/92	1
Iodine deficiency (%)	87 ^a	57 ^{bc}	19	<0.0001
Familial thyroid disease (%)	46 ^a	38 ^b	18	<0.0001
Smokers (%)	22	21	24	0.526
TSH (mIU/L)	1.2 (0.01-8.7)	3.2 (0.01-51.4) ^{de}	1.4 (0.1-9.6)	0.0013
FT4 (pmol/L)	11.4 ± 3.1	9.5 ± 5.1 ^{de}	12.2 ± 2.9	0.537
FT3 (pmol/L)	5.1 ± 1.5	4.6 ± 1.6 ^{de}	4.9 ± 2.1	0.421
AbTg (kIU/L)	31 (3-321)	135 (2-1213) ^{de}	47 (1-476)	0.041
AbTPO (kIU/L)	29 (1-423)	115 (9-2132) ^{de}	28 (4-432)	0.0005
AbTg ⁺	8%	18% ^{de}	12%	0.0001
AbTPO ⁺	7%	35% ^{de}	9%	0.0001
Hypothyroidism, TSH > 4 mIU/L (%)	3	20 ^{fg}	5	<0.0001
Hyperthyroidism, TSH < 0.3 mIU/L (%)	3	4	2	0.172
Thyroid nodules (%)	25 (163/491)	28 (91/236) 0.26 vs. iod def	15 (98/556) ^h	<0.0001
Papillary thyroid cancer (n)	1	6 ^{*^}	1	0.007

$P < 0.05$ by χ^2 : **a** iodine deficient vs. iodine sufficient; **b** SSc vs. iodine sufficient; **c** SSc vs. iodine deficient. Data are expressed as median (range) for TSH, AbTg, and AbTPO. AbTPO = anti-thyropoxidase antibody; AbTg = anti-thyroglobulin antibody; FT3 = free triiodothyronine; FT4 = free thyroxine; $P \leq 0.05$ by the Bonferroni-Dunn test or Mann-Whitney U test (for TSH, AbTg, AbTPO) (**d** SSc vs. iodine deficient; **e** SSc vs. iodine sufficient); $P < 0.05$ by χ^2 , or by Fisher's Exact test (**f** SSc vs. iodine deficient; **g** SSc vs. iodine sufficient; **h** iodine deficient vs. iodine sufficient): * SSc vs. iodine deficient; ^ SSc vs. iodine sufficient.

Table 2. Clinical and laboratory data in SSc patients with (CA-SSc) or without papillary thyroid cancer (SSc).

	<i>SSc</i>	<i>CA-SSc</i>	<i>P</i>
Total	321	6	
Age (years)	55 ± 11	54 ± 12	0.675*
Men/women	27/294	1/5	0.418 [^]
Diffuse/limited	168/153	2/4	0.432 [^]
Anti-antinuclear Ab (+/-)	293/28	5/1	0.429 [^]
Anti-centromere Ab (+/-)	119/202	2/4	>0.999 [^]
Anti-topoisomerase I Ab(+/-)	130/191	2/4	>0.999 [^]
AbTg (+/-)	59/262	3/3	0.084 [^]
AbTPO (+/-)	109/212	5/1	0.021 [^]

ANOVA = *; Fishers's Exact test *P*-Value = [^]