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Increased prevalence of thyroid peroxidase antibodies (TPO-Ab) in women with glomerulonephritis

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Abstract. In a cross-sectional study adjusting for age, gender, and catchment area, the prevalence of thyroid antibodies was assessed in 51 consecutive subjects with biopsy-proven glomerulonephritis and in 112 control subjects admitted for extracorporeal shock-wave lithotripsy treatment for renal stones. Women with glomerulonephritis had both a significantly greater prevalence of thyroid peroxidase antibodies (odds ratio 3.85, 95% confidence interval 1.04–14.3) and an increased prevalence of elevated serum TSH values ($P=0.007$). No such difference was found in men. The prevalence of thyroglobulin antibodies did not differ between the groups. It is suggested that the possibility of an autoimmune thyroid disease should be taken into consideration in patients with glomerulonephritis, particularly in women.

Key words: glomerulonephritis; hypothyroidism; islet-cell antibodies; thyroglobulin antibodies; thyroiditis; thyroid peroxidase antibodies; TSH

Introduction

It is well known that most forms of glomerulonephritis [1] and thyroiditis [2] have an autoimmune aetiology. Thyroiditis is more common in females and in old age, and is known to be associated with other autoimmune diseases, primarily pernicous anaemia and diabetes mellitus type I [3,4]. Furthermore, such autoimmune systemic diseases as SLE (systemic lupus erythematosus) and primary Sjögrens' syndrome are known to have an increased prevalence of thyroid antibodies [5,6]. Glomerulonephritis has not been associated with other non-renal, organ-specific antibodies, but there are scattered case reports of the coexistence of thyroiditis and glomerulonephritis [7,8]. The majority of these cases had a membranous glomerulonephritis and had circulating anti-thyroglobulin (Tg-Ab) or thyroid peroxidase antibodies (TPO-Ab) in serum. In some of these cases deposition of thyroglobulin together with immunoglobulins and complement was demonstrated in the glomeruli by immunofluorescence staining, indicating a possible role for thyroid antigen–antibody immune complex in the development of some forms of glomerulonephritis [9]. In one study of membranous glomerulonephritis it was found that three of 82 consecutive patients had signs of thyroiditis i.e. TPO-Ab, Tg-Ab, and elevated serum TSH level [10]. Membranous and mesangioproliferative glomerulonephritis have been induced in rabbits by parenteral administration of thyroglobulin [11,12].

The prevalence of different antibodies, for example TPO-Ab, Tg-Ab, antibodies to the islet cells of the pancreas (ICA), and intrinsic factor antibodies (IF-Ab), in patients with glomerulonephritis, is not known. In order to assess this question the prevalence of these antibodies was analysed in subjects with different forms of glomerulonephritis and in control subjects, adjusting for the effects of age and gender.

Subjects and methods

Subjects with glomerulonephritis

During the period November 1988 to June 1990 kidney biopsies were performed in 104 patients investigated at the Department of Nephrology, University Hospital of Lund, which is a referral centre for a population of about 750 000 inhabitants in Southern Sweden.

Major inclusion criteria for renal biopsy were proteinuria with an albumin/creatinine clearance ratio of 0.1/ml or more, and/or microscopic haematuria with granular casts in the urinary specimen, and/or a reduction of the glomerular filtration rate by at least 20%. All but one patient (who only had microscopic haematuria with granular casts) met two or more of these criteria.

All renal specimens were assessed morphologically by light-microscopy and for the presence of immune deposits by immunofluorescence-microscopy. Eighty-nine patients had a representative kidney biopsy and 52 of them met the

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morphological criteria for glomerulonephritis. One of these was excluded due to missing interview and blood samples. Thus 51 (34 males and 17 females) were included in the study (Table 1). Their median age was 55 years (range 19–79). The remaining 37 patients had no glomerulonephritis and were excluded from the study.

Control subjects

During the period March 1989 to October 1990 consecutive patients with renal stones admitted to the Department of Urology, University Hospital of Lund for treatment by extracorporeal shock-wave lithotripsy (ESWL) were asked to participate. The catchment area was almost identical to that from which subjects with glomerulonephritis were recruited. Only one of 116 subjects refused to participate. Three had to be excluded, however, one due to missing blood samples, one due to inability to read Swedish, and the third because of suspicion of a glomerulopathy. Included in the study were 112 subjects, 72 males and 40 females. Their median age was close to that of the subjects with glomerulonephritis (Table 1).

Methods

Blood and urine samples were taken shortly before the kidney biopsies or before the ESWL treatment respectively. Sera were stored at −24°C until analysed. A questionnaire on family history of renal and thyroid diseases, previous and present diseases, especially renal and thyroid, smoking habits, present occupation, and pharmaceutical medication was answered by both groups. There were four missing urinary samples among the control subjects, but when investigating the medical records there was no clinical suspicion of glomerulonephritis in these patients and so they were accepted onto the study.

The investigation of the control subjects was approved by the local Ethical committee.

Diagnostic criteria for glomerulonephritis

The morphological classification of the kidney biopsies was done essentially according to the WHO classification (International Classification of Diseases Ninth Revision; ICD-9). IgA-nephropathy was diagnosed as a separate entity on the basis of the demonstration of deposits of IgA in the mesangium. For the diagnosis of extracapillary (crescentic) glomerulonephritis at least 50% crescents were required. The group of proliferative glomerulonephritis included diffuse, focal, and mesangioproliferative types. One of the patients with proliferative glomerulonephritis had sclerotic changes in the current biopsy but had 14 years earlier been found to have a proliferative glomerulonephritis. A clinical classification into primary or secondary glomerulonephritis was based on pre-existing diseases as SLE (two cases), systemic vasculitis (9 cases), malignancy (1 case) or postinfectious (2 cases). There were two patients (1 female and 1 male) with known diabetes mellitus of type II, who had a glomerulonephritis not associated with diabetes.

Diagnostic criteria and characteristics of the control subjects

The diagnosis of renal stones was verified by i.v. urography performed before the ESWL. Renal-stone analysis showed in 41 cases that the stones were composed of calcium oxalate and/or phosphate, in another one it was composed of urate. Forty-eight percent had microscopic haematuria and 13% had proteinuria of + + or less on the Albustix dipstick test. In 11 of the 108 urinary specimens there were findings of occasional finely granulated casts. None of these subjects had a known or suspected glomerular disease, and their serum creatinine levels did not differ from those of the group as a whole. Two of them, however, had borderline proteinuria of + + on the Albustix dipstick test. One male had diabetes mellitus without any urinary findings of nephropathy and another two males had glucosuria.

Other analyses

Serum creatinine was analysed by routine enzymatic methods and serum TSH was analysed with a fluoroimmunometric assay (Delfia®, Pharmacia Diagnostics AB, Sweden). The reference interval for serum creatinine was 60–115 μmol/l and for serum TSH, 0.4–4.0 μU/ml.

Thyroid antibodies

Tg-Ab and TPO-Ab were measured with a sensitive solid-phase immunosorbent radioassay based on the binding of the homologous ^{125}I-labelled antigen. The antibodies were detected by their binding to anti-IgG antibodies covalently coupled to Sepharose as described in detail elsewhere [13]. All samples were assayed in serial dilution until the binding reached the detection limit (the 97.5th percentile). One dilution step (final dilution) before this stage was taken as the

Table 1. Fifty-one subjects with glomerulonephritis and 112 control subjects

	Glomerulonephritis		Controls	
	Age (years)	Serum creatinine	Age (years)	Serum creatinine
Females				
Median	44	90	48.5	71
Range	21–76	67–800	23–84	47–105
Males				
Median	58	154	56	93
Range	19–79	78–815	19–79	71–133

titre. Titres greater than or equal to 1 : 50 were considered as positive. Human Tg was labelled with ^{125}Na (Code IMS 30, Amersham International, Amersham, UK) using the chloramine-T method [14]. Human ^{125}I-labelled TPO was purchased from RSR Limited, Cardiff, UK. The prevalence of TPO-Ab with this method was 14% in a random sample of 50 women from the general population aged 50 and living in the same geographical region in Sweden as our study groups.

Islet cell antibodies

ICA-Ab were analysed only in patients with glomerulonephritis with a two-colour immunofluorescence assay using a monoclonal proinsulin antibody to detect islet B cells, as described in detail elsewhere [15].

Intrinsic factor antibodies

The method was similar to that described and used for analysis of thyroid antibodies except that ^{125}I-labelled intrinsic factor (IF) was used as antigen. Intrinsic factor from porcine gastric mucosa was obtained from Sigma Chemical Company, St Louis, USA.

Statistical methods

Differences in the prevalence of various antibodies in serum between subjects with glomerulonephritis and control subjects were tested with Fischer's exact test. In order to adjust for the age effect, multiple logistic regression was used. Initially, smoking (defined either as present smoking habits or life-time pack-years) was also included in the logistic regression analysis, but it was found to be unrelated to the outcome variables. It was therefore excluded from the final analysis. The risk estimates were expressed as odds ratios (OR). All tests were two-tailed. The term significant is used when $P<0.05$ or the 95% confidence interval (95%CI) excludes 1.

Results

Thyroid antibodies

The prevalence of TPO-Ab was significantly higher among women with glomerulonephritis as compared with the female control subjects (47.1% versus 20%, $P=0.04$; Table 2). This difference was apparent also when the age effect was adjusted for (OR 3.85; 95% CI 1.04–14.3; Table 3). On the other hand, no significant difference in the prevalence of TPO-Ab was found between men with and without glomerulonephritis (11.8% versus 11.1%; Tables 2 and 3).

The somewhat increased prevalence of Tg-Ab among women with glomerulonephritis as compared with their controls (41 versus 30%, Table 2) was not significant ($P>0.5$). Age adjustment did not substantially strengthen this association (OR 1.71; 95% CI 0.46–6.38; Table 3). Among men with glomerulonephritis, 17.6% had Tg-Ab, as compared with 9.7% of the controls (Table 2). This difference was not statistically significant, $P=0.34$. Adjusting for the age effect did not change this (OR 1.93; 95%CI 0.58–6.43; Table 3).

Table 2. Thyroid peroxidase (TPO-Ab) and thyroglobulin antibodies (Tg-Ab) in 51 subjects with glomerulonephritis and 112 control subjects

	Glomerulonephritis		Controls	
	Females ($n=17$)	Males ($n=34$)	Females ($n=40$)	Males ($n=72$)
TPO-Ab				
Negative	9	30	32	64
Positive	8	4	8	8
Tg-Ab				
Negative	10	28	28	65
Positive	7	6	12	7

Table 3. Risk estimates (odds ratios) for presence of thyroid antibodies (TPO-Ab, Tg-Ab) with respect to age and glomerulonephritis, among 57 females and 106 males

	TPO-Ab		Tg-Ab	
	OR	95% CI	OR	95% CI
Females				
Glomerulonephritis	3.85	1.04–14.3	1.71	0.46–6.38
Age (Δ10 years)	1.38	0.95–2.00	1.61	1.10–2.36
Males				
Glomerulonephritis	1.05	0.29–3.84	1.93	0.58–6.43
Age (Δ10 years)	1.16	0.75–1.80	1.29	0.84–1.99

Thyroid Ab were found in other types of glomerulonephritis than membranous (Table 4). Among all 51 subjects with glomerulonephritis, 14 (8 females and 6 males) had TPO- or Tg-Ab. One of the females with glomerulonephritis and thyroid Ab had an SLE.

Thyroid function and previous history of thyroid disease

In total there were nine patients (6 women, 3 men) with serum TSH values elevated above the normal reference range. Six of these nine subjects also had TPO-Ab. There was a higher prevalence of elevated serum TSH in women with glomerulonephritis than in the control group; 29% (5/17) versus 2.5% (1/40), $P=0.007$. Such a difference was not found between the two male groups (5.9% versus 1.4%, $P=0.24$). Of the five women with glomerulonephritis and an elevated serum TSH level, three had TPO- and two had Tg-Ab.

According to their previous history two subjects had had hypothyroidism and two thyrotoxicosis among the 51 subjects with glomerulonephritis. In the control group ($n=112$), one had a known thyrotoxicosis and another man probably had a thyrotoxicosis 27 years ago, but recovered apparantly spontaneously. One control subject had a hypothyroidism. All of these seven subjects with previously known thyroid dysfunctions had both TPO- and Tg-Ab. Two of the subjects

Table 4. Subjects with glomerulonephritis, distribution according to morphological classification and prevalence of thyroid peroxidase antibodies (TPO-Ab) and thyroglobulin antibodies (Tg-Ab)

Morphological diagnosis	N	TPO-Ab	Tg-Ab	TPO-Ab and Tg-Ab
Minimal changes	5	1	1	1
Membranous	7	2	2	2
Mesangiocapillary	2	0	0	0
Proliferative	19	5	5	5
IgA nephritis	9	0	1	0
Extracapillary	7	3	4	3
Endocapillary	2	1	0	0
Total	51	12	13	11

with a known thyroid disease had elevated serum TSH levels.

Islet cell antibodies

No ICA-Ab were found in the glomerulonephritic group.

Intrinsic factor antibodies

Four subjects with glomerulonephritis and two control subjects had IF-Ab. Two of the subjects with glomerulonephritis, both men, had secondary glomerulonephritis due to systemic vasculitis or infectious disease respectively. One woman and one man had a primary glomerulonephritis. The woman had a membranous glomerulonephritis and she was the only one with IF-Ab as well as TPO- and Tg-Ab.

Discussion

This study shows that female subjects with glomerulonephritis have an increased prevalence of autoimmune thyroid disease, demonstrated by both an increased prevalence of TPO-Ab and elevated serum TSH levels. Whether the thyroid antibodies were prevalent before the onset of the glomerulonephritis is not known.

The tests for thyroid antibodies have been described earlier as quite reliable [13]. The prevalence of Tg-Ab in our controls correspond well to those in other studies performed in the same geographical region [6,16]. The prevalence of TPO-Ab in a random sample from the general population of middle-aged women was 14% (U.-B. Ericsson, unpublished results), which corresponds well to the 20% prevalence in the female control subjects. We wanted to compare the prevalence of thyroid antibodies between subjects with and without glomerulonephritis, and therefore decided to choose a control group consisting of patients with much the same selection criteria as the patients with glomerulonephritis.

Since the prevalence of Tg-Ab is higher than TPO-Ab in controls, both in ours and in other control groups [17], it seems plausible that the occurrence of TPO-Ab is a better indication of autoimmune thyroid disease than Tg-Ab.

No significant associations with thyroid Ab were seen for males, although twice as many males as females were included in the present study. This probably rules out strong associations, but as the prevalence of thyroid Ab is quite low among men, weaker associations cannot be excluded.

Including the lowest titre for TPO-Ab, i.e. $\geqslant$ 1:5, did not change the prevalence of these antibodies in females or males with glomerulonephritis. For male control subjects a lowering to the titre $\geqslant$ 1:5 implicated two more subjects. Corresponding changes for the Tg-Ab involved only one more female with glomerulonephritis but five more female control subjects. For males a Tg-Ab titre of $\geqslant$ 1:5 involved four additional subjects with glomerulonephritis and six more of the control subjects.

Due to the low prevalence of IF-Ab and ICA, associations with glomerulonephritis could not be evaluated. A much larger study has to be performed in order to clarify this matter.

Our results could be explained by the loss of self-tolerance in some individuals prone to develop autoimmunity. There is evidence that autoimmune disorders such as SLE, rheumatoid arthritis, and thyroiditis, may initiate several autoimmune phenomena in a cascade-like fashion [3,5,6,17,18]. In our study only one glomerulonephritic subject with thyroid antibodies, also had SLE. Glomerulonephritis, excluding those cases associated with SLE, has not previously been associated with other non-renal, organ-specific antibodies.

The present study shows that thyroiditis was not restricted to membranous glomerulonephritis but was found also in proliferative forms. Notably, four of seven patients with extracapillary glomerulonephritis had thyroid antibodies. Since most of the patients with extracapillary glomerulonephritis have systemic vasculitis, there is perhaps an overrepresentation of thyroiditis among these. Further studies are needed to clarify this. When reviewing the literature we found only two cases reported with extracapillary glomerulonephritis and concurrent thyroiditis [19]. We do not think it is likely that there would be any cross-reactivity between TPO- and MPO-(myeloperoxidase) Ab, as only sera from two patients, out of 27 tested for MPO, also had TPO. Both these patients had an extracapillary glomerulonephritis, one was earlier treated for hypothyroidism and the other one had clinically overt hypothyroidism at the time of kidney biopsy.

We do not believe that the difference in renal function between the two groups, i.e. higher serum creatinine levels in subjects with glomerulonephritis, influenced the prevalence of antibodies. All uraemic patients had a kidney biopsy in the early phase of their disease, and in addition uraemia has not been previously described in the literature as enhancing the prevalence of autoantibodies.

It can be difficult to evaluate thyroid function in renal insufficiency, but the serum TSH level seems to be the best marker for hypothyroidism in patients with decreased renal function [20]. There has been a report of slightly elevated serum TSH levels in uraemic patients [21]. Nevertheless, the subjects with glomerulonephritis in our study were investigated in an early phase of their disease. The patients with a renal insufficiency were all suspected as having a rapidly progressive glomerulonephritis and it is known that a serious illness or acute disease may rather lower the serum TSH level [21]. Thus we conclude that the increased serum TSH levels in the glomerulonephritic subjects were caused by concurrent autoimmune thyroid disorders and not by renal insufficiency.

Since the symptoms of hypothyroidism can easily be concealed by a nephrotic or nephritic syndrome, our results have important clinical implications. We suggest that the possibility of an autoimmune thyroid disease should be taken into consideration in patients with glomerulonephritis, particularly women.

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