Proteinuria in autoimmune thyroid disease

A. P. Weetman, K. Tomlinson1, N. Amos2, J. H. Lazarus, R. Hall and A. M. McGregor

Departments of Medicine and Rheumatology2, University of Wales College of Medicine, Heath Park, Cardiff, CF4 4XN, Wales and Department of Medical Biochemistry¹, Cardiff Royal Infirmary, Cardiff, Wales, UK

Abstract. We have investigated the prevalence of proteinuria in patients with Graves' disease and chronic autoimmune thyroiditis attending a routine thyroid clinic. Using the urine protein creatinine index, proteinuria was found in 29.8% of patients with autoimmune thyroid disease and in 9.5% of patients attending the same clinic but without these conditions. When patients with Graves' disease were treated with ¹³¹I, proteinuria measured by 24 h collections developed in 9 of 14 patients without pre-existing proteinuria and appeared to diminish in 4 patients in whom proteinuria had been present before treatment. The prevalence and fluctuation of proteinuria was independent of thyroglobulin and microsomal antibody levels. We were unable to confirm previous reports of a high prevalence of circulating immune complexes in autoimmune thyroid disease; complexes were detected in only 7.9% of patients and did not correlate with proteinuria. The causes of mild proteinuria in autoimmune thyroid disease are not apparent, but previous case reports suggesting that membranous glomerulonephritis is associated with Graves' disease, albeit rarely, indicate that immunological mechanisms may be implicated.

There have been several single case reports of membranous glomerulonephritis associated with Graves' disease occurring after the initiation of treatment (O'Reagan et al. 1976; Ploth et al. 1978; Reynolds & Bhathena 1979; Horvath et al. 1979) or present from the onset of hyperthyroidism (Jordan et al. 1978, 1981). In all of these cases the occurrence of the nephrotic syndrome pointed to an association between the thyroid and renal lesions, and study of renal biopsies from some of these patients has shown that thyroglobulin and immune complexes can be detected in the glomerulus (Horvath et al. 1979; Jordan et al. 1981).

The development of the nephrotic syndrome in a further patient, treated with carbimazole, from a series of 75 consecutive patients with Graves' hyperthyroidism (unpublished) has prompted us to investigate how commonly proteinuria occurs in Graves' disease and chronic autoimmune thyroiditis. There were two main parts to the study. Firstly, we sought to establish the prevalence of proteinuria in a randomly selected series of 105 patients attending a routine thyroid clinic. For this purpose we made use of the convenient protein creatinine index (Shaw et al. 1983). Secondly, since the nephrotic syndrome has been precipitated by radioiodine as treatment for Graves' disease (O'Reagan et al. 1976; Ploth et al. 1978) sequential 24 h urine protein excretion in 18 patients with Graves' hyperthyroidism treated with ¹³¹I was studied.

Patients and Methods

Protein creatinine index group

One hundred and five patients attending a combined medical and surgical thyroid clinic between 2.00 and 3.00 p.m. provided a sample of urine and blood. There were 11 men and the mean age ± SD for the group was 52.2 ± 13.9 (range 22--75). They were divided into groups with and without autoimmune thyroid disease as shown in Table 1. Graves' disease was defined by initial presentation with hyperthyroidism and diffuse goitre proven by isotope scan and chronic autoimmune thyroid-
Diagnosis and treatment in the 105 patients who provided urine specimens for protein-creatinine index.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No.</th>
<th>Treatment</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1. Autoimmune thyroid disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Graves' disease</td>
<td>59</td>
<td>nil</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>post surgery</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>carbiomal</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$^{131}$I</td>
<td>29</td>
</tr>
<tr>
<td>Chronic autoimmune thyroiditis</td>
<td>25</td>
<td>nil</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>thyroxine</td>
<td>21</td>
</tr>
<tr>
<td>total</td>
<td>84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 2. Non-autoimmune thyroid disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nodular goitre</td>
<td>9</td>
<td>nil</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>surgery</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$^{131}$I</td>
<td>4</td>
</tr>
<tr>
<td>Simple goitre</td>
<td>6</td>
<td>thyroxine</td>
<td>6</td>
</tr>
<tr>
<td>Primary hyperparathyroidism</td>
<td>2</td>
<td>surgery</td>
<td>2</td>
</tr>
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<td>nil</td>
<td>2</td>
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<tr>
<td>Thyroid lymphoma</td>
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<td>surgery</td>
<td>1</td>
</tr>
<tr>
<td>Papillary carcinoma</td>
<td>1</td>
<td>surgery</td>
<td>1</td>
</tr>
<tr>
<td>total</td>
<td>21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1

Diagnosis indicates the most recent form of therapy.

itis by the combination of hypothyroidism and thyroglobulin and/or microsomal autoantibodies. The patients with no previously identified autoimmune disorder were attending for routine follow-up of the conditions listed, diagnosed by clinical presentation and histological findings. Two of these 21 patients proved to have thyroid autoantibodies (see below).

24 h collection groups

Twenty-four hour urine collections were obtained from 18 patients with thyrotoxic Graves' disease aged 54.6 ± 13.8 (range 35–78) who were followed sequentially for up to 15 weeks after $^{131}$I treatment.

In addition, a single 24 h urine collection was obtained from 9 patients aged 35–60 years who required admission for an acute exacerbation of Graves' ophthalmopathy. Three of these were already on prednisone when sampled and 3 had ophthalmic Graves' disease, that is, no past or present biochemical hyperthyroidism.

Assays

Urine samples were stored at -20°C for up to 12 weeks before analysis. Urine protein was measured by the Bio-Rad dye-binding assay (Bio Rad Laboratories, Watford, UK) and creatinine by a Beckman Astor 4 Analyser (Beckman RIC, High Wycombe, UK). Protein creatinine index was calculated as (mg protein/l mmol creatinine/l) x 10 (Shaw et al. 1983). Blood samples were centrifuged within 2 h of collection and serum samples were stored at -70°C until assayed. Thyroglobulin and microsomal antibodies were measured by enzyme-linked immunosorbent assay (Weetman & McGregor 1984). Values greater than 3 s above the mean of a control group (n = 12) were regarded as positive. Circulating immune complexes were measured by two methods described elsewhere; the Clq binding assay (Pussell et al. 1978) and the monoclonal rheumatoid factor binding assay (Schieferli et al. 1981) using the same monoclonal IgM kappa rheumatoid factor. In each assay a standard curve of heat-aggregated IgG and positive and negative serum samples were included. Results were expressed as a percentage of the maximum binding of the radio-labelled protein assessed by precipitation with 20% trichloroacetic acid. Serum free thyroxine (FT$_4$) was measured using a routine radioimmunoassay (Amerlex, Amer sham, UK).

Statistics

Analysis was performed by the $\chi^2$ test (protein creatinine index data), the Wilcoxon Rank test (comparing immune complex levels in patients with or without proteinuria) and Student's t-test (comparison of $^{131}$I dosage).

Results

Prevalence of proteinuria using the protein-creatinine index

The ranges of the protein-creatinine index results are shown in Fig. 1. All except 2 of the patients without autoimmune thyroid disease had values below 100: the exceptions were a patient with primary hyperparathyroidism and a patient with a toxic adenoma receiving antihypertensive medication. However, 30 of the 84 patients with autoimmune thyroid disease had values greater than 100. Review of the case records failed to reveal any other cause of proteinuria in this group. The excess of patients with an index greater than 100 was significant compared to the group without autoimmune thyroid disease ($\chi^2 = 4.27, P < 0.05$).

All of the patients with chronic autoimmune thyroiditis and proteinuria were euthyroid on the basis of FT$_4$ measurement at the time of sampling. Of the patients with Graves' disease and proteinuria, 12 (63%) had received $^{131}$I, 3 (16%) were thyrotoxic and 2 each had received carbimazole...
Proteinuria in ophthalmopathy

Three of the 9 patients with severe ophthalmopathy had proteinuria measured by in-patient 24 h urine collections. These were a patient who was euthyroid after $^{131}$I (0.35 g/day), a patient with ophthalmic Graves' disease (0.38 g/day), and a patient euthyroid after carbimazole who was receiving prednisone (0.8 g/day).

Proteinuria following $^{131}$I

In 4 of the 18 patients followed sequentially, a full 24 h urine sample was not obtained prior to

Circulating immune complexes were found in 6 of 79 patients with autoimmune thyroid disease by the rheumatoid factor binding assay and in 3 by the Clq binding assay (Fig. 3). None of these patients had proteinuria and there was no significant difference in percentage binding by either assay when patients with proteinuria were compared with those without proteinuria ($P > 0.1$).
Levels of circulating immune complexes measured by the Clq binding assay (ClqBA) and rheumatoid factor binding assay (RFBA). The established upper limits of normal for these assays are ClqBA; 4% binding (equivalent to 40 μg heat aggregated IgG/ml) and RFBA; 14% binding (equivalent to 32 μg heat aggregated IgG/ml). The groups studied were healthy normal controls (N), patients with Graves' disease (G) and autoimmune hypothyroidism (H) and patients without autoimmune thyroid disease (O). Subjects with proteinuria are shown as solid symbols; these without proteinuria as open symbols.

Fig. 3.

Treatment. However, in these patients Albustix (Ames) measurement before 131I revealed no proteinuria and they were therefore allotted to the group without proteinuria (i.e. less than 0.1 g/day) prior to 131I. On this basis 4 of the 18 patients (22.2%) had proteinuria prior to 131I.

As shown in Fig. 4, there was a tendency for protein excretion to decrease following 131I in the 4 patients with established proteinuria whereas in 9 patients without measurable proteinuria before therapy, this developed between 5 and 10 weeks after 131I. In the other 5 patients proteinuria remained less than 0.1 g/day throughout the period of study. There was no significant difference (P > 0.1) between the dose of 131I given to patients who developed proteinuria (669 ± 318 mBq, mean ± SD; range 300–1110) compared to those who did not develop proteinuria (521 ± 189 mBq; range 259–555).

Thyroid autoantibodies were not detected in 5 patients before or after 131I; 3 in the group which developed proteinuria and 2 in the group which never had proteinuria. For simplicity, circulating immune complex measurements were grouped into those before 131I (week 0), those at 4–6 weeks (week 5) and those at 9–11 weeks (week 10). Of the 9 patients in whom they were measured, significant levels of immune complexes were found in one subject and fell after 131I; this patient did not develop proteinuria. There was no obvious change in percentage binding after 131I by either assay in the other patients (Fig. 5).

Discussion

These results show a high prevalence of mild proteinuria in patients with autoimmune thyroid disease. We found that in our series of routine thyroid clinic patients the protein creatinine index was below 100 in all but 2 of the subjects without autoimmune thyroid disease and in these patients a likely cause for proteinuria was evident (hyper-
either prior to or at sampling, were less likely to have proteinuria and 2) \(^{131}\text{I}\) caused the appearance of proteinuria in 9 patients and diminished proteinuria in 4 patients who had this prior to treatment. In 4 of the 9 patients who developed proteinuria after \(^{131}\text{I}\), their assignment to this group was based on Albustix measurement. Although not entirely satisfactory, this method is unlikely to have missed proteinuria of the magnitude found in two cases (0.5 and 1.25 g/day after \(^{131}\text{I}\)).

The prevalence of proteinuria in autoimmune thyroid disease has not been previously assessed using large numbers of patients and modern methods. The older literature however, suggests that proteinuria is indeed a feature of autoimmune thyroid disease. In the extensive review by Sattler (1952) data from several studies, totalling over 300 patients with Graves' disease, indicated that albuminuria was found in about 11% of cases and the author concluded that this was a true complication of the disease. (Sattler also furnished details of three probable cases of the nephrotic syndrome due to glomerulonephritis in Graves' disease).

The cause of the mild proteinuria we have found is not clear. It seems unlikely to be related to serum tension in one and hyperparathyroidism in the other. In contrast, over 35% of the patients with autoimmune thyroid disease had indices over 100 and 29.8% were greater than 150. This excess of patients with an elevated protein-creatinine index was confirmed by the 24 h urine collections performed as baseline for the investigation of \(^{131}\text{I}\) effects, and extended to include patients with Graves' ophthalmopathy.

Treatment was associated with proteinuria in two ways: 1) patients treated only with carbimazole,
protein concentration which is usually normal in these patients, especially when euthyroid, and a predisposition to urinary tract abnormalities has not been documented in these conditions. So-called benign or transient proteinuria related to stress may explain some cases but the thyroid status of the majority of patients was normal and stable. Clearly mild forms of the glomerulonephritis which rarely produces the nephrotic syndrome in Graves' disease may be responsible. Deposition of circulating immune complexes in the glomerulus (Germuth et al. 1977) currently seems an unlikely mechanism for this and indeed, none of the patients with immune complexes had proteinuria nor did complexes appear after $^{131}$I treatment. The low prevalence of circulating immune complexes found in our patients contrasts with reports that 27–55% of patients with chronic autoimmune thyroiditis and Graves' disease are positive by the Clq binding assay and over 90% by the Raji cell assay (Cano et al. 1976; Bogner et al. 1979; Brohee et al. 1979; Hopf et al. 1979; Van der Heide et al. 1980; Endo et al. 1983). This difference may be due in part to assay variations and patient selection, but even in the patients in whom complexes were detected, the amounts we have found were not large. The formation of immune complexes in situ on the glomerular basement membrane (Van Damme et al. 1978) is a more likely mechanism for glomerular damage. This could account for the effects of treatment on proteinuria since the increase in circulating antigen and, later, autoantibody levels after $^{131}$I could enhance in situ complex formation, whereas carbimazole would lower both antigen and antibody levels (Weetman & McGregor 1984). However, there was no obvious correlation between autoantibody levels and proteinuria and 3 patients developed proteinuria after $^{131}$I but did not have either antibody. A potential role for TSH receptor antibodies remains to be assessed.

In conclusion, we have found significant though mild proteinuria in over a quarter of patients with autoimmune thyroid disease. This did not appear to be related to thyroid status but persisted after treatment; $^{131}$I therapy for Graves' disease induced transient proteinuria in some patients. The cause of these changes is not clear but previous reports of several patients with membranous glomerulonephritis associated with Graves' disease suggest an immunological mechanism in at least some patients. In addition we have found a far lower prevalence of circulating immune complexes in autoimmune thyroid disease than previously reported and there was no relationship between such complexes and proteinuria.

Acknowledgments

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References


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