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Urinary Glycosaminoglycans Excretion in Graves' Disease

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Urinary excretion of glycosaminoglycans was measured in 10 patients with pretibial myxoedema, 7 of whom also had thyroid-associated ophthalmopathy, and in 3 additional patients with ophthalmopathy but no skin changes. Total uronic acid excretion was raised above control levels in only 2 patients, who had both eye and skin disease of recent onset. In these patients excretion was initially three times the control level but declined sharply in subsequent months. This decline was in the absence of effective treatment or spontaneous improvement and would appear to reflect the natural history of the disease. These data show that although glycosaminoglycans excretion may be disturbed in Graves' disease, it provides an unreliable reflection of clinical status and of the effectiveness of treatment. ***Key words: myxoedema; ophthalmopathy; hyaluronic acid.***

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Pretibial myxoedema affects up to 5% of patients with Graves' hyperthyroidism, sometimes after correction of the endocrine condition (1, 2). Most patients are middle-aged women who also suffer from thyroid-associated ophthalmopathy (TAO). Winand (3) reported that urinary excretion of acid mucopolysaccharides (glycosaminoglycans, GAG) is increased in TAO. More recently, however, Kahaly recorded that patients with inactive TAO excreted similar amounts of GAG to controls; he also suggested that changes in the patients' condition with therapy were reflected in their GAG excretion (4).

In monitoring urines from patients with pretibial myxoedema, we have found that high levels of uronic acid associated with TAO can decline even in the absence of effective therapy or spontaneous improvement, presumably as part of the natural progression of the disease. This finding has important implications for attempts to use GAG excretion levels to monitor disease activity.

MATERIALS AND METHODS

Patients and urine collection

Details of the patients, all female, are given in Table I. Patients 1–10 had pretibial myxoedema, with or without TAO; patients 11–13 had recently diagnosed thyrotoxicity with no changes in their skin. Urine was collected over 24-h periods without preservatives. After the volume had been measured, aliquots were stored at −20 °C. Control urines were collected from 10 normal women of similar age to the patients (55 ± 2 y)(SEM).

Uronic acid assay

Uronic acid concentrations were measured directly in triplicate samples of urine diluted 1:100 with distilled water after brief centrifugation to remove particulate debris (5). Aliquots (0.4 ml) were boiled with concentrated sulphuric acid-borate (2.4 ml) for 5 min and then cooled. The samples were checked for discoloration, and any significant absorbance at this stage was subtracted from the final reading. Meta-hydroxydiphenyl reagent (Kodak, Liverpool) was added (50 µl) to produce a pink colour after vigorous mixing (6). The absorbance was then read at 520 nm against a standard curve prepared with glucurono-lactone at 0–30 µg/ml. Creatinine concentration was also measured by the standard alkaline picrate method and uronic acid excretion was expressed as the ratio of uronic acid/creatinine contents (UA/C as mg/g). Polymeric GAG was precipitated from acidified urine (pH 5). Aliquots (3 ml) were diluted with 3 ml of distilled water in glass centrifuge tubes and mixed with 0.2 ml 5% aqueous cetyl trimethylammonium bromide. After standing overnight at 4°C, the tubes were centrifuged at 2,000 rpm for 15 min. The precipitates were washed with 95% ethanol saturated with sodium chloride, dried and redissolved in 1.0 ml distilled water by incubation at 37°C for 30 min. Aliquots of this solution (0.4 ml) were measured using the uronic acid assay described above.

Hyaluronic acid assay

The initial and final urine samples from patients 1 and 2, and samples from 2 controls, were also assayed for hyaluronic acid by I^{125}-radiometric assay using a standard kit (Pharmacia AB, Uppsala, Sweden).

RESULTS

Patients 1 and 2 had UA/C levels several times those of the controls, while the remainder were within the control range (Table I). The 13 patients had a mean UA/C level of 568 ± 123 (SEM) compared with 450 ± 45 in the controls (not significant; $p > 0.05$; Wilcoxon rank sum test.). Monitoring of patient 1 throughout treatments with para-aminobenzoate and then with octreotide, neither of which improved the condition of her skin or eyes, showed that the levels had declined to those of controls by the time octreotide treatment had begun and remained stable or decreased only slightly thereafter (Fig. 1). The UA/C levels in patient 2 fell dramatically just before an attempted treatment with a single injection of octreotide (Fig. 1).

Table I also shows that the 2 patients (1 and 2) with raised uronic acid levels had normal levels of precipitable GAG in their urine, although this GAG was high in patient 3 (without TAO). The thyrotoxic patients 11–13, without skin changes, had normal levels of precipitable GAG and uronic acid. The initial samples from patients 1 and 2 contained 654 and 620 µg of hyaluronic acid, respectively, and their final samples 501 and 1593 µg, compared with 664 and 546 µg in the 2 controls.

DISCUSSION

Our data confirm that patients with active TAO, of recent origin, may have grossly raised urinary GAG excretion, but suggest that high excretion declines spontaneously (3, 4). Both skin and eye abnormalities then stabilise but persist. The distinguishing features of patients 1 and 2 may merely be that their eye and skin symptoms were still developing at the time the measurements were made. This natural history may be important in attempts to assess the success of new treatments.

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Table I. *Details of patients and their urinary glycosaminoglycans (GAG) excretion relative to creatinine (C) in a 24-h period compared with mean values (±SEM) from 10 controls*

Initital values are shown for patients 1 and 2. TAO; thyroid-associated ophthalmopathy, UA: uronic acid, UA/C: uronic acid/creatinine.

Patient	Age	Skin condition	TAO	C (g)	GAG (mg)	UA (mg)	UA/C
1	64	Active; both legs	+	0.89	1.73	1451	1636
2	42	Active;? left leg	+	0.94	1.70	1335	1419
3	48	Stable	−	0.94	1.34	444	472
4	59	Stable	−	0.74	1.52	336	454
5	68	Stable	−	0.78	10.35	203	261
6	45	Severe	+	1.85	3.15	536	289
7	69	Mild	+	1.68	4.79	495	295
8	58	Severe	+	1.23	2.82	430	351
9	58	Moderate/severe	+	0.69	1.16	173	250
10	58	Mild	+	1.29	4.04	398	305
11	65	Normal	+	0.43	0.53	181	436
12	26	Normal	+	0.50	1.83	297	654
13	50	Normal	+	0.57	1.10	325	571
Controls	55±2	Normal	−	1.28±0.1	2.43±0.29	578±107	450±55

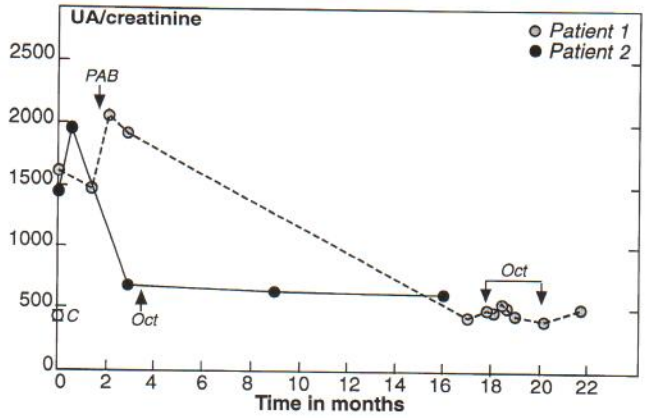

Fig. 1. Sequential values for uronic acid/creatinine in the urine of patients 1 and 2. The times of treatments with octreotide (Oct) and para-aminobenzoate (PAB) are indicated. The mean control value is shown on the left (*C*).

Chang et al. (7) attributed a 30% fall in urinary GAG in 6 patients to a 1-week treatment with octreotide, but without sequential measurements on a group of similar untreated patients this conclusion may be invalid. The number of our patients reflects the relative rarity of pretibial myxoedema among patients with hyperthyroidism and the uncommon referral of such patients to dermatologists in the UK. Our first 5 patients were used to study the component in their serum which stimulates GAG secretion by pretibial and other fibroblasts *in vitro* (8). It seems significant that, by the time of the final urine samples in Fig. 1, sera from Patients 1 and 2 had lost this capacity and only stimulated fibroblast GAG secretion to the same extent as controls.

The urinary GAG is raised in mucopolysaccharidoses, rheumatoid arthritis, scleroderma, psoriasis, diabetic cheiroarthropathy, and after severe burns or major surgery (5, 9, 10). The GAG may originate from the skin or from other connective tissue sites via the plasma, the predominant GAG polymer in normal urine being chondroitin sulphate (5, 11). Hyaluronic acid accumulates to many times its normal concentrations in the skin and eyes in Graves' disease (12, 13), but normal urine contains only trace amounts of low molecular weight fragments which can pass through the kidneys (14). The possibility that such fragments could account for the high levels of uronic acid in patients 1 and 2 had no support from the hyaluronic acid assays, which showed hyaluronic acid as around 1% of the total uronic acid in the urines of both these patients and of controls.

For the purposes of monitoring the patients' clinical state, use of uronic acid/creatinine levels is quicker and more reliable than the measurements of polymeric GAG previously used. Twenty-four-hour collections are necessary because excretion of some GAGs follows circadian patterns (15) and use of the GAG/creatinine ratio is some safeguard against incomplete collection. However, because we recorded raised levels in only a minority of patients and found that even their levels declined spontaneously, gross GAG excretion seems to be an unreliable indicator of disease activity or of the effectiveness of treatment.

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