

A Case of Hashimoto's Thyroiditis Presenting with Chronic Upper eye Lid Retraction and Positive Calsequestrin and Collagen XIII Antibodies

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The pathogenesis of thyroid-associated ophthalmopathy (TAO) and the mechanism for its unique link with thyroid autoimmunity are poorly understood, in part due to the absence of specific diagnostic clinical signs and confirmatory "gold standard" laboratory tests. We report a patient who highlights the potential clinical role for eye muscle and collagen XIII antibody testing in the diagnosis and management of thyroid-related eye, eyelid and orbital disorders. A healthy euthyroid woman member of the Thyroid Research Laboratory presented with the incidental finding of positive calsequestrin and collagen XIII antibody tests carried out in the context of our research. She was pregnant at the time. Examination revealed marked upper eyelid retraction (UER) and a small goitre. Seven months after an uneventful pregnancy, which was 12 months after the initial blood test, she presented with overt hypothyroidism. Thyroglobulin and microsomal antibodies were strongly positive (25,600, > 25,600, respectively) and TSH was 40. We then tested the earlier serum sample for thyroid antibodies, which were positive at a low titre (1600, 1600, respectively) indicating that she had Hashimoto's thyroiditis at that time. The hypothyroidism persisted, requiring long term management with L-thyroxine. UER can occur in the absence of other features of ophthalmopathy except for mild proptosis in patients with Hashimoto's thyroiditis

in whom antibodies targeting eye muscle or collagen XIII antibodies are detected in about 50% of cases. Overall about 20% of patients with Hashimoto's thyroiditis have eye signs. Calsequestrin antibodies are specific and sensitive markers for extra ocular and upper eyelid muscle inflammation and damage in patients with thyroid autoimmunity and collagen XIII antibodies appear to be good markers for the congestive ophthalmopathy subtype of TAO.

Key Words: Autoimmunity, Eye muscle, Antibodies, Thyroid-associated ophthalmopathy, Calsequestrin, Collagen XIII, Eyelid retraction

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Introduction

The pathogenesis of thyroid-associated ophthalmopathy (TAO) and the mechanism for its unique link with thyroid autoimmunity are poorly understood, in part due to the absence of specific diagnostic clinical signs and confirmatory tests.¹ A popular hypothesis for the development of ophthalmopathy is autoimmunity against the TSH-receptor (TSH-r) in the orbit, although this has not been proven. Chronic upper eyelid retraction, which sometimes occurs as a dominant feature of ophthalmopathy or as an isolated ab-

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normality, is a common and related orbital disorder.^{2,3} Recent evidence that antibodies targeting the major calcium binding protein calsequestrin are specific and sensitive markers of eye muscle⁴⁻⁶ and upper eyelid^{7,8} muscle damage has highlighted the need for diagnostic antibody tests in ophthalmopathy. We report here a seminal case of a euthyroid woman with chronic upper eyelid retraction and positive calsequestrin and collagen XIII antibodies who developed Hashimoto's thyroiditis and hypothyroidism 22 months later. We review the role for eye muscle antibody testing in the diagnosis and management of thyroid-related eye and orbital disorders.

Materials and Methods

The ELISA has been described in previous publications from this laboratory⁵⁻⁸ and is standard. Briefly, highly purified rabbit skeletal muscle calsequestrin, which has 97% homology with human calsequestrin, was supplied by Dr. Nicole Beard (ANU, Canberra, Australia) and recombinant human collagen XIII was provided by Drs Taina Pihlajaniemi Dr Tu Min (Oulu University, Finland) and (In preliminary studies, the assay was optimised in respect to antigen concentration and serum dilution, for each antigen, as follows; 0.25 µg/mL for collagen XIII and 0.5 µg/mL for calsequestrin and the optimal serum dilution was 1:25 for both antigens. Secondary antibody was a goat anti-human IgG at a dilution of 1 in 2000. A positive test was taken as an OD > the upper limit of normal for 30 healthy males. Hence, the upper limit of normal was 194 for calsequestrin and 174 for collagen XIII, antibodies.

Other tests

Plasma free thyroxine (fT4) and TSH, serum thyroglobulin and thyroid peroxidase antibodies were measured by Barratt and Smith Pathology, Sydney, Australia, using commercial kits according to the manufacturers' instructions. TSH-r antibodies, determined as TBII, were measured at the Pathology Department of Westmead Hospital Sydney Australia by one of

us (GM), using commercial kits according to the manufacturer's instructions.

Case report

In September 2005, a 26 year old female member of the Thyroid Research Group tested her serum for eye muscle and collagen XIII antibodies in the context of the group's research. Calsequestrin and collagen XIII antibodies were positive. She was pregnant and otherwise well at the time and had no past or family history of thyroid or other autoimmunity.

Eye examination

Examination revealed marked UER and lag, which tended to be worse in the afternoon than morning, and which persisted throughout the study period, but no other signs of ophthalmopathy. The severity of upper eyelid dysfunction was assessed at the 12 o'clock position and quantified as an eyelid activity score (EAS), where 1 = upper lid margin is just above the upper limbus (showing the "whites" of the eye), 2 = limbus eyelid distance ≥ 1.5 mm but < 2 mm and 3 = limbus eyelid distance ≥ 2 mm, for each eye, for an EAS of 0-6, for 1 or 2 eyes. She had an EAS of 4. Further examination revealed that she had a small soft goitre. Serum TSH and free T4 levels were normal (Table 1). In May 2006 she delivered a normal term female baby in December 2006. Six months after delivery, which was 12 months after testing positive for eye muscle and collagen XIII antibodies, she noted that she had become very fatigued, had gained 10 kg weight and was intolerant to cold. On examination she was found to be hypothyroid and her thyroid was now enlarged, firm and irregular. EAS was now 2. TSH was 40, fT4 and fT3 low normal and thyroglobulin and microsomal (thyroid peroxidase) antibodies were strongly positive at very high titres (25,600, > 25,600, respectively) (Table 1). Eye muscle and collagen XIII antibodies were retested on a third serum sample

Table 1. Calsequestrin and collagen XIII antibodies, thyroid antibodies and thyroid function tests in a patient with Hashimoto's thyroiditis who presented with chronic upper eye lid retraction

| Date | Orbital antibodies* | | Thyroid antibodies† | | | Thyroid function‡ | |
|-------------|---------------------|------------|---------------------|----------|--------|-------------------|-------------|
| | CSQ | CollXIII | TSH-r | TPO | Tg | ft4 (pmol/L) | TSH (mIU/L) |
| Sept. 2005 | <u>198</u> | <u>199</u> | Neg | 1600 | 1600 | 14 | 3.8 |
| May 2006 | NT ss | NT | NT | > 25,600 | 25,600 | 10.8 | 40 |
| August 2006 | 46 | 61 | Neg | NT | NT | 11 | 23 |

* CSQ = calsequestrin, CollXIII = type XIII collagen, measured in enzyme linked immunosorbent assay (ELISA). Positive tests (>194 for calsequestrin and >174 for collagen XIII) are shown underlined; † TSH-r = TSH-receptor, TPO = thyroid peroxidase, Tg = thyroglobulin, measured in ELISA using commercial kits according to the manufacturer's instructions. ‡ T4 = Free thyroxine (Normal range: 10-25 pmol/L), TSH = thyroid stimulating hormone (Normal range: 0.3-4.0 mIU/L); ^dNT = Not tested

taken in August 2006 and were now all negative (Table 1). We then tested for thyroid antibodies on serum taken at the time of eye muscle antibody testing (September 2005). In retrospect, both antibodies had been positive, at titres of 1:600, at that time which, in association with the small goitre, confirmed the initial diagnosis of Hashimoto's thyroiditis.

Thyroid ultrasonography, carried out only when hypothyroid, revealed characteristic features of end stage Hashimoto's thyroiditis (not shown). TSH-R antibodies were not detected in either serum sample, from September 2005 and August 2006.

Discussion

We report the case of an apparently normal member of the Thyroid Research Laboratory with persistent upper eyelid retraction and positive calsequestrin and collagen XIII antibodies and, in retrospect, Hashimoto's thyroiditis, who developed hypothyroidism 6 months after an uneventful pregnancy, which was 12 months after testing positive for calsequestrin and collagen XIII antibodies. Thus, positive serum eye muscle antibodies and chronic upper eyelid retraction were the presenting features of her Hashimoto's thy-

roiditis. We presume that the thyroiditis had been suppressed during pregnancy then rebounded in the late post partum period. Although it is possible that her post pregnancy hypothyroidism represented the hypothyroid phase of post partum thyroiditis we feel that this is not so for the following reasons; there was no hyperthyroid phase that preceded the hypothyroidism, she had a goitre and positive thyroid antibodies before pregnancy, serum thyroid antibody titres were very high and the hypothyroidism has been persistent.

The major calcium binding protein calsequestrin is now shown to be the main target of the autoimmune reactions of the eye muscle component of TAO.¹ Calsequestrin is expressed 4.7 times more in eye muscle than in other skeletal muscle⁹ which could partly explain the localisation of skeletal muscle inflammation in the orbit and upper eyelids. Calsequestrin antibodies are detected in approx. 90% of patients with recent onset eye muscle inflammation⁴⁻⁶ and in 60% of patients with Graves' hyperthyroidism and dominant chronic upper eyelid retraction.⁷ Calsequestrin (and flavor protein) antibodies are also linked to eye signs in patients with transient (sub acute, silent) and progressive

(Hashimoto's) thyroiditis^{7,8} as demonstrated in our patient. From our clinical studies we estimate that approximately 20% of patients with Hashimoto's thyroiditis and transient thyroiditis have mild ophthalmopathy, manifest mainly as upper eyelid swelling, lag and retraction, at the time of diagnosis. Antibodies targeting collagen XIII are linked to active ophthalmopathy, early eye disease and, particularly, orbital connective tissue inflammation.⁶ Although antibodies targeting the TSH-receptor (TSH-R) expressed in the orbit are proposed as the most likely mechanism for the initiation of orbital inflammation in patients with Graves' hyperthyroidism¹⁰⁻¹² the eye and upper eyelid muscle reactions seem to be separate and, as in this case, unrelated to the presence of TSH-R antibodies.

Calsequestrin and collagen XIII antibody tests are helpful for ophthalmologists, who are referred patients with a variety of eye and eyelid disorders some of which may be linked to thyroid disease. The best use of the antibody tests is to follow patients with Graves' hyperthyroidism without eye signs in whom the new production of calsequestrin antibodies can be taken to indicate the onset of eye muscle inflammation, with the possibility of early treatment.

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