

# HISTOPATHOLOGIC ANALYSIS OF PALPEBRAL CONJUNCTIVA IN THYROID-RELATED ORBITOPATHY (AN AMERICAN OPHTHALMOLOGICAL SOCIETY THESIS)

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BY Don O. Kikkawa MD

## ABSTRACT

**Purpose:** To examine the histopathology of palpebral conjunctiva in patients with thyroid-related orbitopathy. Based on previously published anecdotes, the hypothesis is that conjunctiva shows increased inflammation and fibrosis.

**Methods:** This was a comparative case series. Superior palpebral conjunctiva was examined from two groups. The study group consisted of 20 patients undergoing thyroid-related upper eyelid retraction surgery. The control group consisted of 18 patients undergoing ptosis repair. Specimens were processed and stained using hematoxylin and eosin and trichrome. Histopathologic grading was performed using light microscopy. Main outcome measures were degree of inflammation and fibrosis, mast cell infiltration, and fibroblast count.

**Results:** The two groups did not differ with regard to age or gender. Mean degree of inflammation was 1.4 (95% CI: 0.9, 1.9) for the control group and 1.7 (95% CI: 1.3, 2.1) for the study group. Relative intensity of blue from trichrome staining mean was 134.3 (95% CI: 130.3, 138.3) for the control group and 138.6 (95% CI: 133.7, 143.6) for the study group. The Mann-Whitney test showed no difference between groups in inflammation ( $P=.17$ ), relative blue intensity ( $P=.11$ ), degree of mast cell infiltration ( $P=.61$ ), and fibroblast count ( $P=.45$ ). Point estimates show a trend toward greater inflammation in the study group.

**Conclusions:** While there is a trend toward higher inflammation in the study group, the superior palpebral conjunctiva of patients with thyroid-related orbitopathy is largely spared from autoimmune changes. This has implications in surgical approaches to eyelid retraction repair.

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## INTRODUCTION

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### HISTORICAL PERSPECTIVE

While Aëtius of Amida (502-575 AD), a Byzantine physician, may have been the first to record observation of a patient with goiter and exophthalmos,<sup>1</sup> it was the writings of English physician Caleb Parry (1755-1822) that characterized the disease by reporting seven patients with heart failure and “bronchocele of the thyroid.”<sup>2</sup> The first of his seven patients also demonstrated marked exophthalmos. Interestingly, Parry’s written records were not published until 3 years after his death in 1825, by his son.

Subsequently, several physicians made independent observations. Robert Graves (1796-1853), an Irish physician, reported three female patients who had marked swelling of the thyroid gland with palpitations. A fourth case of exophthalmos was conveyed to him by his colleague, William Stokes.<sup>2</sup> This fourth patient was described as having eyes enlarged so much that they were “incapable of closing.” In 1840, Karl von Basedow (1799-1854), a German physician, described the triad of exophthalmos, goiter, and palpitations, known as the Merseburg triad, from where von Basedow hailed.<sup>3</sup> von Basedow also gave the most detailed descriptions of the eye changes and recognized that the bulging of the eye was not due to the eye changes itself, but to the increase in tissue behind the eye.<sup>4</sup>

Parry, Graves, and von Basedow are all recognized as forerunners in the characterization of this disease; however, both Parry and von Basedow had some imprecision in their original diagnoses. Parry thought the thyroid enlargement was from a “bronchocele.” von Basedow was convinced that the association of goiter and exophthalmos was an atypical presentation of mycobacterial infection.<sup>2</sup> In terms of precedence, Parry was clearly the first to describe the disease and von Basedow the last, but in 1862, the French Academy of Medicine honored Graves with naming rights.<sup>3</sup>

In recent times, nomenclature differences still exist. Because the typical eye findings of proptosis, eyelid retraction, and strabismus can be seen in patients without the diffuse toxic goiter, non-eponymous names have been coined, including the term *thyroid-related orbitopathy*. For purposes of this thesis, this term will be used, which recognizes this fact, in addition to the autoimmune effects on the entire orbit.<sup>5</sup>

### PATHOPHYSIOLOGY

Thyroid-related orbitopathy is one manifestation of a systemic autoimmune process that targets multiple organs in the body, including the thyroid gland, orbital contents, skin, and soft tissues of the face and pretibial region.<sup>6-8</sup> Hyperthyroidism occurs in approximately 90% of patients, and the other 10% are either euthyroid or hypothyroid.<sup>9</sup>

Principal interchanges between the orbital fibroblast and the immune cells that infiltrate the retrobulbar tissues are thought to be the driving force in the pathogenesis of thyroid-related orbitopathy.<sup>9,10</sup> Immunomodulatory mechanisms that govern, in particular, the deposition of glycosaminoglycans appear to be a significant factor in the development of the clinical aspects of this disease.<sup>11</sup> The underlying pathogenesis is unknown; however, several mechanisms have been proposed.<sup>12-16</sup> The thyrotropin receptor,<sup>17</sup> interleukins,<sup>18</sup> insulinlike growth factors,<sup>15,19</sup> cyclooxygenase,<sup>20</sup> and prostaglandins<sup>21,22</sup> have all been implicated in the autoimmune process.

From the Division of Ophthalmic Plastic and Reconstructive Surgery, Department of Ophthalmology, University of California San Diego, La Jolla, California.

## THYROID-RELATED ORBITOPATHY—SITE-SPECIFIC CHANGES

### Extraocular Muscles

Pathologic changes in the retrobulbar tissues have been previously studied.<sup>23-26</sup> Enlargement of the extraocular muscles (Figure 1) is one of the most common abnormalities in patients with thyroid-related orbitopathy.<sup>6</sup> Deposition of extracellular matrix is one of the main causes of the increased volume.<sup>27</sup> Circulating antibodies to extraocular muscles were once thought to be causal in the pathogenesis of the thyroid-related orbitopathy.<sup>28,29</sup> However, they are now thought to be secondary to damage from the inflammatory process, and the main cause of increase in muscle size is thought to be hyaluronan production from the orbital fibroblast.<sup>10</sup>



**FIGURE 1**

Axial computer tomographic scan image of patient with thyroid-related orbitopathy showing enlarged extraocular muscles (arrow points to right medial rectus muscle).

Histopathologic analysis of the orbital tissues and extraocular muscles shows infiltration, primarily with inflammatory cells and interstitial cells.<sup>6,30</sup> Within the individual extraocular muscles, the muscle fibers are separated by increased quantities of extracellular matrix, glycosaminoglycans, and interstitial fluid within the connective tissue. Early in the active inflammatory stage the muscle fibers are intact.<sup>31</sup> Mast cells have also been implicated in the disease process.<sup>6,14</sup>

As the muscles enlarge, the clinical manifestations worsen. The orbital apex becomes crowded and, in addition to the proptosis, strabismus and compressive optic neuropathy can occur. Since the most frequent muscles involved are the inferior and medial rectus muscles, the most common deviations are hypotropia and esotropia (with limitations in the opposite fields of gaze).

### Orbital Fat

The bony confines of the orbit dictate the space available to the soft tissues, and when the tissues swell from either increased fat volume or muscle enlargement, the globe displaces anteriorly. This accentuates congestion, leading to impaired vascular egress and lymphedema, which can further worsen proptosis. Clinically, it is well known that some patients with thyroid-related orbitopathy have normal extraocular muscle size and manifest only increased orbital fat volume (Figure 2).<sup>32</sup> In some patients, proptosis appears to be a combination of both increased muscle and increased fat volume.<sup>10</sup>

Adipogenesis, or increased orbital fat production, is also thought to be caused by the orbital fibroblast, which has multilineage potential. The ability of the orbital fibroblast to either undergo adipogenesis or increase hyaluronan production seems to be related to its expression of the surface glycoprotein marker Thy-1.<sup>10</sup> Thy-1 positive cells invest the extraocular muscles and constitute the majority of orbital fibroblasts. Thy-1 negative cells are capable of adipogenesis and comprise the minority of cells. Under certain conditions, stimulation of the Thy-1 negative cells by autoantibodies to the thyrotropin receptor (TSHr) and insulinlike growth factor 1 (IGF-1) receptor causes adipogenesis, leading to the increase in orbital fat volume.<sup>10</sup>



**FIGURE 2**

Axial computed tomographic scan image of patient with thyroid-related orbitopathy showing increased retrobulbar fat (arrow points to increased fat volume in intraconal space between right lateral rectus and optic nerve).

### **Eyelids**

Eyelid retraction is one of the clinical hallmarks of thyroid-related orbitopathy.<sup>33</sup> The typical scleral show of both the upper and lower eyelids (Figure 3) is essentially diagnostic of the disease.



**FIGURE 3**

Photo of 39-year-old man with thyroid-related orbitopathy and typical eyelid retraction of all four eyelids. Note lateral flare of both upper eyelids.

The lid retractors consist of the levator palpebrae superioris and Müller's muscle. Both appear to be involved in the disease process. The levator palpebrae superioris muscle has been shown to display fibrosis, striated muscle atrophy, and fatty infiltration.<sup>34</sup> Müller's muscle has also been studied and shown to be grossly enlarged with fibrosis, fatty infiltration, and mast cell infiltration.<sup>35</sup>

Serum markers against intracellular proteins may exist in patients with chronic upper lid retraction. Gopinath and colleagues<sup>36</sup> measured serum antibodies against calsequestrin, flavoprotein, G2s, and collagen XIII in patients with eyelid retraction. They found a

prevalence of 80% of antibody positivity for calsequestrin and 40% for collagen XIII, both significantly greater than normal levels. These findings may be similar to that seen in the extraocular muscles in that they are not causal, but are more indicative of damage that has occurred from the autoimmune attack.

### Ocular surface—tear film and conjunctiva

The bulbar and palpebral conjunctiva represent the majority of the adnexal ocular surface area with lesser contributions from the upper and lower tarsal plates. The integrity of the ocular surface is dependent not only on the underlying health of the conjunctiva but also on the condition of the tear film.

Inflammatory infiltration of the lacrimal excretory system affects both volume of the tear production and tear composition. Thyroid-related orbitopathy is no exception. Baker and colleagues<sup>37</sup> collected reflex tears in smokers and patients with thyroid-related orbitopathy and analyzed the specimens with electrophoresis and mass spectrometry. Composition differences were noted with increased expression of zinc-alpha-2-glycoprotein and lactoferrin in patients with thyroid-related orbitopathy and smokers compared to nonsmokers. They were unable, however, to correlate tear TSHr activity with clinical activity score. In another study, Khalil and associates<sup>38</sup> studied the levels of secretory IgA and lysozyme in patients with thyroid-related orbitopathy vs controls and noted a higher ratio of IgA to lysozyme.

Clinical involvement of the conjunctiva is frequently described as one of the manifestations in thyroid-related orbitopathy. Chemosis, conjunctival injection and dellen have all been described. Both chemosis and caruncular swelling are part of the individual criteria for the clinical activity score proposed by Mourits.<sup>39</sup>

Histopathologically, however, the conjunctiva has not been extensively studied. Studies of the conjunctiva have focused on its involvement in superior limbic keratoconjunctivitis,<sup>40</sup> conjunctivochalasis,<sup>41</sup> and cell morphology using impression cytology.<sup>42</sup> Of particular interest is the clinical implication of the conjunctiva in the pathogenesis and treatment of upper eyelid retraction.<sup>32</sup> Surgeons have suspected clinically that the conjunctiva is involved. One study described the occurrence of fibrosis of the conjunctival substantia propria using a trichrome stain.<sup>43</sup> Only three specimens were examined, however, and there were no controls. We were unable to find any other studies in the literature that specifically examined the palpebral conjunctiva for fibrosis or inflammation in this disease process.

### DISEASE COURSE

Rundle was one of the first physicians to describe the clinical course of this disease.<sup>44</sup> The natural history is one of progressive deterioration over several months to a plateau, then a gradual slower improvement over a longer period of time. The return to normal, however, is rare. The deterioration phase has been termed the *dynamic* or *inflammatory* phase and the improvement phase has been termed the *static* phase.<sup>45</sup> Intervention during the inflammatory phase is typically supportive unless vision-threatening complications, such as optic neuropathy or severe corneal exposure, occur. Reconstructive surgery typically occurs during the static phase of the disease.

### TREATMENT

As described, orbital manifestations of thyroid-related orbitopathy include a variety of ocular signs and symptoms, such as proptosis, upper eyelid retraction, optic neuropathy, and strabismus.<sup>33</sup> The severity of these signs and symptoms varies, and many spontaneously resolve over time. The management of ocular surface disease in thyroid-related orbitopathy is based on symptoms. Early in the disease, ocular lubricants can be of help. Punctal plugs may assist with the volumetric aqueous deficit.

Some patients, however, do experience significant disfigurement, life impact and mood disturbance.<sup>7,46</sup> Surgical rehabilitation of these patients occurs in a staged fashion, with omission of stages if not indicated. Orbital decompression is typically performed first, followed by extraocular muscle surgery second, and eyelid surgery last.<sup>47</sup>

The treatment for thyroid-related eyelid retraction is primarily surgical.<sup>46</sup> Pharmacologic treatment has been tried with varying success and has included botulinum A toxin and guanethidine.<sup>48,49</sup> Indications for eyelid retraction repair include symptomatic dry eyes and exposure keratopathy. The surgical approach to upper eyelid retraction has primarily revolved around two approaches: the anterior transcutaneous approach (Figure 4) with levator recession<sup>50</sup> and the posterior transconjunctival approach with combined Müller's/levator recession.<sup>51</sup>

One newer variation involves an anterior skin incision combined with a transverse conjunctival incision (Figure 5) to help release the lid retractors.<sup>41</sup> This transverse conjunctival incision is also thought to release fibrosis, further allowing the eyelid to drop. Some complications with this technique have been described, including full-thickness eyelid fistulas. Tears can penetrate through the conjunctival incision and find their way through the recessed eyelid tissues and exit through the skin. Such fistulous tracts can become epithelialized and remain open if not repaired. Furthermore, flattening of the central eyelid contour (Figure 6) may result from this technique, leading to a suboptimal aesthetic result.<sup>52</sup>

Other potential complications of eyelid retraction surgery include overcorrection of the eyelid retraction leading to ptosis, undercorrection of the eyelid retraction, eyelid contour abnormalities, and diminished aqueous tear production. George and colleagues<sup>53</sup> studied patients who underwent upper lid retraction repair via the transconjunctival approach. Preoperative and postoperative basal and reflex tear testing was performed and was noted to be reduced in 11 of 24 cases. It was hypothesized that injury to the lacrimal secretory apparatus, from either the incision or dissection, was the cause of the decreased tear production.

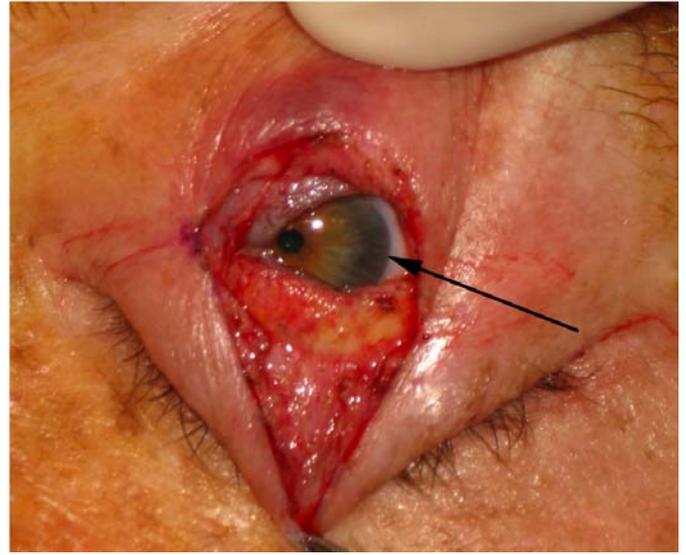
Due to the potential problems that can occur with a transverse conjunctival incision, some questions exist. For example, Is the transverse conjunctival incision necessary to release conjunctival fibrosis that an anterior retractor recession alone will not address?

Furthermore, there may be several compelling reasons to maintain the anatomic integrity of the conjunctiva: (1) less risk of complications (full-thickness lid fistulas), (2) less potential for injury to lacrimal secretory apparatus, and (3) less risk of flattening of the central lid curvature.



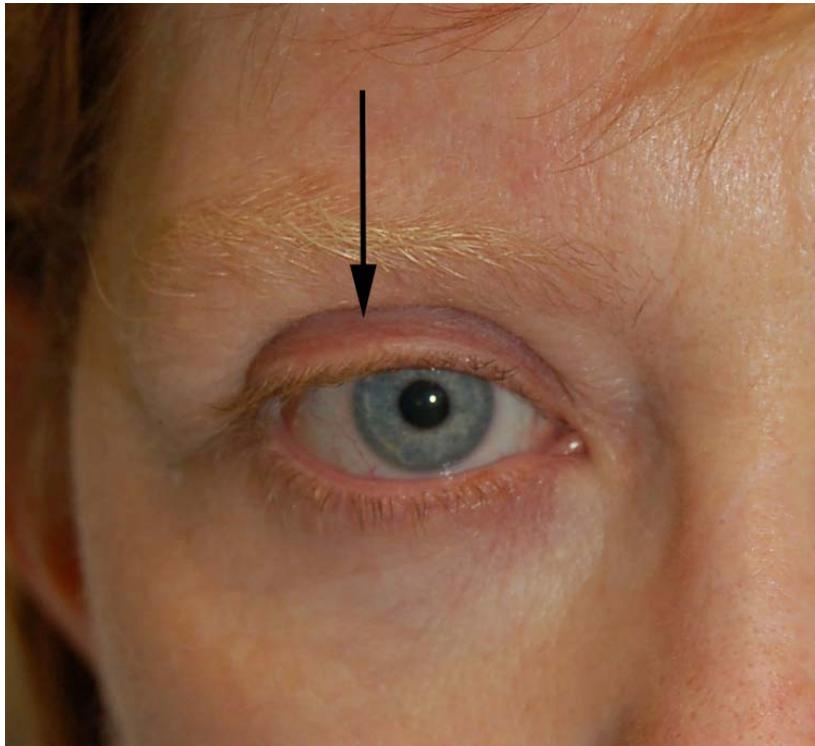
**FIGURE 4**

Intraoperative photo of anterior transcutaneous approach to eyelid retraction. Long arrow points to intact conjunctiva with black corneal protector visible through conjunctiva. Short arrow points to recessed edge of levator and Müller's muscle.



**FIGURE 5**

Intraoperative photo of anterior transcutaneous approach to eyelid retraction with combined transverse conjunctival incision (photo courtesy of David B. Lyon, MD). Note visible cornea through the blepharotomy (arrow).



**FIGURE 6**

Patient after undergoing upper eyelid retraction repair with full-thickness blepharotomy. Note central flattening of eyelid (arrow).

## GOALS OF THIS STUDY

This study was undertaken to perform a histopathologic analysis of the palpebral conjunctiva in patients with thyroid-related orbitopathy and to compare the findings to a control group of patients without autoimmune orbitopathy undergoing routine ptosis repair. Based on previously published anecdotal evidence, the hypothesis is that the conjunctiva does show increased inflammatory activity and fibrosis compared to controls.

## METHODS

This study was approved by the University of California San Diego Human Subjects Protection Program. A prospective nonrandomized comparative case series was undertaken. Two groups of patients were studied. The study group consisted of 20 patients diagnosed as having thyroid-related orbitopathy based on previously published criteria.<sup>33</sup> Ten patients (50%) were treated previously with radioactive iodine for control of hyperthyroidism. Three patients (15%) had prior surgical thyroidectomy. The control group had no underlying systemic disease and consisted of 18 patients undergoing conjunctival Müller's muscle resection for visually significant ptosis (Table 1). Patients with a history of previous ocular or eyelid surgery, contact lens use, conjunctival cicatrizing or allergic disorders, lid margin disease, autoimmune diseases, or prolonged use of ophthalmic medications, including corticosteroids, mast cell stabilizers, and ocular decongestants, were excluded from the study.

<b>CHARACTERISTIC</b>	<b>CONTROL GROUP</b>	<b>STUDY GROUP</b>
Gender (M/F)	5/13	6/14
Number of eyelids	18	20
Age (years)	56.1 ± 12.6	53.6 ± 7.7
Medical history		
History of RAI therapy	0	10
Current levothyroxine	0	9
Current methimazole	0	4

RAI, radioactive iodine.

## SURGICAL PROCEDURES

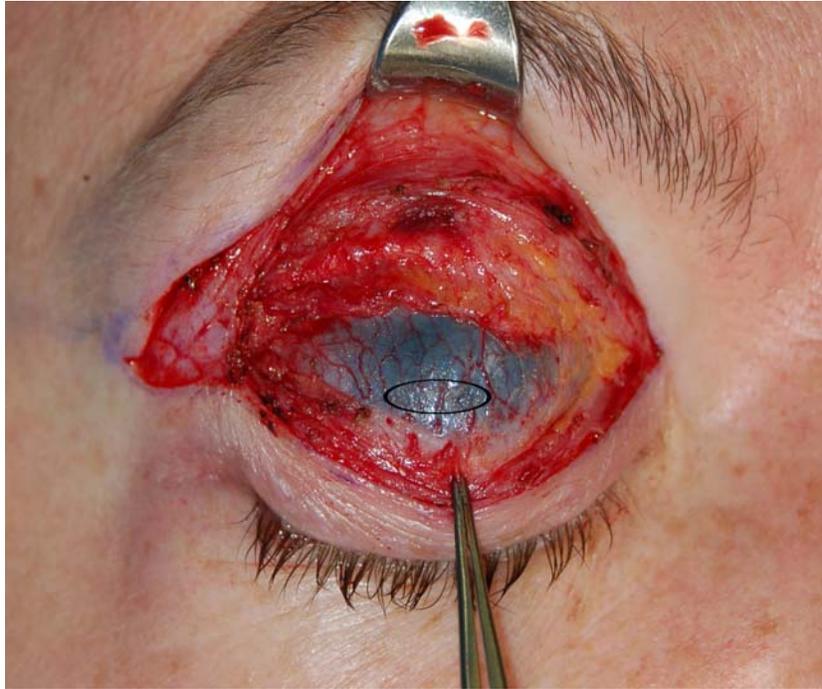
Standard informed consent for the proposed surgical procedure was obtained from all participants. Standard general anesthesia or monitored anesthesia care was utilized. No alterations were otherwise made in the standard of care.

For the control group, ptosis surgery was performed using a posterior approach that has been previously described.<sup>54</sup> Preoperatively, patients had a positive response to phenylephrine indicating a contractile Müller's muscle. During surgery, just above the central tarsus, 8 to 10 mm of conjunctiva and Müller's muscle is excised and typically discarded. Histopathologic examination of the typically discarded tissue was performed.

For the study group, all patients had eyelid retraction, defined by an upper lid margin to reflex distance (MRD) of greater than +6 mm, and were symptomatic with dryness and/or exposure keratopathy. All patients were in the stable phase of disease, defined by unchanged clinical measurements (MRD, exophthalmometry, and extraocular motility) for at least 6 months, when the eyelid retraction surgery was performed. Eyelid retraction surgery was performed anteriorly through an upper lid crease incision. The levator palpebrae superioris and Müller's muscle were recessed from the upper tarsal border in a similar manner previously described.<sup>55</sup> After the recession of both the levator palpebrae superior and Müller's muscle in a single complex, the isolated superior palpebral conjunctiva was then visible. A similar-sized specimen of central palpebral conjunctiva was obtained for biopsy just above the tarsus, corresponding to the same area of conjunctiva in the control group (Figure 7). One patient that had active disease who required early intervention for optic neuropathy was studied (Figure 8). During orbital decompression, conjunctival sampling was obtained.

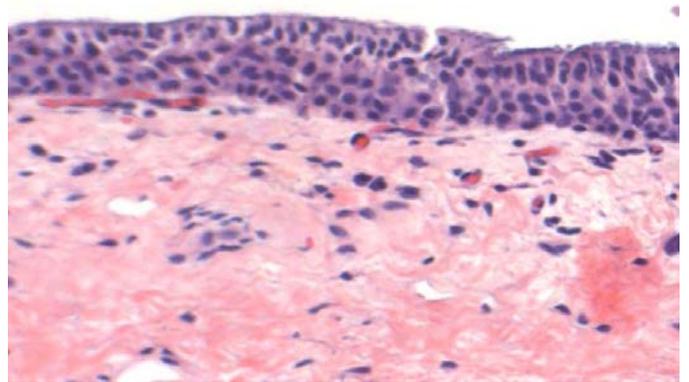
## SPECIMEN PROCESSING

After harvesting of the conjunctiva during the surgical procedure, the specimen was gently unfolded and laid flat on a piece of perforated nonstick dressing. The specimen was then placed in 10% neutral buffered formalin for fixation. The specimens were then removed and oriented with the epithelium facing up and embedded in paraffin. Microtome sections (5 to 6 µm) were made longitudinally (parallel to the long axis). The sections were then stained with hematoxylin-eosin and Masson trichrome. Specimens were grouped and stained together to ensure uniformity of staining. The slides were then examined under light microscopy in collaboration with two independent experienced board certified pathologists who were masked with regard to the group origin of the specimen.



**FIGURE 7**

Intraoperative photo of site where palpebral conjunctival sampling for this study is performed (oval).



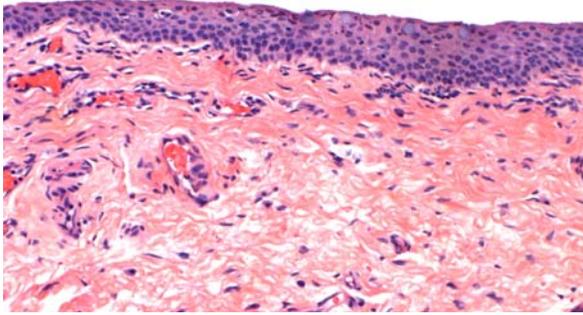
**FIGURE 8**

Left, Photo of a 63-year-old woman with active orbitopathy who underwent orbital decompression for thyroid-related orbitopathy. Note chemosis, eyelid swelling, and proptosis. Right, Conjunctival specimen of patient shown at left (hematoxylin and eosin, 10×10 original magnification). Inflammation is grade 1.

### **SPECIMEN GRADING**

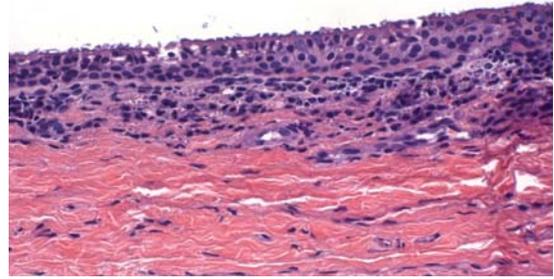
The degree of conjunctival inflammation was graded on an advancing scale from 0 to 4 (Figures 9 through 13). Masson trichrome–stained slides were used for determination of fibrosis in the subepithelial plane, while hematoxylin-eosin-stained slides were used for determination of inflammation from the presence of inflammatory cells. Also, for each specimen individually, the quantity of mast cells was counted in 10 high-powered fields (400× magnification), and the averages were recorded (Figure 14).

Only one specimen (eyelid) per patient was graded. For patients that had bilateral procedures done, the side that was graded was chosen randomly. For the patient with orbital decompression, only one side was studied.



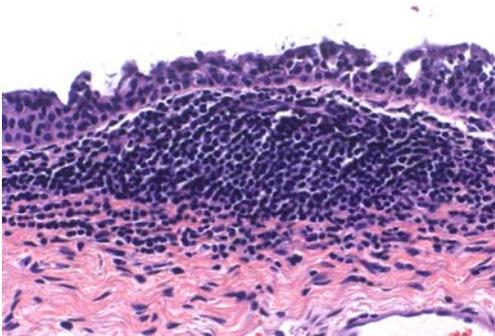
**FIGURE 9**

Control group patient 2, right eye. Inflammation, grade 0 (hematoxylin and eosin, 10×20 original magnification). Minimal inflammatory infiltrate is visible in substantia propria.



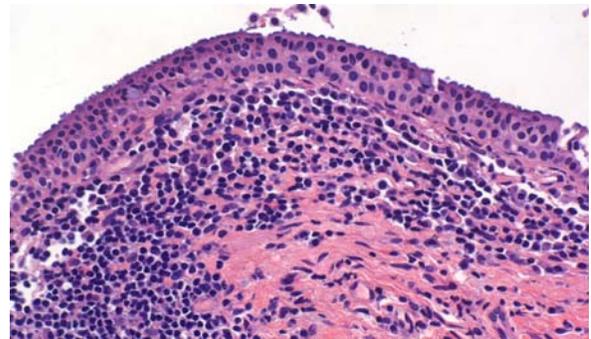
**FIGURE 10**

Control group patient 5, left eye. Inflammation, grade 1 (hematoxylin and eosin, 10×20 original magnification). Mild inflammatory infiltrate is visible in substantia propria.



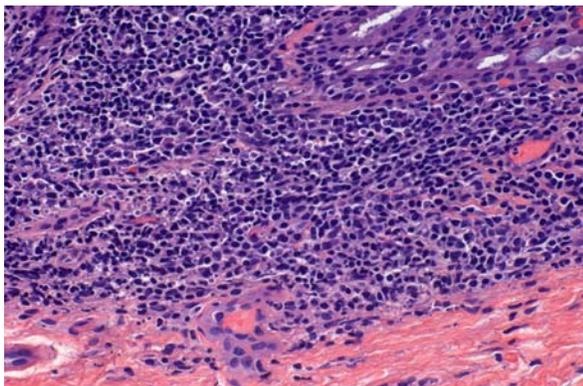
**FIGURE 11**

Study group patient 3, right eye. Inflammation, grade 2 (hematoxylin and eosin, 10×20 original magnification). Moderate inflammatory infiltrate is visible in substantia propria.



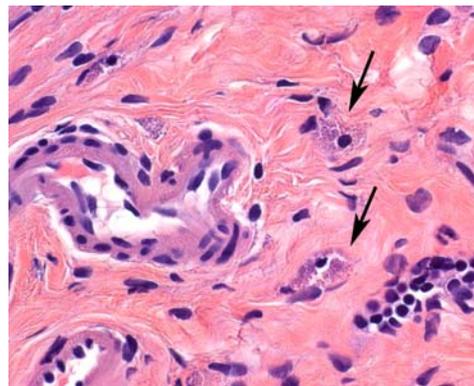
**FIGURE 12**

Control group patient 8, left eye. Inflammation, grade 3 (hematoxylin and eosin, 10×20 original magnification). Marked inflammatory infiltrate is visible in substantia propria.



**FIGURE 13**

Control group patient 7, right eye. Inflammation, grade 4 (hematoxylin and eosin, 10×20 original magnification). Severe inflammatory infiltrate is visible in substantia propria.

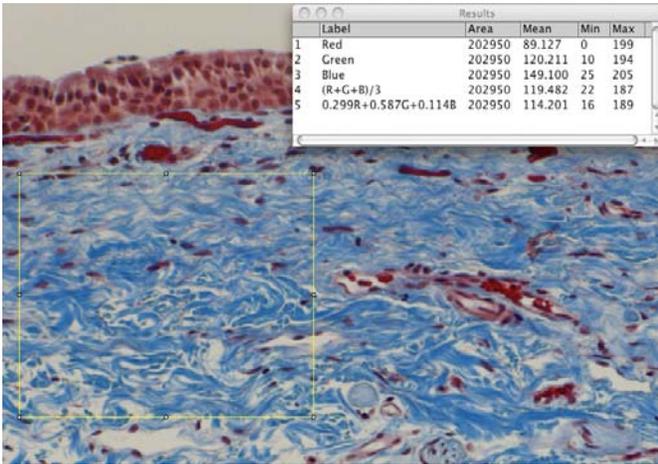


**FIGURE 14**

High-powered view of mast cells (arrows) (hematoxylin and eosin, 10×40 original magnification).

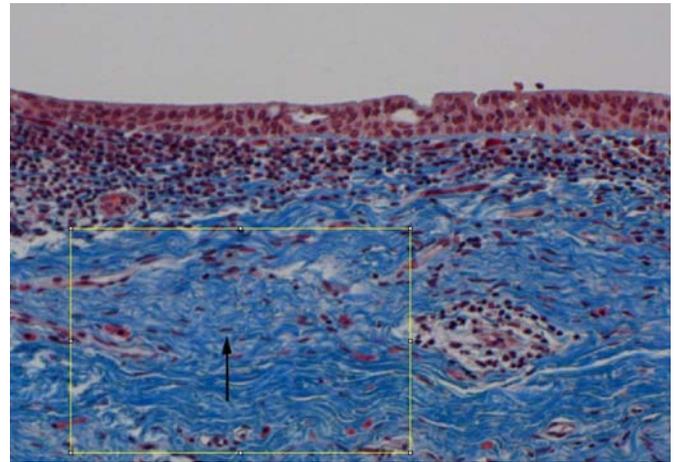
**QUANTITATIVE FIBROSIS ASSESSMENT**

Objective grading of fibrosis was done by examining each specimen in two ways. High-quality digital images of the trichrome-stained slides were made using standard photomicrograph techniques. The area examined was confined to the central portion of the substantia propria, measuring 1260 × 960 μm. First, the relative intensity of the blue stain was measured using Image J software (W. Rasband, Research Services Branch, National Institute of Mental Health, Bethesda, Maryland) by extracting the blue component from the RGB brightness value formula where intensity (V) equals 0.299R+0.587G+0.114B or (R+G+B)/3. The numeric value of the relative intensity of blue was then recorded (Figure 15). Second, quantitative measurements of the fibroblast population were made for each specimen also using Image J software. Only cells that were spindle-shaped and those cells not associated with vascular channels, nerves, or muscle were counted (Figure 16). A total of three areas were examined for each specimen, and the counts were averaged and recorded.



**FIGURE 15**

Study group patient 5, right eye. Relative blue intensity value measurement using Image J software. Area in yellow corresponds to sampling area (trichrome, 10×10 original magnification). Inset data set shows red, green, and blue values. The blue value of 149.1 was recorded for this specimen.



**FIGURE 16**

Fibroblast quantitative assessment using Image J software. Arrow points to spindle-shaped cell counted as a fibroblast in the substantia propria. Area outlined in yellow corresponds to sampling area (trichrome, 10×10 original magnification).

**RESULTS**

The study population characteristics are summarized in Table 1. The study group consisted of 20 specimens from 20 patients. There were 6 men (6 specimens) and 14 women (14 specimens) in the study group with an age range of 42 to 71 years (mean 53.6 ± 7.7). Ten patients had a history of radioactive iodine treatment. Nine had a history of smoking, and 5 were smoking at the time of surgery. The time from diagnosis of disease to the date of surgery ranged from 10 to 152 months (mean 37.3 ± 39.6). All patients were in the stable phase of their disease and had stable ophthalmic examinations with unchanged eyelid levels and exophthalmometry readings at least 6 months before surgery. No patient had chemosis, conjunctival injection, or caruncle edema. Twelve patients were euthyroid, 5 were hyperthyroid, and 3 were hypothyroid at the time of surgery. Ten (50%) had a history of undergoing prior radioactive iodine treatment. Nine patients were taking oral levothyroxine and four patients were taking oral methimazole.

The control group consisted of 18 patients with 18 specimens. There were 5 men (5 specimens) and 13 women (13 specimens) with an age range of 39 to 67 years (mean 56.1 ± 12.6). Seventeen patients had no prior history of any thyroid problems, and one patient had a remote history of a thyroid nodule being removed 20 years prior. Five of the 18 patients had serum thyroid studies done within 2 years of surgery, and all 5 were found to be euthyroid. There were no statistically significant differences between the study group and the control group with respect to age or gender. One control patient was found to have a high degree of fibrosis. Upon further questioning, it was determined that this patient previously had a long history of contact lens use but had since stopped wearing them. This patient was subsequently excluded from the study.

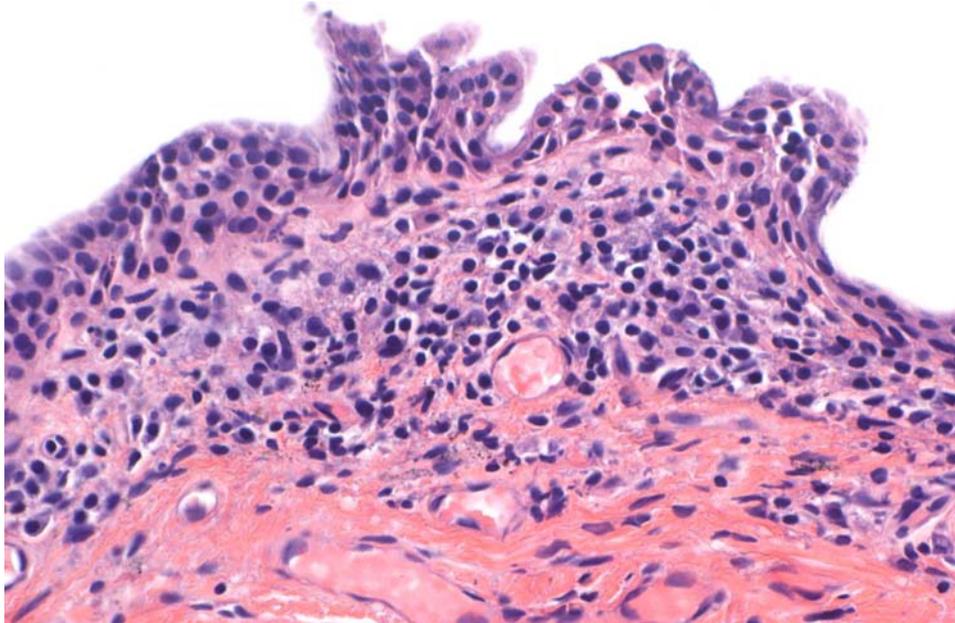
**CONTROL GROUP HISTOPATHOLOGY**

Eighteen specimens were examined after processing and staining. There were 8 specimens from right eyelids and 10 specimens from left eyelids. Light microscopy of hematoxylin-eosin-stained slides showed multifocal inflammation throughout the interstitial tissue of the palpebral conjunctiva. The infiltrating inflammatory cells consisted primarily of lymphocytes, but plasma cells and mast cells were also observed. On the scale of 0 to 4, the mean grade of inflammation was 1.4 (95% CI: 0.9, 1.9).

In examination for fibrosis, trichrome staining was utilized. The mean relative value for blue intensity was 134.3 (95% CI: 130.3,

138.3).

Under light microscopy, 10 high-powered fields were examined, and the number of mast cells were counted and averaged. The mean number of mast cells observed per high-powered field was  $1.3 \pm 1.6$ . Figure 17 shows a representative specimen from the control group. For fibroblast count, the mean was 27.3 (95% CI: 23.4, 31.2). The results for the control group are summarized in Table 2.

**FIGURE 17**

Control group patient 4, left eye.  
Inflammation, grade 1 (hematoxylin and eosin, 10×20 original magnification).

<b>TABLE 2. CONTROL GROUP: GRADING OF INFLAMMATION, RGB RELATIVE INTENSITY, MAST CELLS/HPF, AND FIBROBLAST COUNT</b>				
<b>CONTROL GROUP PATIENT/EYE</b>	<b>INFLAMMATION</b>	<b>RGB (RELATIVE INTENSITY)</b>	<b>MAST CELLS/HPF</b>	<b>FIBROBLAST COUNT</b>
1/OS	1	129.7	0.6	23
2/OD	1	133.3	0.2	17
3/OD	1	127.2	0.2	31
4/OS	0	131.8	0.2	32
5/OS	1	132.2	0.1	18
6/OS	4	159.4	6.2	22
7/OS	3	146.1	3.2	37
8/OD	1	134.7	2.2	42
9/OD	1	129.6	0.2	15
10/OS	2	141.2	0.8	24
11/OS	2	133.9	1.9	33
12/OD	1	122.8	2.6	19
13/OS	1	127.6	1.8	35
14/OD	2	135.9	0.2	40
15/OD	0	129.5	0.4	22
16/OS	1	126.3	0.2	19
17/OS	1	142.2	0.8	29
18/OD	3	134.1	1.4	33

HPF, high-powered field; RGB, red, green, blue.

**STUDY GROUP HISTOPATHOLOGY**

Twenty specimens were examined after processing and staining. There were 11 specimens from right eyelids and 9 specimens from left eyelids. The appearance of the conjunctiva in the thyroid-related orbitopathy group when stained with hematoxylin-eosin was similar to that of the negative controls. Multifocal areas of inflammation were noted with none of the specimens showing diffuse involvement. The inflammatory cellular composition was varied, consisting primarily of lymphocytes but also displaying the presence of plasma cells and mast cells. On the scale of 0 to 4, the mean grade of inflammation was 1.7 (95% CI: 1.3, 2.1).

In examination for fibrosis, trichrome stains were again utilized. The mean relative value for blue intensity was 138.6 (95% CI: 133.7, 143.6).

After examination of 10 high-powered fields per specimen, the mean number of mast cells observed per high-powered field was 1.5 +/- 1.1. For fibroblast count, the mean was 27.6 (95% CI: 23.5, 31.7). The results from the study group are summarized in Table 3.

The correlation between duration of disease and grades of inflammation and fibrosis was also examined. Poor correlation was noted between the duration of ocular disease prior to conjunctival biopsy and the degree of inflammation ( $r = 0.04, P=.43$ ) (Figure 18).

**TABLE 3. STUDY GROUP: GRADING OF INFLAMMATION, RGB RELATIVE INTENSITY, MAST CELLS/HPF, AND FIBROBLAST COUNT**

STUDY GROUP PATIENT /EYE	INFLAMMATION	RGB (RELATIVE INTENSITY)	MAST CELLS/HPF	FIBROBLAST COUNT
1/OS	2	151.5	1.0	17
2/OD	2	139.6	1.3	26
3/OD	4	147.1	4.2	41
4/OS	2	121.1	2.0	12
5/OD	0	149.1	0.3	36
6/OS	1	129.2	0.9	25
7/OS	2	133.0	0.8	39
8/OD	2	126.9	3.4	32
9/OD	0	157.4	0.5	29
10/OD	1	140.2	1.8	13
11/OD	1	136.9	0.6	17
12/OS	1	135.7	0.8	23
13/OD	2	149.2	2.4	22
14/OD	1	122.5	1.6	26
15/OD	2	125.3	0.8	19
16/OS	1	123.1	3.2	32
17/OS	2	149.7	2.4	39
18/OD	3	150.2	1.8	33
19/OS	2	147.2	0.2	27
20/OS	2	136.5	0.2	43

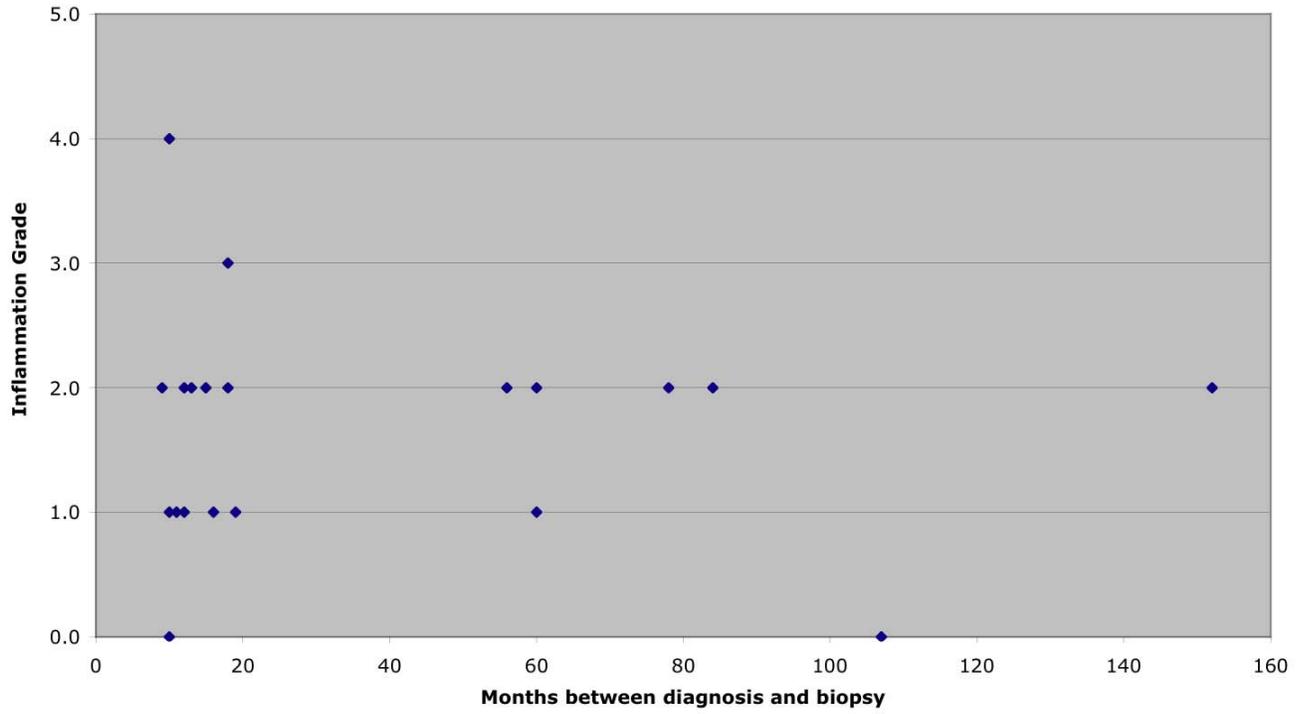
HPF, high-powered field; RGB, red, green, blue.

**COMPARISON BETWEEN GROUPS**

Several comparisons were made between the study and control groups. Using the Mann-Whitney test, comparisons were made between groups for grades of inflammation, grades of fibrosis, and mast cells per high-powered field. For inflammation, there was no statistical difference ( $P=.17$ ). For relative blue intensity, there was no statistical difference ( $P=.11$ ). For fibroblast counts, there was no statistical difference ( $P=.45$ ). For mast cells per high-powered field, a Student *t* test was used. There was no statistical difference ( $P=.61$ ).

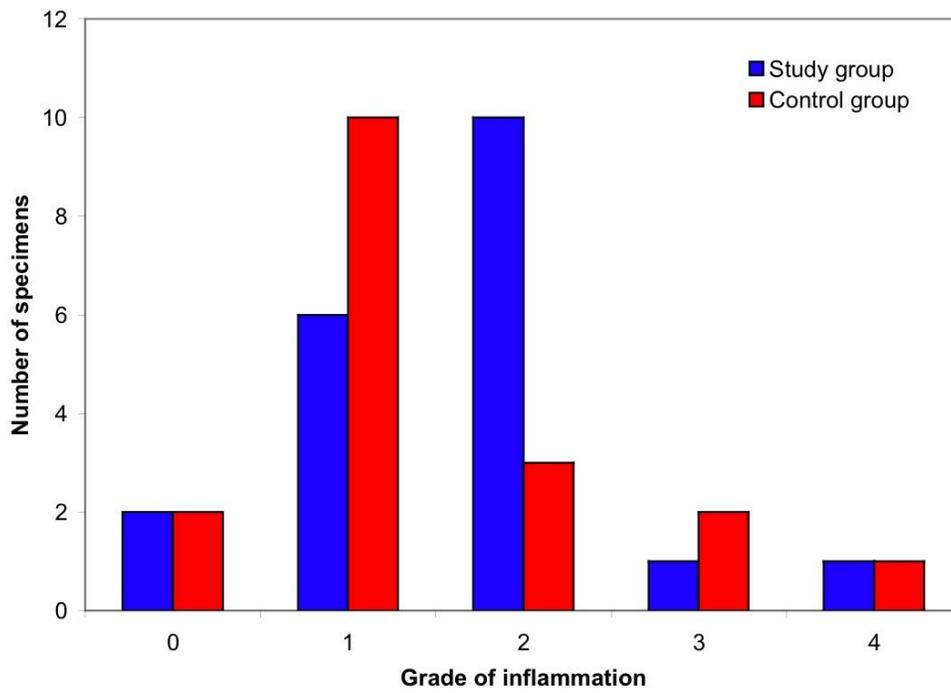
Figure 19 depicts the number of specimens vs grade of inflammation. When looking at Figure 19, it appears that a higher percentage of patients in the study group have a higher grade of inflammation. A subgroup analysis was performed to determine if there was any correlation between a history of radioactive iodine and inflammation. Using the Mann-Whitney test, for inflammation, there was no difference between groups ( $P=.29$ ).

Using point estimates, for a grade of inflammation greater than or equal to 2, the study group was 0.59 at the 95% CI and the control group was 0.35, indicating a trend toward more inflammation in the study group.



**FIGURE 18**

Disease duration vs degree of inflammation for study group.



**FIGURE 19**

Histogram of degree of inflammation vs number of eyes for both study and control groups.

## DISCUSSION

The pathophysiology of thyroid-related orbitopathy is not well understood, but progress is being made. It is generally believed that an autoimmune inflammatory cellular infiltration activates orbital fibroblasts and eventually leads to an increase in orbital volume, by either ground substance deposition or adipogenesis, causing proptosis.<sup>4-8</sup> The molecular mechanisms are unclear, but several possibilities have been proposed.<sup>17-22,28,29</sup>

Several investigators have previously examined histopathologic changes in specimens from patients with thyroid-related orbitopathy. In an exenterated specimen of a patient with Graves disease, Hufnagel and colleagues<sup>24</sup> showed massive extraocular enlargement due to endomysial fibrosis, mucopolysaccharide deposition, and perivascular lymphocytic and plasmacytic infiltration. Matos and coworkers<sup>26</sup> studied specific changes in the extraocular muscles. They found weak cellular reactions and only scattered inflammatory cells, suggesting that the inflammation was localized and not equally distributed throughout the muscle. Interestingly, fibrosis in muscle fibers was found in only one case, and the duration of disease in this case was only 7 months, indicating a somewhat unpredictable nature. Our study showed a similar pattern, with palpebral conjunctival fibrosis, as judged by trichrome staining, being no more severe in thyroid patients than in controls. This was further quantified with fibroblast counts, which were also similar between groups.

Orbital infiltration with mast cells has been noted with increased numbers in both animal studies<sup>27</sup> and patients with Graves disease.<sup>26</sup> Mast cells are increased in a variety of conditions, including chronic inflammation, edema and lymphedema, healing wounds, and in the stroma of benign and malignant tumors.<sup>26</sup> While some hypothesize that mast cells play an integral role in fibroblast proliferation and resultant proptosis,<sup>14</sup> others feel that the presence of mast cells represents a nonspecific response in association with the chronic inflammatory process and connective tissue proliferation and is not of etiologic significance.<sup>6,26</sup> Our study showed no difference between groups, but there was a correlation between increased numbers of mast cells and degree of inflammation in both groups. When point estimates with confidence intervals are applied, some differences are apparent. When looking at the group of patients with an inflammation grade of 2 or higher, there is a definite trend toward higher inflammation in the study group.

There was a wide range of time from onset of disease to surgery in our study group, from 10 to 152 months (mean  $37.3 \pm 39.6$ ). It is definite that our sample population does not encompass nor represent every phase of this chronic disease. All patients were clinically stable; there was a sampling bias against the inflammatory and in favor of the quiescent phases of the disease. Despite this, we did not find any correlation between the duration of disease and the degree of inflammation or fibrosis. It is likely that more exuberant cellular reactions occur at earlier stages of the disease.<sup>30</sup> However, one would expect to see fibrosis later in the chronic phase, particularly if there was significant prior inflammation to cause upper eyelid retraction. No increase in fibrosis was noted. A greater cross section of patients might have been examined if varying stages of disease were studied; however, there are few indications for surgery in the inflammatory stage of the disease. Surgery in the inflammatory phase is primarily limited to orbital decompression, and it is typically indicated in cases of optic neuropathy and/or exposure keratopathy. We did study one patient who underwent acute orbital decompression in the active phase. There was minimal inflammation and no increase in fibrosis noted. Primary eyelid surgery is rarely performed in this setting, in this subset of patients; hence the opportunity to obtain tissue would have been limited. It is also possible that sampling error might have played a role. Many studies show a patchy inflammation, and it is possible that the affected areas might have been missed during the tissue sampling.

Radioactive iodine (RAI) treatment for hyperthyroidism has been shown to clinically worsen orbitopathy in selected patients.<sup>56</sup> Oral prednisone has been shown to reduce the risk of worsening inflammation in patients with preexisting orbitopathy. Fifty percent of our study group had a history of RAI treatment, and because of this reported association, an analysis was done to see if there was any worsening in this subgroup. We were unable to find any differences; however, our subgroup was small.

Eyelid retraction in thyroid-related orbitopathy can have multiple causes. Inflammatory infiltration of both the levator palpebrae superioris and Müller's muscle has been described.<sup>34,35</sup> Fixation duress from a tight inferior rectus muscle can also cause upper lid elevation and typically improves with recession of the inferior rectus muscle.<sup>57</sup> Proptosis itself can worsen lid retraction and lateral flaring.<sup>58</sup> Finally, conjunctival fibrosis has been noted in one study,<sup>31</sup> but only three specimens were examined and were not compared to controls.

Clinically, eyelid retraction surgery is challenging and especially difficult in some instances. In these cases, it may be necessary to release the conjunctiva. George Bartley,<sup>59</sup> in his discussion of Elnor's manuscript "Graded Full Thickness Anterior Blepharotomy for Upper Eyelid Retraction," further reinforces this, noting that sometimes the lid would "not drop until all layers of the eyelid had been transected." Furthermore, Bartley<sup>59</sup> stated that his own investigation into the ultrastructural histology of the conjunctiva showed that it "disappointingly, appeared unremarkable." It may be that because of the intimate relationship of Müller's muscle to the conjunctiva, there are adherent fibers that are clinically indistinct and unable to be dissected, necessitating that the surgeon transect the conjunctiva to fully release the eyelid. In our own experience, we have found this to be the rare exception rather than the rule, and we keep the conjunctiva intact in the vast majority of cases. Furthermore, as mentioned previously, there are compelling reasons to maintain the integrity of the conjunctiva.

This study does have some limitations. First, the sample size is small and may be inadequate to detect differences between the two populations. Second, the conjunctival specimens were limited in size and may not be representative of the ocular surface as a whole. Thus, sampling bias may occur and the area examined may not include areas of involvement. Third, the control group was not entirely "normal" in that ptosis was present and may not represent the true undiseased state. Acquired ptosis has been associated primarily with aponeurotic changes in the levator and not the conjunctiva. However, we could find no literature to support that the conjunctiva

shows any pathologic findings in these patients. Fourth, as mentioned, because of the diversity of multiphasic intervals of this disease, our sampling might not occur at the times of peak inflammation or fibrosis. It is possible that studying larger numbers of patients in each phase of the disease may uncover differences between groups. Finally, it is also possible that in our study group, the time from disease onset to surgery was insufficient for fibrosis to develop. Nonetheless, to our knowledge, this is the first study to histopathologically examine the palpebral conjunctiva of thyroid-related orbitopathy patients against controls, and we were unable to demonstrate any statistical difference between patients in the static phase of thyroid-related orbitopathy and the control group.

## CONCLUSION

The histopathology of the superior palpebral conjunctiva has been studied in patients in the static phase of thyroid-related orbitopathy and compared to a nonautoimmune control group undergoing surgery for ptosis. Although there is a trend toward greater inflammation in the thyroid-related orbitopathy group, we did not find any statistical differences in inflammation, fibrosis, mast cell infiltration, or quantitative fibroblast count in the substantia propria between the two groups, suggesting that the palpebral conjunctiva is largely spared in thyroid-related orbitopathy.

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## REFERENCES

1. Perros P, Kendall-Taylor P. Natural history of thyroid eye disease. *Thyroid* 1998;8:423-425.
2. Werner SC. The eye changes of Graves' disease: overview. *Mayo Clin Proc* 1972;47:969-974.
3. Weetman AP. Graves disease 1835-2002. *Horm Res* 2003;59(suppl 1):S114-118.
4. Hennemann G. Historical aspects about the development of our knowledge of morbus Basedow. *J Endocrinol Invest* 1991;14:617-624.
5. Rootman J, Dolman PJ. Thyroid orbitopathy. In: Rootman J, ed. *Diseases of the Orbit. A Multidisciplinary Approach*. 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 2003:169-175.
6. Riley F. Orbital pathology in Graves' disease. *Mayo Clin Proc* 1972;47:975-986.
7. Bartley GB. The epidemiologic characteristics and clinical course of ophthalmopathy associated with autoimmune thyroid disease in Olmsted County, Minnesota. *Trans Am Ophthalmol Soc* 1994;92:477-588.
8. Kiljanski J, Nebes V, Stachura I, Kennerdell JS, Wall JR. Should Graves' disease be considered a collagen disorder of the thyroid, skeletal muscle and connective tissue? *Horm Metab Res* 1995;27:528-532.
9. Weetman AP. Thyroid-associated eye disease: pathophysiology. *Lancet* 1991;338:25-28.
10. Khoo TK, Bahn RS. Pathogenesis of Graves' ophthalmopathy: the role of autoantibodies. *Thyroid* 2007;17:1013-1018.
11. Bahn RS. Cytokines in thyroid eye disease: potential for anticytokine therapy. *Thyroid* 1998;8:415-418.
12. Heufelder AE. Pathogenesis of ophthalmopathy in autoimmune thyroid disease. *Rev Endocr Metab Disord* 2000;1:87-95.
13. Hatton MP, Rubin PA. The pathophysiology of thyroid-associated ophthalmopathy. *Ophthalmol Clin North Am* 2002;15:113-119.
14. Ludgate M, Baker G. Unlocking the immunological mechanisms of orbital inflammation in thyroid eye disease. *Clin Exp Immunol* 2002;127:193-198.
15. Smith TJ, Hoa N. Immunoglobulins from patients with Graves' disease induce hyaluronan synthesis in their orbital fibroblasts through the self-antigen, insulin-like growth factor-I receptor. *J Clin Endocrinol Metab* 2004;89:5076-5080.
16. Konuk O, Hondur A, Akyurek N, Unal M. Apoptosis in orbital fibroadipose tissue and its association with clinical features in Graves' ophthalmopathy. *Ocul Immunol Inflamm* 2007;15:105-111.
17. Boschi A, Daumerie C, Spiritus M, et al. Quantification of cells expressing the thyrotropin receptor in extraocular muscles in thyroid associated orbitopathy. *Br J Ophthalmol* 2005;89:724-729.
18. Cao HJ, Wang HS, Zhang Y, Lin HY, Phipps RP, Smith TJ. Activation of human orbital fibroblasts through CD40 engagement results in a dramatic induction of hyaluronan synthesis and prostaglandin endoperoxide H synthase-2 expression. Insights into potential pathogenic mechanisms of thyroid-associated ophthalmopathy. *J Biol Chem* 1998;273:29615-29625.

19. Douglas RS, Gianoukakis AG, Kamat S, Smith TJ. Aberrant expression of insulin-like growth factor-1 receptor by T cells from patients with Graves disease may carry functional consequences for disease pathogenesis. *J Immunol* 2007;178:3281-3287.
20. Konuk EB, Konuk O, Misirlioglu M, Menevse A, Unal M. Expression of cyclooxygenase-2 in orbital fibroadipose connective tissues of Graves' ophthalmopathy patients. *Eur J Endocrinol* 2006;155:681-685.
21. Smith TJ. The putative role of prostaglandin endoperoxide H synthase-2 in the pathogenesis of thyroid-associated orbitopathy. *Exp Clin Endocrinol Diabetes* 1999;107(Suppl 5):S160-163.
22. Smith TJ, Parikh SJ. HMC-1 mast cells activate human orbital fibroblasts in coculture: evidence for up-regulation of prostaglandin E2 and hyaluronan synthesis. *Endocrinology* 1999;140:3518-3525.
23. Wegelius O, Ashoe-Hansen G, Lamberg BA. Retrobulbar connective tissue changes in malignant exophthalmos. *Acta Endocrinol (Copenh)* 1957;25:452-456.
24. Hufnagel TJ, Hickey WF, Cobbs WH, Jakobiec FA, Iwamoto T, Eagle RC. Immunohistochemical and ultrastructural studies on the exenterated orbital tissues of a patient with Graves' disease. *Ophthalmology* 1984;91:1411-1419.
25. Chen RJ, Kagoshima T, Ishii Y, Hori S, Inoue Y. Histopathological observations on orbital fatty tissue in dysthyroid ophthalmopathy. *Nippon Ganka Gakkai Zasshi* 1990;94:846-855.
26. Matos K, Nose V, Manso PG, et al. Correlation between clinical and histological analyses in retroocular connective tissues and extraocular muscles from patients with Graves' ophthalmopathy. *Endocr Pathol* 2000;11:185-194.
27. Ashoe-Hansen G, Iversen K. Influence of thyrotrophic hormone on connective tissue; pathogenetic significance of mucopolysaccharides in experimental exophthalmos. *Acta Endocrinol (Copenh)* 1951;8:90-96.
28. Salvi M, Scalise D, Stolarski C, et al. Upper eyelid retraction in the absence of other evidence for progressive ophthalmopathy is associated with eye muscle autoantibodies. *Clin Immunol Immunopathol* 1995;74:44-50.
29. Hiromatsu Y, Fukazawa H, How J, Wall JR. Antibody-dependent cell-mediated cytotoxicity against human eye muscle cells and orbital fibroblasts in Graves' ophthalmopathy—roles of class II MHC antigen expression and gamma-interferon action of effector and target cells. *Clin Exp Immunol* 1987;70:593-603.
30. Tallstedt L, Norberg R. Immunohistochemical staining of normal and Graves' extraocular muscle. *Invest Ophthalmol Vis Sci* 1988;29:175-184.
31. Heufelder AE. Involvement of the orbital fibroblast and TSH receptor in the pathogenesis of Graves' ophthalmopathy. *Thyroid* 1995;5:331-340.
32. Nunery WR, Martin RT, Heinz GW, Gavin TJ. The association of cigarette smoking with clinical subtypes of ophthalmic Graves' disease. *Ophthal Plast Reconstr Surg* 1993;9:77-82.
33. Bartley GB, Gorman CA. Diagnostic criteria for Graves' ophthalmopathy. *Am J Ophthalmol* 1995;119:792-795.
34. Grove AS Jr. Upper eyelid retraction and Graves' disease. *Ophthalmology* 1981;88:499-506.
35. Cockerham KP, Hidayat AA, Brown HG, Cockerham GC, Graner SR. Clinicopathologic evaluation of the Mueller muscle in thyroid-associated orbitopathy. *Ophthal Plast Reconstr Surg* 2002;18:11-17.
36. Gopinath B, Adams CL, Musselman R, Tani J, Wall JR. Antibodies against caldesmon and type XIII collagen are good markers for chronic upper eyelid retraction. *Ocul Immunol Inflamm* 2007;15:81-88.
37. Baker GR, Morton M, Rajapaska RS, et al. Altered tear composition in smokers and patients with Graves ophthalmopathy. *Arch Ophthalmol* 2006;124:1451-1456.
38. Khalil HA, De Keizer RJ, Bodelier VM, Kijlstra A. Secretory IgA and lysozyme in tears of patients with Graves' ophthalmopathy. *Doc Ophthalmol* 1989;72:329-334.
39. Mourits MP, Prummel MF, Wiersinga WM, Koornneef L. Clinical activity score as a guide in the management of patients with Graves' ophthalmopathy. *Clin Endocrinol (Oxf)* 1997;47:9-14.
40. Passons GA, Wood TO. Conjunctival resection for superior limbic keratoconjunctivitis. *Ophthalmology* 1984;91:966-968.
41. de Almeida SF, de Sousa LB, Vieira LA, Chiamollera MI, de Nadai Barros J. Clinic-cytologic study of conjunctivochalasis and its relation to thyroid autoimmune diseases: prospective cohort study. *Cornea* 2006;25:789-793.
42. Ozkan SB, Söylev MF, Vahapoglu H, Can D, Arsan AK, Duman S. Evaluation of conjunctival morphology in thyroid associated eye disease by use of impression cytology. *Acta Ophthalmol Scand* 1997;75:145-147.
43. Elner VM, Hassan AS, Frueh BR. Graded full-thickness anterior blepharotomy for upper eyelid retraction. *Arch Ophthalmol* 2004;122:55-60.
44. Hales IB, Rundle FF. Ocular changes in Graves' disease. A long term follow-up study. *Q J Med* 1960;29:113-126.
45. Prummel MF, Wiersinga WM. Medical management of Graves ophthalmopathy. *Thyroid* 1995;5:231-234.
46. Farid M, Roch-Levecq C, Brody BL, Granet DB, Levi L, Kikkawa DO. Psychological disturbance in Graves ophthalmopathy. *Arch Ophthalmol* 2005;123:491-496.
47. Shorr N, Seiff SR. The four stages of surgical rehabilitation of the patient with dysthyroid orbitopathy. *Ophthalmology* 1986;93:476-483.
48. Gay AJ, Wolkstein MA. Topical guanethidine therapy for endocrine lid retraction. *Arch Ophthalmol* 1966;76:364-367.
49. Uddin JM, Davies PD. Treatment of upper eyelid retraction associated with thyroid eye disease with subconjunctival botulinum toxin injection. *Ophthalmology* 2002;109:1183-1187.
50. Woog JJ, Hartstein M, Hoening J. Adjustable suture technique for levator recession. *Arch Ophthalmol* 1996;114:620-624.

51. Ben Simon G, Mansury AM, Schwarz RM, Modjtahedi S, McCann JD, Goldberg RA. Transconjunctival Mueller muscle recession with levator disinsertion for correction of eyelid retraction associated with thyroid related orbitopathy. *Am J Ophthalmol* 2005;140:94-99.
52. Looi AL, Sharma B, Dolman PJ. A modified posterior approach for upper eyelid retraction. *Ophthal Plast Reconstr Surg* 2006;22:434-437.
53. George JL, Tercero ME, Angioi-Duprez K, Maalouf T. Risk of dry eye after muellerectomy via the posterior conjunctival approach for thyroid-related upper eyelid retraction. *Orbit* 2002;21:19-25.
54. Dresner SC. Further modifications of the Mueller's muscle conjunctival resection for blepharoptosis. *Ophthal Plast Reconstr Surg* 1991;7:114-122.
55. Older JJ. Surgical treatment of eyelid retraction associated with thyroid eye disease. *Ophthalmic Surg* 1991;22:318-322.
56. Bartalena L, Marcocci C, Bogazzi F, et al. Relation between therapy for hyperthyroidism and the course of Graves' ophthalmopathy. *N Engl J Med* 1998;338:73-78.
57. Hamed LM, Lessner AM. Fixation duress in the pathogenesis of upper eyelid retraction in thyroid orbitopathy. A prospective study. *Ophthalmology* 1994;101:1608-1613.
58. Lemke BN. Anatomic considerations in upper eyelid retraction. *Ophthal Plas Reconstr Surg* 1991;7:158-166.
59. Elner VM, Hassan AS, Frueh BR. Graded full-thickness anterior blepharotomy for upper eyelid retraction. *Trans Am Ophthalmol Soc* 2003;101:67-73;discussion 73-75.