Histopathological and electron microscopic study for different grades of inferior oblique muscle overaction

Momen M Hamdi1
Golzamine R El-Hawary1
Nadia G El-Hefnawy2
Manal I Salman2
1Ophthalmology Department, 2Pathology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt

Purpose: To correlate between the clinical degree of inferior oblique muscle (IO) overaction and the histopathological changes of the muscle.

Settings: Departments of Ophthalmology and Pathology, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Patients and methods: Biopsies from 12 IO muscles were taken during strabismus surgery for cases of IO muscle overaction. One biopsy from a normal IO was taken as a control. All samples were examined under light microscopy and transmission electron microscopy.

Results: In light microscopy, all cases showed histopathological changes, in the form of degenerated and regenerating muscle fibers, increased fibrofatty infiltration, and mild variability of muscle fiber size. Hypertrophied nerve bundles also appeared in biopsies from patients with grade II and grade III IO overaction. Endomysial and perimysial fibrosis, mononuclear inflammatory infiltrates, and focal fatty infiltration were seen in biopsies from cases of grade III IO overaction. In electron microscopy, ultrastructural examination revealed an increased number of mitochondria associated with some degree of mitochondrial pleomorphism. Hypercontracted fibers, vacuoles, and fat droplets were also noticed.

Conclusion: IO overaction is always accompanied by histopathological changes that differ in severity according to the clinical grading of the overaction. Changes in nerve fibers can also occur in severe cases.

Keywords: inferior oblique, electron microscope, overaction, histopathological, muscle fibers

Introduction
Disturbances of ocular motility often include inferior oblique (IO) muscle overaction, which, clinically, is classified into primary and secondary types.1 The primary type is of unknown cause and is usually bilateral, whereas the secondary type is usually unilateral.1 A secondary cause is weakness of IO muscle’s direct antagonist, the superior oblique muscle, which may be congenital or acquired. IO overaction might also be due to the weakness of IO muscle’s contralateral synergist, the superior rectus muscle.2-3

IO overaction can be graded clinically from I to III. Grade I presents mild overaction, only noticed in the extreme “up-in” gaze. Grade II presents moderate overaction, seen in the “adducted” gaze. Grade III shows severe overaction seen in the primary gaze.4

Biopsy can be easily taken from the IO muscle without causing any undesirable results during myectomy. This facilitates histopathological examination.5
The extraocular muscles (EOMs) are comprised of striated muscle fibers, separated by connective tissue (epimysium). Each fiber has a sheath (the sarcolemma). The fiber unit contains the contractile tissue (the myofibrils), the nucleus, and the ground substance (the sarcoplasm) which contain the organelles. Histologically, EOMs, with a unique two-fiber system, differ from the other skeletal muscles. Resembling the usual skeletal muscle is the “fibrillenstruktur” fiber, which contains small, well-organized myofibrils surrounded by abundant sarcoplasm, large concentrations of mitochondria, and a nucleus that is usually situated peripherally. Each sarcomere has an orderly tubular (T) system. The unique striated muscle fiber found in the EOMs is the “felderstruktur” fiber, containing large, partially fused myofibrils embedded in scanty cytoplasm, a virtual absence of concentrations of mitochondria, and a nucleus that is usually located centrally. The sarcomeres are nearly devoid of a T system. Electron microscopy of both types of fibers reveals their histological differences. Light microscopy of a transverse section of an EOM shows the “fibrillenstruktur” fibers staining palely with regularly spaced myofibrils, while the “felderstruktur” fibers stain more densely, with irregularly clumped myofibrils.

The present study aimed to investigate the histopathological and electron microscopic changes that occur in different grades of IO overaction and correlate them to the degree of overaction.

Patients and methods

The study included biopsies of twelve IO muscles, collected during strabismus surgery from seven patients (unilateral biopsy from two and bilateral biopsies from five). All cases were chosen from the strabismus specialized clinic at Ain Shams University Specialized Hospital, Cairo, Egypt.

The cases belonged to two clinical groups, moderate and severe (grade II and III, respectively), since grade I is not a candidate for surgical intervention. For comparison, a biopsy was taken from a normal IO muscle of an enucleated eye. Among the above findings, endomysial and perimysial fibroses (Figure 1C) were noted in three of the twelve biopsies (25%). Prominent hypertrophied nerve bundles (Figure 1A–D) were seen in six of the twelve biopsies (50%).

Table 1 presents the clinical features and the histopathological findings of all cases.

Histopathological findings

Light microscopic features

Mild to moderate variability in muscle fiber size was seen in all biopsies (Figure 1A). Degenerated fibers, hypercontracted fibers (Figure 1B), and regenerating fibers (Figure 1C) were seen in seven out of twelve biopsies (58.3%). Many round fibers (Figure 1A) were seen in three of the twelve biopsies (25%), and focally split fibers were seen in two of the twelve biopsies (16.6%). Focal fatty infiltration of the fibers was noted in one of the twelve biopsies (8.33%). Mononuclear inflammatory cell infiltrate (Figure 1D) was noted in three of the twelve biopsies (25%). Endomysial and perimysial fibroses (Figure 1C) were noted in three of the twelve biopsies (25%). Prominent hypertrophied nerve bundles (Figure 1A–D) were seen in six of the twelve biopsies (50%).

Among the above findings, endomysial and perimysial fibrosis, mononuclear inflammatory infiltrate, and focal fatty infiltration were seen in biopsies from cases of grade III only (25%), whereas the rest of the findings were seen in
biopsies from cases of both grade II and grade III, but they were more profound in grade III.

Electron microscopic features

Many fibers contained numerous mitochondria. The mitochondria showed mild pleomorphism and dense matrix (Figure 2A). They formed subsarcolemmal aggregates or were found dispersed between the myofilaments (Figure 2B and C).

Some fibers were hypercontracted and contained many vacuoles, including lipid ones (Figure 2D). Disarray of myofilaments along with focal loss was also noted in some fibers. Some fibers showed large vesicular nuclei with prominent nucleoli (Figure 2E). Degenerated mitochondria with vacuolation were also noted by higher magnification (Figure 2F).

These findings were noted in biopsies from cases of both clinical grades II and III, but they were more profound in grade III.

**Discussion**

Vertical strabismus can result from primary and secondary IO overaction. Suitable biopsies can be taken easily from this muscle without adverse effects. This allows its histopathological examination by light and electron microscopy.3

The current study included twelve biopsies of IO muscles from patients complaining of unilateral or bilateral vertical strabismus of different etiologies. Nine out of twelve biopsies (75%) belonged to grade II and three out of twelve biopsies (25%) belonged to grade III. All biopsies examined showed histopathological and ultrastructural abnormalities.

**Table 1 Clinical features of the patients**

<table>
<thead>
<tr>
<th>Case no</th>
<th>Diagnosis and grade of overaction</th>
<th>Surgical procedure</th>
<th>Histopathological findings (LM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alternating accommodative esotropia with bilateral IO overaction, grade II (Rt &gt; Lt)</td>
<td>Bilateral IO myectomy (Rt &gt; Lt)</td>
<td>Hypertrophied nerve bundles, few scattered degenerated muscle fibers</td>
</tr>
<tr>
<td>2</td>
<td>Bilateral IO overaction, Rt grade II, Lt grade III</td>
<td>Bilateral IO myectomy (Lt &gt; Rt)</td>
<td>Endomyosal fibrosis, perimysial fibrosis, moderate variability in muscle fiber size</td>
</tr>
<tr>
<td>3</td>
<td>Bilateral IO overaction, grade II (Lt &gt; Rt)</td>
<td>Bilateral IO myectomy (Lt &gt; Rt)</td>
<td>Hypertrophied nerve bundles, mild variability in muscle fiber size, scattered degenerated fibers</td>
</tr>
<tr>
<td>4</td>
<td>Infantile alternating esotropia</td>
<td>Bilateral medial recession</td>
<td>Hypertrophied nerve bundles, mild variability in muscle fiber size, scattered degenerated fibers</td>
</tr>
<tr>
<td>5</td>
<td>Lt third nerve palsy with contralateral IO overaction, grade III</td>
<td>Lt lateral recession</td>
<td>Slight increase in number of mitochondria</td>
</tr>
<tr>
<td>6</td>
<td>Lt IO overaction, grade III</td>
<td>Lt IO myectomy</td>
<td>Endomyosal fibrosis, mild variability in muscle fiber size, focal infiltration by chronic inflammatory cells, great increase in the mitochondria, fatty infiltration of the muscle fibers (lipid vacuoles)</td>
</tr>
<tr>
<td>7</td>
<td>Infantile alternating esotropia</td>
<td>Bilateral medial recession</td>
<td>Mild variability in muscle fiber size, focal splitting of muscle fibers, degenerated hypercontracted fibers, many rounded fibers, and increased number of mitochondria</td>
</tr>
</tbody>
</table>

**Abbreviations:** IO, inferior oblique; IR, inferior rectus; LM, light microscope; Lt, left; Rt, right.
The striking histopathological features included: degenerated fibers and regenerating ones; mild to moderate variability in muscle fiber size, along with hypercontracted fibers; some round fibers; and focal splitting of some others. Ultrastructural examination also revealed degenerative changes in the fibers along with regenerating ones with large vesicular nuclei. Similar findings were reported by Meyer et al., Martinez et al., and Spencer and McNeer in their studies of primary and secondary IO overaction. They found by light microscopy the presence of granular fibers with sarcoplasmic masses, and also noted that some fibers showed various stages of atrophy. Moreover, in 1992, Choi and Chang studied overacting IO muscle by light and electron microscope and noted variability of muscle fiber size with vacuolation and degeneration.

More profound degenerative changes were also noted in biopsies from grade III in the current research, since we found focal fatty infiltration and perimysial and endomysial fibrosis in these cases.

The persistent contraction of the IO muscle due to the loss of equilibrium resulting from the weakness of its antagonist...
may result in such degenerative changes in the muscle fibers. Consequent activation of satellite cells may follow, with the appearance of new regenerating fibers resulting in the variability in the size of muscle fibers.9

Focal mononuclear inflammatory cell infiltrate was also noted in some cases with grade III in the current study. This may be related to an inflammatory response following necrosis of some fibers.

Vacuoles, including fat vacuoles, were also noted in some cases. Supporting these results, other authors have found by ultrastructural examination increased vacuolation.1,3,7,8 Meyer et al claimed that most of these vacuoles represented dilated endoplasmic reticulum or empty mitochondria.1

Mitochondrial aggregates and pleomorphism were also profound findings in the current study, mainly in grade III. In agreement with this finding, Meyer et al,1 Choi and Chang,3 and Spencer and McNeer4 concluded that mitochondrial aggregation and degeneration were the striking features in their studies. This finding may result from the degenerative and regenerative changes in the fibers. It may also result from the relative abundance of singly innervated muscle fibers (SIF), which are characterized by mitochondrial abundance.

Hypertrophied nerve bundles were also seen in some cases in the present study. No similar results were obtained from other studies. However, this finding may be related to an increase of the number of SIF innervated fibers in the hypertrophied overacting IO muscle. These SIFs are fast, twitch-generating, and specialized for intense oxidative metabolism and fatigue resistance.8 In addition, Berard-Badier et al in 1978 confirmed on the presence of subsarcolemmal inclusions of granular fibrillar aggregates.9

Conclusion
From this study it could be concluded that overactive IO muscles may result in such degenerative changes in the muscle fibers. Consequent activation of satellite cells may follow, with the appearance of new regenerating fibers resulting in the variability in the size of muscle fibers.9

Focal mononuclear inflammatory cell infiltrate was also noted in some cases with grade III in the current study. This may be related to an inflammatory response following necrosis of some fibers.

Vacuoles, including fat vacuoles, were also noted in some cases. Supporting these results, other authors have found by ultrastructural examination increased vacuolation.1,3,7,8 Meyer et al claimed that most of these vacuoles represented dilated endoplasmic reticulum or empty mitochondria.1

Mitochondrial aggregates and pleomorphism were also profound findings in the current study, mainly in grade III. In agreement with this finding, Meyer et al,1 Choi and Chang,3 and Spencer and McNeer4 concluded that mitochondrial aggregation and degeneration were the striking features in their studies. This finding may result from the degenerative and regenerative changes in the fibers. It may also result from the relative abundance of singly innervated muscle fibers (SIF), which are characterized by mitochondrial abundance.

Hypertrophied nerve bundles were also seen in some cases in the present study. No similar results were obtained from other studies. However, this finding may be related to an increase of the number of SIF innervated fibers in the hypertrophied overacting IO muscle. These SIFs are fast, twitch-generating, and specialized for intense oxidative metabolism and fatigue resistance.8 In addition, Berard-Badier et al in 1978 confirmed on the presence of subsarcolemmal inclusions of granular fibrillar aggregates.9

References