Role of rectal biopsy in predicting response to intrasphincteric botulinum toxin injection for obstructive symptoms after a pullthrough operation

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Objective: Our aim was to correlate the pathological results and clinical response in patients who underwent botulinum toxin (BT) injection for obstructive symptoms (OS) after a pullthrough operation for Hirschsprung’s disease (HD).

Methods: Between August 2002 and February 2006, 16 of 107 HD patients (15%) were referred with persistent OS after pull-through (PT) operation in this center. They underwent rectal biopsy and BT injection in the internal sphincter. Their responses to BT injection were evaluated by the constipation score before, and at 1, 3 and 8 months after the injection, and anorectal manometry (ARM) before and at 2 weeks, and 1 and 8 months after the injection. The association between response to BT and acetylcholinesterase (AChE) staining of rectal biopsy was also assessed.

Results: Fourteen of 16 patients (87%) had improvement in bowel function after 2 weeks, and two patients did not respond at all. Six of the 14 patients with early response had recurrence of symptoms after 2–3 months. Eight patients with normal ganglia and negative AChE had good response with no recurrence on follow-up. However, 4 of 6 recurrences were neurogenic dysfunctions and 2 were intestinal neuronal dysplasia (2-4+AChE). Two patients with no response had an aganglionic segment (4+AChE). Four of 6 patients with recurrence showed improvement with BT re-injection and only 2 did not improve.

Conclusion: A higher degree of AChE staining is associated with lack of response to BT injection. This is also a test for predicting the severity of neurogenic dysfunction in the intestinal wall.

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Patients with Hirschsprung’s disease (HD) usually have a favorable outcome after surgery; however, obstructive symptoms (OS) recur after surgical correction in 11%–42% of cases.1,2 These symptoms range from mild constipation to severe episodes of abdominal distension and enterocolitis. Many patients respond to laxatives, dietary manipulation and other supportive measures,4 but those who fail should be managed with redo-pullthrough (PT), posterior sphincter myectomy (PM) or botulinum toxin (BT) injection. BT injection is less invasive compared with other methods and is therefore an attractive option but elicits a variable response.5,6 This study was performed to determine whether pathological examination of rectal biopsies could predict clinical response in patients who were treated with BT injection.

Methods

Between August 2002 and February 2006, 107 cases of HD underwent a PT operation at our center. Sixteen patients were referred to the bowel management clinic because of unresponsive post-PT OS. Nine patients with OS had constipation, 5 had abdominal distension and 2 had enterocolitis.

This study was approved by the ethics committee of the Shiraz University of Medical Sciences and parental consent was taken. All patients were evaluated by history, physical examination, anorectal manometry (ARM) and full-thickness rectal biopsy (proximal to the previous anastomosis) at the time of BT injection. Rectal biopsies were stained for acetylcholinesterase (AChE) by the modified Karnovsky–Roots method and with hematoxylin–eosin (H&E).7 Histology of tissue specimen obtained during previous surgery was reviewed.

Botulinum toxin (5 units/Kg) (Dysport, Ipsen, UK) was injected intrasphincterically at four sites (3, 6, 9 and 12 o’clock) under general anesthesia.8,9
AChE staining predicts response to botulinum toxin

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The constipation score, which was noted by the parents, was recorded at baseline and at one month and 8 months after BT injection; the score was evaluated by nurses and other pediatric surgeons in the bowel management clinic.

Anorectal manometry was performed under oral midazolam sedation (0.8 mg/Kg) using standard water-perfused system (Uromic Samba, Medkonsult, Czech Republic) and the results were evaluated by anorectal software analyzer (AMA version 3.11). Resting pressures were recorded before, and at 2 weeks, 1 month, and 8 months after BT injection. A decrease in the resting pressure of at least 30 mmHg or 10% of baseline pressure was considered significant.

All patients received the same protocol of treatment that consisted of polyethylene glycol (1 g/Kg/day) and sena extract syrup (Tulid Daru, Iran); disimpaction and dilatation were done if needed. Patients were followed up for 8 months. Resolution of OS or significant changes in the score for at least 1 month was considered as a positive response to BT injection.

Statistical analysis

Data were analyzed with repeated measures ANOVA. Matched-pair related observations were analyzed using the Wilcoxon signed rank test; Eta correlation test was used for correlation statistics between AChE staining and the response to BT. Repeated measure ANOVA was used for statistical changes in manometry and constipation score during the follow-up periods. A p value <0.05 was considered significant.

Results

Sixteen of 107 children (10 boys, 6 girls) with persistent OS and a mean age of 5.4 (SD 2.1; range 2–9) years were included in this study. Of the 16 patients, 13 had undergone the Soave and 3 the Duhamel pull-through operation. The mean time interval between developing OS and receiving BT injection was 4.2 (SD 2.5; range 2–7) years. The response to BT injection did not correlate with age, gender, time interval or type of operation in this study. These patients had normal ganglion cells (on H&E staining) after PT as seen on reviewing their previous pathology reports.

Results of pathological investigations

Eight of the 16 children had normal ganglion cells and were –AChE in the pre-BT injection biopsy. Four children had a reduced number of normal ganglia and neurogenic dysfunction (up to 2+ AChE); 2 had giant ganglia and intestinal neuronal dysplasia (IND) (2+ AChE); and 2 had no ganglion cells (>2+ AChE). (Table 1)

The constipation score in patients with good-response considered significant.

Table 1: Classification of neurogenic dysfunction according to AChE staining and ganglia

<table>
<thead>
<tr>
<th>Number of ganglion cells</th>
<th>Absent</th>
<th>&lt;4 normal shape</th>
<th>&gt;8 or bizarre and giant</th>
</tr>
</thead>
<tbody>
<tr>
<td>AChE staining grade - features</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 - No proliferation of AChE+ fibers in lamina propria</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>1 - Slight proliferation of thin fibers only at bottom of lamina propria</td>
<td>+</td>
<td>+</td>
<td>_</td>
</tr>
<tr>
<td>2 - Obvious proliferation in the lower part of lamina propria</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>3 - AChE+ fibers extend to the tip of lamina propria</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>4 - AChE+ fibers proliferate diffusely, run transversely, make network</td>
<td>+</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Categorization</td>
<td>HD</td>
<td>ND</td>
<td>Normal</td>
</tr>
</tbody>
</table>

HD Hirschsprung disease; ND Neurogenic dysfunction; IND Intestinal neuronal dysplasia

Table 2: Comparison of resting rectal pressure and constipation score between responder and non-responder groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before</th>
<th>2 weeks</th>
<th>1 month</th>
<th>8 months</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting rectal pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good response (n=8)</td>
<td>67.1 (8.0)</td>
<td>36.4 (4.7)</td>
<td>37.2 (4.9)</td>
<td>43.3 (3.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Recurrence (n=6)</td>
<td>66.0 (1.3)</td>
<td>38.0 (4.3)</td>
<td>54.0 (14.3)</td>
<td>54.0 (10.8)</td>
<td>0.5</td>
</tr>
<tr>
<td>Non-responders (n=2)</td>
<td>64.0 (4.4)</td>
<td>36.0 (9.1)</td>
<td>53.0 (11.0)</td>
<td>56.0 (10.6)</td>
<td>0.4</td>
</tr>
<tr>
<td>Constipation score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good response (n=8)</td>
<td>20.7 (7.4)</td>
<td>9.3 (4.8)</td>
<td>11.0 (7.0)</td>
<td>10.8 (7.1)</td>
<td>0.01</td>
</tr>
<tr>
<td>Recurrence (n=6)</td>
<td>26.0 (8.0)</td>
<td>13.0 (11.5)</td>
<td>14.0 (9.2)</td>
<td>15.0 (8.4)</td>
<td>0.1</td>
</tr>
<tr>
<td>Non-responders (n=2)</td>
<td>23.0 (8.4)</td>
<td>12.5 (3.5)</td>
<td>14.5 (3.2)</td>
<td>18.5 (6.5)</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Improved from a pre-injection value of 20.7 (7.4) to 10.8 (7.1) in 8 months (p=0.01); in cases with recurrence from 26.0 (8.0) to 15.0 (8.4) (p=0.1) and in those with no response from 21.6 (13.7) to 16.3 (8.3) (p = 0.2) (Table 2).

Fourteen patients (87%) did not have any obstructive symptoms after 2 weeks; 2 patients had no improvement at all. Six of 14 patients who had any early response developed recurrence of symptoms after 2–3 months. The 6 patients with recurrence and 2 with no response underwent BT re-injection (in the same doses) 1–3 times during a 6-month period, but only 4 of those with recurrence showed improvement. Two patients with recurrence, and those with no response underwent redo-pullthrough but one of each still did not improve.

Eight patients who were negative for AChE with normal ganglia, had good response to BT injection with no early recurrence. Patients with recurrence (n=6) included 4 with neurogenic dysfunction and 2 with IND; 3 of the patients with neurogenic dysfunction and 1 with IND had good improvement after BT re-injection. One patient with IND and one with an aganglionic segment did not respond even to re-PT.

There were no local infectious complications or fecal or urinary incontinence during this study.

The correlation value (CV) between AChE staining as a predictor variable and the response to BT as an independent variable was 0.69.

**Discussion**

Our study showed that AChE staining of punch mucosal rectal biopsies could be used as a test for predicting the severity of neurogenic dysfunction in the intestinal wall. This study implies that AChE has an important role in predicting the response to intrasphincteric BT injection for OS after a PT operation. A higher degree of AChE staining is associated with the lack of response to BT injection.

Obstructive symptoms occur in 11%–42% of children even after a technically excellent operation for HD. These symptoms should be managed classically after considering all possible causes. Clinical assessment, ARM and rectal biopsy should be used to work up these patients before internal sphincter achalasia is considered as the cause of the OS.

However, proximal aganglization should be ruled out; this can remain following a PT operation or may be acquired. Some surgeons do not accept the concept of ‘acquired’ aganglization but ischemia may be postulated as a cause for ganglion cell loss following the first PT. Errors in reporting (pathologist’s experience, inadequate tissue in the sample) may be another cause, as in two of our patients.

Langer et al. studied the effect of BT on patients with OS after PT operation and reported different results, and Minkes et al. reported that BT has effects similar to myectomy. If symptoms persist despite a fall in resting pressure, a non-sphincteric cause should be ruled out, but none of these studies tried to find the pathological factors that might have played a role in the response to BT injection.

Two patients with recurrence in our study had IND. Nakao et al. reported negative AChE results in IND. However, many investigators believe that AChE is weakly positive (submucosal vessels and base of the lamina propria) with giant and ectopic ganglion cells in these associated groups of HD. Four of our patients had a reduced number of ganglion cells and were positive for AChE staining. These may have been due to severe intramural neurogenic dysfunction because of ischemia or chronic changes following surgery.

The majority of our patients were referred with constipation (9 of 16) which started immediately or in the month after PT; the symptoms had been present for several years. A high anal resting pressure accompanied by ineffective rectal peristalsis is the cause of constipation. However, many of these patients outgrew this problem or the need for myectomy and injections of BT. Langer et al. stated that recurrence is due to inadequate doses of BT or an incorrect injection site. In our study, all patients had a significant reduction in resting pressure especially in the first month after BT injection, although a few developed recurrence. The reason why many patients with recurrence would respond to re-injection can be explained by the study of Pamphlett et al. They reported that BT not only affects chemical denervation and ameliorates many dysautonomic problems but also affects compensatory autonomic nerve sprouting in the denervated region.

In this study, we found that the presence of normal ganglia was inversely related to the degree of AChE positivity. The staining of hypertrophied nerves in the intestinal wall demonstrates the absence of ganglia or presence of neurogenic dysfunction. Our study shows that if neurogenic function is in order, the effect of BT is acceptable but in cases with IND and neurogenic dysfunction, more time and BT re-injection are needed. The aganglionic segment does not respond to BT injection and should be removed. Rectal mucosal biopsy for AChE staining is a non-invasive and easily performed test. The degree of AChE staining showed an inverse correlation (CV=0.69) with the clinical response in patients with OS who underwent BT injection. Therefore, full-thickness rectal biopsy and its complications can be avoided in those...
who are candidates for treatment with BT.

We recommend punch mucosal biopsy in every patient with OS following PT as a non-invasive test. The specimen should be subjected to AChE staining. The higher the degree of AChE staining, the greater the chances of non-responsiveness. AChE staining demonstrates the severity of neurogenic dysfunction in the intestinal wall. However, further investigations with larger samples might be needed to evaluate different aspects of this test more accurately.

References


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