Congenital Megacolon : Diagnosis Using Size of Submucosal Nerve Trunk as a Criteria

SURASAK SANGKHATHAT, M.D.*,
KAMOLNATE TADTAYATHIKOM, M.D.*,
SAKDA PATRAPINYOKUL, M.D.*,
WINYOU MITARNUN, M.D.**

Abstract

Cholinergic hyperinnervation in submucosa of aganglionic segment of Hirschsprung's disease has been described. However, objective measurement of the nerve fibers was insufficiently reported.

Objectives : To study the amount and size of hypertrophic submucosal nerve trunks in the aganglionic segment of Hirschsprung's disease, semiquantitatively and evaluate the possibility of using the size as objective diagnostic criteria.

Material and Method : Thirteen specimens from aganglionic segments from Hirschsprung's disease and six specimens from the age-matched control subjected without colonic innervation disorders were studied with the S-100 immunohistochemical technic. The submucosal nerve trunks were counted per ten HPF and measured in width, semiquantitatively. Fiber sizes were stratified into small (<20 micrometers), medium (20-40 micrometers), and large (>40 micrometers). Maximum fiber size in each specimen was also recorded.

Results : Average density of submucosal nerve trunk in the aganglionic segment and the control were 12.5 and 3.4 fibers per ten HPF, respectively. The large nerve trunks were detected in 12 of 13 aganglionic specimens and none was detected in the controls. The maximum fiber size in aganglionic segments was 78.2 micrometers, compared to 24.1 micrometers in the control group. The sensitivity and specificity to set the presence of hypertrophic nerve trunk, larger than 40 micrometers as a diagnostic criteria for Hirschsprung's disease were 92.3 and 100 per cent, respectively. The data suggest the use of this size as an objective diagnostic criterion, which may be valuable in rectal suction biopsy specimens.

Key word : Hirschsprung's Disease, Enteric Nervous System

SANGKHATHAT S, PATRAPINYOKUL S, TADTAYATHIKOM K, MITARNUN W
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* Pediatric Surgery Unit, Department of Surgery,
** Department of Pathology, Faculty of Medicine, Prince of Songkla University, Songkhla 90112, Thailand.

The "gold standard" diagnostic tool for congenital megacolon (Hirschsprung's disease) is the full-thickness rectal biopsy to demonstrate the absence of ganglion cells in the enteric nervous plexus[1,2]. The technique is currently employed in the pediatric surgical service at Songklanagarind Hospital. The procedure usually requires general anesthesia and hospitalization.

Rectal suction biopsy (RSB) is a less invasive technique, which can be done as a bedside procedure. The tissue obtained by this method contains only mucosa and submucosa. To read positive for congenital megacolon, absence of ganglion cells must be shown in at least 60 sections[3]. Although positive readings from combined evidence of increased staining intensity with acetylcholinesterase (AChE) can alleviate that difficulty, RSB together with AChE immunohistochemical staining has been accepted by various institutes as a simple and reliable diagnostic tool. Increased acetylcholinesterase activity is found in the hypertrophic nerve trunk in submucosa, lamina propria and muscularis mucosae from patients with Hirschsprung's disease[4]. The limitation of this technique is that the enzyme loses its activity during storage. Rapid transfer of fresh tissue under moist and cold preservation is, therefore, necessary[5].

Evidence of cholinergic fiber hypertrophy and hyperinnervation has been defined in the aganglionic segment of Hirschsprung's disease. The hypertrophic nerve trunk can be identified in the routine hematoxylin-eosin stain. Quantitative measuring of each fiber can be aided by immunohistochemical techniques. S-100 protein is a neural specific staining method which can be processed in formalin-preserved or paraffin-embedded specimens[6,7]. Although the technique is not specific to the cholinergic nerve, it stains well in the hypertrophic nerve trunk and helps in qualitative and quantitative examinations. This study aimed to compare the size and number of hypertrophic submucosal nerve trunks between the aganglionic segment of Hirschsprung's disease and the control, in order to evaluate the possibility of using the fiber size as an objective diagnostic criterion.

MATERIAL AND METHOD

Aganglionic segments of rectum were obtained at pull-through operation or by full-thickness rectal biopsy from thirteen infants with Hirschsprung's disease. Cases with long segment aganglionosis and total colonic aganglionosis were not included in this study. Age of the patients at the time of the procedures ranged from one month to eight years. To simulate the RSB, each specimen was dissected into a piece of tissue containing only mucosa and submucosa. For the control group, 6 specimens of colon were prepared from age-matched patients without a colonic innervation disorder.

All specimens were embedded in paraffin blocks. Sections of 5 micrometers thickness were stained with hematoxylin-eosin (H&E) to confirm aganglionosis. Immunohistochemical studies with S-100 antibody (Zymed laboratories, Inc., San Francisco, California) were carried out. Deparaffinized sections were incubated overnight with 1:20 primary antibody followed by a thirty-five minute bath with 1:200 secondary antibody. Counterstain was done with Mayer's hematoxylin.

The morphology of S-100 stained nerve trunk was examined by one observer. Nerve trunks were counted per ten high power fields (HPF) and stratified into small (less than 20 micrometers) trunk, medium (20-40 micrometers), and large (more than 40 micrometers) fibers. The width of each fiber was measured in perpendicular direction at its widest point. The maximum nerve trunk size in each specimen was also recorded. Measurement was done under high power field, with an objective micrometer.

The mean was calculated for the density of each fiber size stratification. An unpaired Student *t*-test was performed to determine the difference between average size-stratified densities between the Hirschsprung's disease group and control group.

RESULT

Hematoxylin & Eosin stain

Myenteric ganglia were present in all cases in the control group and no cases in the congenital megacolon group. Submucosal ganglia were not found in all cases of both groups.

S-100 immunohistochemistry

Average nerve trunk density in congenital megacolon group was significantly more than the control group. ($p < 0.01$). In the control group, discrete nerve trunks were found in the submucosa (Fig. 1A). Average identifiable nerve trunk was 3.4 fibers per 10 HPF. Average density of small and medium trunks was 2.2 and 1.2 fibers per 10 HPF,

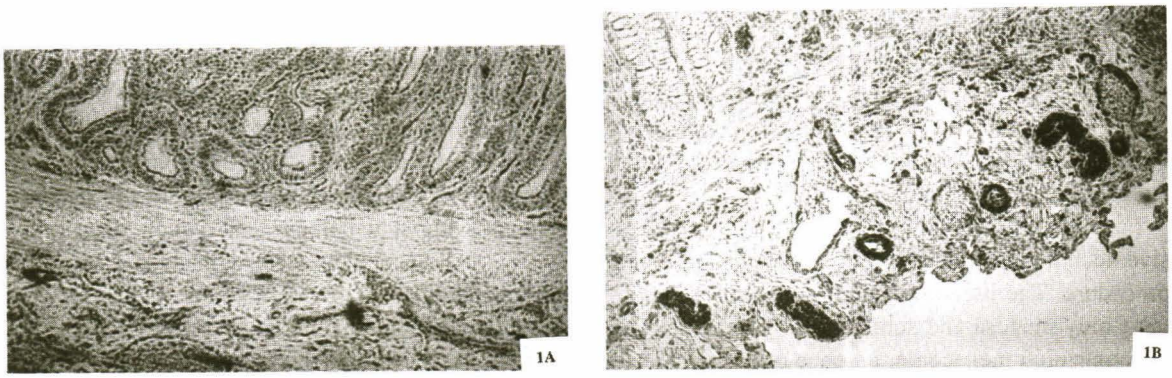

Fig. 1. Discrete nerve trunks were detected in submucosa of ganglionic colon from a control patient (A), whereas hypertrophic trunks were found in aganglionic bowel from a patient with congenital megacolon (B).

Table 1. Densities and maximum fiber sizes of submucosal nerve trunk.

Group	Density of fiber (fibers per 10 HPF)			Maximum fiber size (micrometers)
	Small (<20 micrometers)	Medium (20-40 micrometers)	Large* (>40 micrometers)	
Congenital megacolon	4.9	5.6	2.0	78.27
Control	2.2	1.2	0	24.17

* Large fibers were detected in 12/13 sections from congenital megacolon and 0/6 from control group.

respectively. The maximum trunk size ranged from 15 to 37.5 micrometers (mean 24.2 micrometers). No nerve trunk larger than 40 micrometers was detected in any studied specimen. (Table 1)

In the congenital megacolon group, hypertrophic submucosal nerve trunks were found in all cases. The average nerve trunk density was 12.5 trunks per 10 HPF. The average density of small, medium and large trunks was 4.9, 5.6 and 2.0 fibers per 10 HPF, respectively. The maximum trunk size ranged from 40 to 125 micrometers (mean 78.3 micrometers). (Fig. 1B) The large trunk was detected in twelve of thirteen specimens.

The sensitivity and specificity to set the presence of hypertrophic nerve trunk larger than 40 micrometers as a diagnostic criterion for Hirschsprung's disease were 92.3 and 100 per cent, respectively. The negative predictive value is 85.7 per cent.

DISCUSSION

The full-thickness rectal biopsy to determine the absence of ganglia in myenteric plexus is a reliable and familiarized method to confirm the diagnosis of congenital megacolon[1,2]. However, the procedure is invasive and requires general anesthesia. Practically, a rectal biopsy can be performed just before the colostomy in a case whose contrast enema and/or manometric study have already suggested congenital megacolon[8]. Only when those results are doubtful, a frozen section study is requested and the operation has to be terminated if the biopsy result shows positive ganglion cells.

Rectal suction biopsy is a less invasive, bedside tissue sampling method. The procedure usually yields a small tissue piece, of which only mucosa and submucosa are included. Because the histologic identification of nerve cells in submucosal

plexus is more difficult than in the myenteric plexus, up to sixty serial sections are required to prove the absence of ganglion cells which means 'positive' for Hirschsprung's disease[3]. In this study, no submucosal ganglion could be detected in the ganglionic segments of the control group whereas the myenteric ganglia were present in all.

An alternative mean to diagnose Hirschsprung's disease by the demonstration of increased acetylcholinesterase (AChE) activity was introduced by Myer-Ruge in 1972[4]. This histochemical technique can be applied well to the RSB specimen. The simplicity and high accuracy have made the procedure one of the standard diagnostic tools employed by various institutes in western countries. In Thailand, Chentanez et al reported satisfactory experience with AChE staining by a modification of Lake's method[9]. The group also proposed a new classification of staining patterns of AChE in RSB tissue of Hirschsprung's disease[10]. Although the AChE staining is an accepted staining method for RSB specimens, the technique requires fresh and cool preservation of tissue[5]. Moreover, the high cost of this method (600 - 1,000 Baht per case) prohibits the test as a routine screening tool in Thailand.

The innervation pattern in the submucosa of Hirschsprung's disease, studied with the immunohistochemical technique has been reported[6,7,11]. There are techniques in which the staining can be processed in formalin-fixed or paraffin-embedded specimen. Monoclonal antineurofilament antibodies have been tested in colonic tissue from congenital megacolon by Kluck et al[11]. The study reported heavily stained hyperplastic axon bundles in aganglionic segments. The nerve bundles in aganglionic region were more numerous and larger in diameter. Immunostaining using antibody to S-100 protein is a transmitter-nonspecific neural staining technique that has been employed in demonstrating innervation pattern of the gastrointestinal tract. A comparison of S-100 staining in RSB specimens between congenital megacolon and unaffected control by Robey et al showed mucosal nerve proliferation in all cases with congenital megacolon, contrary to 32 per cent in the control group[6]. Although there is some overlap in the range of measurement, the average submucosal nerve trunk thickness in the congenital megacolon and colonic neuronal dysplasia groups were significantly larger than the unaffected specimens. Monforte-Munoz measured the submucosal nerve trunk semi-quantitatively and found increased caliber in aganglionic segment[7]. Their study also suggested that a nerve trunk larger than 40 micrometers was specific for aganglionosis, and size stratification in our study was cut at that the diameter.

The present results revealed that S-100 neural staining facilitates the identification and measurement of a nerve bundle. Hypertrophy of the submucosal nerve trunk in RSB-simulated aganglionic tissue from cases with Hirschsprung's disease is consistent with previous documentation. Semiquantitative measurement showed that twelve of thirteen aganglionic segments were positive for the large trunk, whereas, none was found in the control group. The medium and small trunks could be found in both the Hirschsprung's disease and control groups although the average amount in the Hirschsprung's disease group was higher. The evidence suggests the possibility of using the presence of a large nerve trunk in the submucosa as a positive diagnostic criteria for congenital megacolon. A trial with real-RSB specimens in a larger size of population needs to be done to determine the clinical value of the test.

SUMMARY

The histology of the nerve trunk in the submucosa of the colon from pediatric subjects with Hirschsprung's disease was studied with S-100 immunohistochemical technique. Semiquantitative measuring revealed the specificity of fibers larger than 40 micrometers with the aganglionic segment, suggesting the use of this size as an objective criterion to diagnose Hirschsprung's disease.

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ลำไส้ใหญ่โป่งพองแต่กำเนิด : การวินิจฉัยโดยใช้ขนาดของใยประสาทชั้นใต้เยื่อบุผิวเป็นเกณฑ์

สุรศักดิ์ สังขทัต ณ อยุธยา, พ.บ., ศักดา ภัทรภิญโญกุล, พ.บ.*,*
กมลเนตร ตัณยาธิคม, พ.บ., วิญญ มิตรานันท์, พ.บ.***

ได้ศึกษาขนาดของใยประสาทชั้นใต้เยื่อบุผิวในลำไส้ใหญ่ส่วนที่ขาดเซลล์ปมประสาท 13 ตัวอย่างเทียบกับลำไส้ใหญ่จากกลุ่มควบคุมที่อายุใกล้เคียงกัน พบความหนาแน่นของใยประสาทในลำไส้ที่มีพยาธิสภาพเทียบกับกลุ่มควบคุมเป็น 12.5 และ 3.4 หน่วยต่อพื้นที่ตามลำดับ ใยประสาทขนาดโตกว่า 40 ไมโครเมตรพบได้ 12 ใน 13 ตัวอย่างเนื้อเยื่อจากกลุ่มที่เป็นโรค ในขณะที่ไม่พบเลยในกลุ่มควบคุม ขนาดของใยประสาทที่โตที่สุดในกลุ่มที่เป็นโรควัดได้ 78.2 ในขณะที่วัดได้ 24.1 ในกลุ่มควบคุม

ข้อมูลที่ได้แนะว่าขนาดของใยประสาทในชั้นใต้เยื่อบุผิวอาจใช้เป็นเกณฑ์วินิจฉัยโรคลำไส้ใหญ่โป่งพองโดยกำเนิดได้ การศึกษาทางคลินิกในขนาดตัวอย่างที่ใหญ่ขึ้นจะทำให้ได้ข้อมูลเกี่ยวกับความเที่ยงตรงที่ชัดเจนกว่านี้

คำสำคัญ : ลำไส้ใหญ่โป่งพองแต่กำเนิด, ใยประสาท, โรคเฮิร์ทซปรุง

สุรศักดิ์ สังขทัต ณ อยุธยา, ศักดา ภัทรภิญโญกุล,
กมลเนตร ตัณยาธิคม, วิญญ มิตรานันท์
จดหมายเหตุทางแพทย์ ฯ 2545; 85: 250–255

* หน่วยกุมารศัลยศาสตร์, ภาควิชาศัลยศาสตร์,
** ภาควิชาพยาธิวิทยา, คณะแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์, สงขลา 90112