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Histopathological perspective of the pulled-through colon in Hirschsprung disease: Impact on clinical outcome

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ABSTRACT

Background: The outcome in HD has not been always satisfactory even after a technically sound operation.
Purpose: To define the characteristic histopathological features of the pulled-through colon in patients with HD, and it is impact on clinical outcome.
Patients and methods: The study included patients with HD who underwent surgical repair between 2010 through 2016. The proximal margin of resected bowel segments (which corresponds to the pulled through colon) was subjected to detailed histopathological examination by two experienced pathologists.
Based on the frequency of postoperative attacks of HAEC (fever, vomiting, abdominal distention, fluid offensive stools), cases included in the study were divided into two groups: Group A, those with less frequent attacks of HAEC; and Group B, those with recurrent attacks of HAEC (more than 3).
Results: The study included 35 patients (25 in group A; and 10 in group B). Their age ranged from 0.2 to 144 months (median 6 months).
Comparing the histopathological findings in the two clinical groups, we have found that Group B (recurrent attacks of HAEC) had significantly more frequent focal disarray of nerve bundles and thicker nerve bundle diameter. Also, histopathological features of acute inflammation were more prevalent in examined specimens from group B.
Conclusion: Several histopathological features of the examined bowel specimens in HD, other than presence or absence of ganglion cells, are indicative of postoperative functional outcome. These include the thickness and maturity of nerve bundles, in addition to the presence of histopathological features of acute inflammation.
Level of evidence: This is a case control study (level III evidence).

Hirschsprung disease (HD), also known as congenital megacolon, is characterized by the absence of ganglion cells in the myenteric and submucosal plexuses of the intestine [1,2]. The incidence of HD is approximately 1:5000 live births [1–5]. Moreover, HD is relatively the most common cause of intestinal obstruction in the neonatal period [2–7].

The diagnosis of HD is usually based on clinical history, radiological studies, anorectal manometry, and particularly depends on histopathological examination of rectal biopsy [6]. The gold standard for the diagnosis is the absence of ganglion cells in the submucosal and myenteric plexuses of the bowel on histological examination [8]. Despite the increased knowledge about HD, significant complications continue to be associated with the different surgical procedures [7]. The most common complications are recurrence of constipation and enterocolitis that may develop in up to 40% of patients [6]. These findings prompted further investigation into why patients with good operations would have poor outcomes [8]. In this study, we correlated histopathological findings of excised colonic specimens during pull-through operations with clinical outcomes.

1. Patients and methods

The study included patients with HD who underwent surgical repair at the Pediatric surgery department between 2010 through 2016. Diagnosis of HD was based on clinical presentation, characteristic contrast enema, and confirmed by histopathological examination of rectal biopsy.

Our surgical technique was the 'Soave' repair whether transanal or abdominal-assisted pull-through. In the lithotomy position, and after applying the anal retractor, we place the circumferential incision 2 cm above the dentate line. We proceed with submucosal excision of the rectum for 3–5 cm before we shift to full thickness excision of the bowel. We tend to leave a relatively shorter muscle cuff which is also incised posteriorly. Our upper limit for bowel excision is usually 5–10 cm above the macroscopic transitional zone.

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Table 1
Summary of the demographic data and the gross pathology of excised specimens of the 35 cases of HD included in the study.

Variables	Median age in months (IQR)	Male	Female	Specimen length in cm (mean ± SD)	Narrow segment length in cm (mean ± SD)	Wide segment length in cm (mean ± SD)
Group A ($N = 25$)	5.5(2.6–12.0)	16(64.0%)	9(36.0%)	16.8 ± 5.8	7.5 ± 5.1	9.3 ± 4.1
Group B ($N = 10$)	7.5(4.3–25.5)	9(90.0%)	1(10.0%)	14.9 ± 3.9	6.1 ± 2.1	8.8 ± 2.9
P-value	0.315[a]		0.218[b]	0.353[c]	0.403[c]	0.748[c]

Group A: less frequent attacks of HAEC.
Group B: recurrent attacks of HAEC (more than 3).
[a] Mann Whitney test.
[b] Fisher's Exact test.
[c] Independent t-test.

We excluded patients with extreme form of the disease (total colonic aganglionosis), as well as those with postoperative technical complications (strictures at the coloanal anastomosis/ rolled muscle cuff) that would affect the functional outcome. Patients were contacted by phone, informed about the study, and asked to attend the outpatient (follow-up) clinic. Included patients were subjected to a questionnaire to assess their bowel function after the corrective surgery. We used part of the scoring system proposed by ElSawaf et al. [9] which involves the assessment of bowel function mainly concentrating on Hirschsprung Associated Enterocolitis (HAEC). Diagnosis of HAEC depends on the following symptoms: fever; vomiting; abdominal distention, fluid offensive stools. According to the frequency of postoperative attacks of HAEC, patients included in the study were divided into two groups [9]: Group A included those with less frequent attacks of HAEC (zero to 3 attacks of HAEC in a follow-up period of at least one year); and Group B included those with recurrent attacks of HAEC (more than 3 attacks/year). The study was conducted after internal review board approval (IRB 00006379).

1.1. Histopathological examination of the resected bowel segment during corrective surgery for cases of HD included in the study

All specimens were assessed grossly by measuring the whole length of the specimen (resected bowel), the length of the proximal 'wide' segment and the length of the distal 'narrow' part. Specimens were opened to assess for any mucosal abnormalities. Sections were taken as usual from the distal and proximal ends of the resected bowel segment. The proximal margin of the resected bowel which corresponds to the pulled through colon (assumed to be healthy) was our main concern in this study and was subjected to detailed histopathological examination. The examination involved full bowel circumference; two sections (2 serials) were prepared, stained by Hematoxylin and Eosin (H&E) and examined by light microscope. In addition, the width of the submucosal and myenteric nerve bundles was measured using image analyzer Leica, Germany Q 500 MC program. During the image analyzer assessment, the apparent largest submucosal and myenteric nerve bundles were selected for the measurement with 3 width measurement readings taken along the nerve bundle length, then the mean was calculated.

Regarding the retrospective cases included in the study: patients' files were revised, blocks and slides were retracted, new slides were prepared from the proximal margin of the resected bowel (2 sections). Histological examination was performed similar to prospective cases.

Examination was done by two experienced pathology consultants who were blinded regarding the distribution of cases in the two clinical groups.

1.2. Statistical analysis

Statistical analysis was performed using Mean and SD values for normally distributed data. Median, IQR, and range values were calculated for continuous data with nonnormal distribution. Fisher's Exact test was used for comparison of categorical variables between groups A and B; Receiver Operating Characteristic (ROC) curves were used to calculate 'limit' values for the nerve bundle diameter to discriminate between the two clinical groups. For all results, a p-value <0.05 was considered statistically significant.

2. Results

The study included 35 patients with the diagnosis of HD who underwent surgical correction at the pediatric surgery unit. Their age ranged from 0.2 to 144 months (median 6 months). The male to female ratio was 2.5:1. Group A with less frequent attacks of HAEC included 25 cases (71.4%), while the remaining 10 cases (28.6%) were included in group B (more than 3 attacks of HAEC). Table 1 summarizes the demographic data and the gross pathological features of excised specimens in both groups.

2.1. Histopathological examination of the proximal margin of the resected colonic segment

Ganglion cells were present in the proximal margin of all cases included in the study. Most ganglion cells were mature polygonal cells

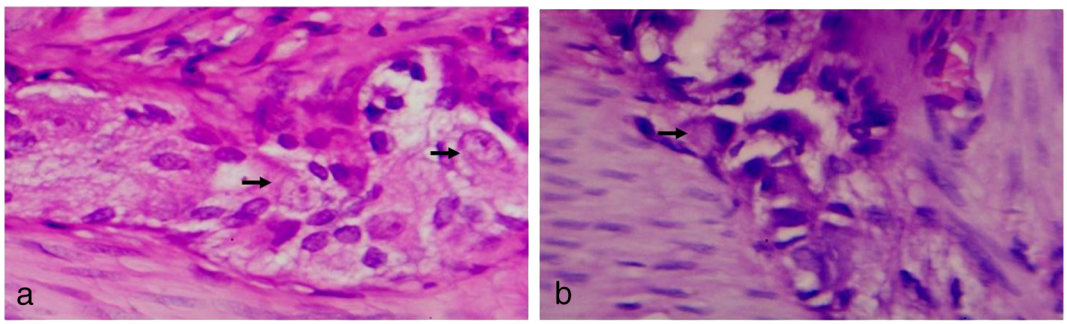

Fig. 1. (a) Normal looking ganglion cells (arrows) in myenteric nerve bundle: the cells have abundant eosinophilic granular cytoplasm and eccentric vesicular nuclei with conspicuous eosinophilic nucleoli (arrow). (b) Immature ganglion cells (arrow) in myenteric nerve bundle: smaller cell size and hyperchromatic nuclei. H&E, ×400.

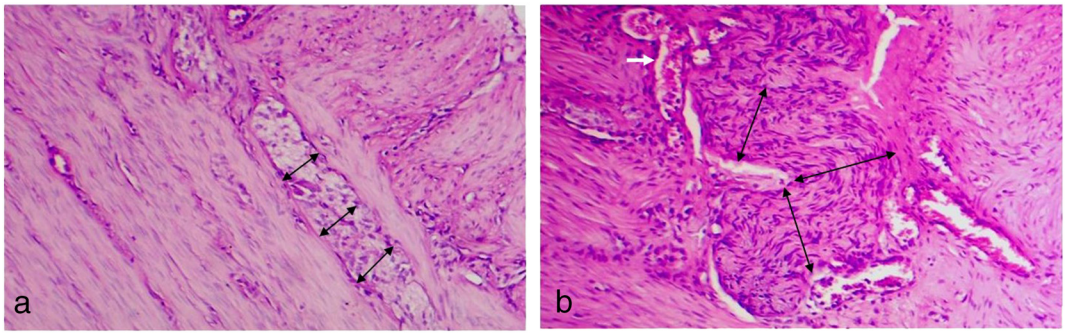

Fig. 2. (a) Normal looking myenteric nerve bundle (double arrowhead) arranged linearly between the inner circular and the outer longitudinal muscle fibers. (b) Abnormal myenteric nerve bundle (double arrowhead): hypertrophic (thick), unmyelinated, irregular arrangement (focal disarray), prominent congested blood vessels (white arrow) (H&E, ×100).

with abundant eosinophilic granular cytoplasm and eccentric vesicular nucleus with conspicuous eosinophilic nucleoli (Fig. 1a). Few were immature, smaller in size with hyperchromatic nuclei (Fig. 1b).

Normally, myenteric nerve bundles are arranged linearly between the inner circular and outer longitudinal muscle fibers (Fig. 2a). In HD, they can appear irregular, grouped and dissecting between hypertrophied disorganized muscle fibers (Fig. 2b); this is known as focal disarray. In our study, myenteric nerve bundles showed focal disarray in 28.6%. Also, prominent congested blood vessels were seen around these abnormal looking nerve bundles. Moreover, these nerve fibers appeared mostly unmyelinated in 65.7% with a mean diameter of 65.8 ± 16.2 μm (range between 40.5 and 117.2 μm). On the other hand, submucosal nerve bundles showed a mean diameter of 36.0 ± 10.4 μm (range between 11.5 and 62.8 μm).

Chronic inflammatory cells are usually present in examined colonic specimens of HD owing to chronic fecal stasis. However, we also detected histopathological features of acute inflammation (acute inflammatory cells, cryptitis, and crypt abscesses) in 40% of examined specimens.

Comparing the histopathological features of the proximal margin of the resected bowel in the two clinical groups (Table 2), we have found that Group B (recurrent attacks of HAEC) had significantly more frequent focal disarray of nerve bundles and thicker nerve bundle diameter. Also, features of acute inflammation were more prevalent in examined specimens from group B. Limit values for nerve bundle diameter were calculated using (ROC) curves to discriminate between the two clinical groups (Fig. 3), which were found to be 68 for the myenteric and 40 for the submucosal nerves. Statistically, the diagnostic performance of the myenteric bundle diameter for discrimination between the two groups was more significant.

3. Discussion

Despite the advancement of the surgical management of Hirschsprung disease, still some patients suffer from postoperative functional problems even after a technically sound operation. Some patients will still have disturbances of bowel function such as constipation, enterocolitis, and functional obstructive symptoms. To avoid postoperative fecal incontinence, pediatric surgeons should be very careful to preserve the anal canal mucosa by starting their dissection 1–2 cm above the dentate line [10]. Starting the dissection at a lower level (closer to the dentate line) may endanger the potential for fecal continence by damaging the anal canal mucosa responsible for the sensory component of continence after removing the rectum [10]. On the other hand, starting the dissection at a higher level may increase the liability for postoperative obstructive symptoms and enterocolitis that has been described as a nonpreventable and nonpredictable complication [10]. However, it has been suggested that one of the important causes for postoperative obstructive symptoms may be related to transitional zone pull-through [10–13]. The pathophysiology of the disease is more complex, and abnormalities of both adrenergic and cholinergic fibers are demonstrable in the aganglionic bowel; histochemical stain for acetylcholine esterase can highlight a marked increase in thick nerve fibers throughout the lamina propria and muscularis mucosa. Moreover, it was found that the ganglion cells in the proximal

Table 2
Comparison between histopathological features of proximal margin of resected colon in operated cases of Hirschsprung Disease.

	Group A ($N = 25$)	Group B ($N = 10$)	P-value	OR (95% CI)
Myenteric nerve bundle				
Mean diameter (μm)	59.1 ± 16.3	84 ± 18.1	0.001[a,d]	--
Arrangement				
- Focal disarray	4 (16%)	6 (60%)	0.016[c,d]	7.9(1.5–41.3)
- Organized	21(84%)	4 (40%)		
Myelination				
-Unmyelinated	15(60%)	8 (80%)	0.43[c]	2.78(0.5–15.3)
- Myelinated	10(40%)	2 (20%)		
Submucosal nerve bundle				
Mean diameter (μm)	33.2 ± 9.1	43 ± 10.7	0.01[a,d]	--
Histopathological features of acute inflammation (abundant acute inflammatory cells, cryptitis, crypt abscess)				
Present	6 (24%)	8 (80%)	0.002[b,d]	--
Absent	19(76%)	2(20%)		

Group A (those with less frequent attacks of HAEC); and group B (those with more than 3 attacks of HAEC/year).
[a] Independent t-test.
[b] Chi square test.
[c] Fisher's Exact test.
[d] Statistically significant.

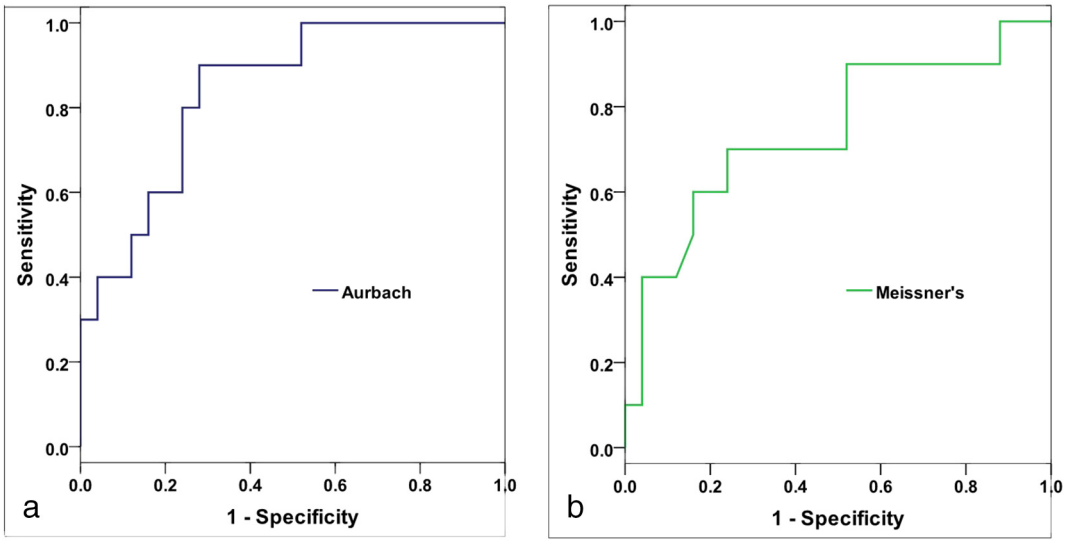

Fig. 3. Receiver Operating Characteristic (ROC) curves used to calculate limit values for nerve bundle diameter to discriminate between the two clinical groups. (a) ROC curve for myenteric plexus: limit value 68; area under the curve 0.840; 95% confidence interval 0.705–0.975. (b) ROC curve for submucosal plexus: limit value 40; area under the curve 0.742; 95% confidence interval 0.548–0.936.

healthy colon of HD diagnosed patients may still fail to react for colonic neuropeptides such as vasoactive intestinal peptide and others [4,13,14] . Therefore, more research in the histopathological features of HD should be considered.

This study was designed to determine histopathological parameters that would correlate to the patient's postoperative morbidity and mortality. This has been done through analysis of histopathological features of the assumed healthy ganglionic proximal margin of the resected colon and correlating the findings with the clinical outcome. Based on the frequency of postoperative HAEC, cases included in the study were divided into two groups: Group A, those with less frequent attacks of HAEC; and Group B, those with recurrent attacks of HAEC (more than 3 attacks). The histopathological findings that were found to correlate with poor outcome in operated cases of HD were: increased nerve bundle thickness, focal disarray of nerve bundles (clustered, disorganized instead of the normal parallel arrangement), unmyelinated nerve bundles, and presence of features of acute inflammation in examined specimens.

Nerve hypertrophy (increased nerve bundle thickness) has been used to diagnose Transitional-Zone pull-through in operated cases of HD; a cutoff value of 40 μm has been described. Some scholars considered this value for both submucosal and myenteric nerve plexus [15]; however, Rosai reported this value to submucosal plexus only [16]. Serio et al. reported that the submucosal nerve plexus is finer, and the ganglia are smaller than those of the myenteric plexus [17]. In our study, we differentiated between myenteric and submucosal nerve bundles during the measuring process. Although both nerve bundles were hypertrophied, myenteric nerve bundles were thicker and showed more significant correlation with the clinical outcome. This may be explained by the fact that myenteric nerve bundles are more concerned with bowel motility, while the submucosal nerves have mainly secretory function. Therefore, it is quite expected that if this limit value (40 μm) has been described for submucosal plexus, a higher limit value would be expected for the myenteric plexus. In our study, the estimated limit value for submucosal nerve plexus hypertrophy was similar to the reported standard value (40 μm). However, the limit value for the myenteric plexus was higher as expected (68 μm). We believe that detailed description of the measuring process is necessary to interpret any discrepancy that may appear between different studies. Also, it is important to differentiate between myenteric and submucosal plexus when considering these limit values.

Regarding the unmyelinated immature looking nerve bundles, it is well known that the vagus nerve contains three types of fibers: highly myelinated type A fibers (which have low activation thresholds); lightly myelinated type B fibers; and unmyelinated type C fibers (which have high activation thresholds). Type A fibers are heavily myelinated and therefore most sensitive to activation. Therefore, the unmyelinated nerve bundles that were evident in 65.7% of the assumed healthy ganglionic colonic segment may also play a role in decreased colonic motility and constipation recurrence following corrective surgery as they need a higher activation threshold compared to the normally myelinated myenteric nerve bundles. Other scholars have referred to the significance of unmyelinated nerve bundles in examination of specimens in HD as well [14,18].

Besides the hypertrophy and unmyelination of myenteric nerve bundles, we observed some other pathological features that included prominent congestion associated with focal disarray of nerve bundles. Searching through the literature, we have found other studies reporting on the possibility of fibromuscular dysplasia in the arteries located in the transitional zone [19]. Lastly, the presence of histopathological features of acute inflammation was more common in specimens from group B with recurrent attacks of HAEC. We do agree that the presence of acute inflammatory cells is more probably a result from HAEC rather than a causative factor. However, this may indicate that these patients are susceptible for developing postoperative attacks of enterocolitis in the future which is one of the most important causes for postoperative morbidity and mortality. This goes with the observation that cases who present with more severe preoperative attacks of enterocolitis are more susceptible for developing these attacks after surgery.

Kapur and Kennedy extensively studied surgical pathology considerations for diagnosing transitional zone pull-through in operated cases of HD. They discussed several histopathological features: subcircumferential aganglionosis, myenteric hypoganglionosis, abnormal extrinsic hypertrophic innervation, and intestinal neuronal dysplasia type B. These require special expertise and may be liable to significant subjective interpretation [13]. Kapur questioned the value of submucosal nerve hypertrophy (limit 40 μm) as a single indicator for diagnosis of transitional zone pull-through [20]. The limit value of 40 μm may be valid at less than 1 year of age but may need to be adjusted for older age and according to the location of the biopsy through different parts of the large intestine [20]. Instead, Kapur recommended a multidisciplinary approach for managing these cases; and not to depend on pathological studies alone but rather incorporate clinical, radiological, and manometric studies in the algorithm of management [20]. In our report, we studied different pathological

features of nerve bundles rather than considering a single feature (submucosal nerve thickness). This included the myenteric nerve thickness, unmyelination, nerve bundle disarray, and surrounding congestion. Together, this context of abnormal histopathological features would strengthen the value of pathological examination which is a major component in managing cases of HD.

Our study is limited by the small sample size. Also, we did not perform intraoperative frozen section assessment of the resected bowel, which has now been recommended as the standard of care in several centers all over the world. Further studies using frozen section examination are needed to see whether these changes persist throughout the bowel or are a transitional zone phenomenon [21] that would comply surgeons to keep moving more proximally in their dissection. However, our findings would suggest the importance of a more detailed histopathological analysis of excised specimens in HD (whether frozen or paraffin sections), and not just depend on presence or absence of ganglion cells in examined specimens. This may prove to be useful in choosing the level of colonic resection during operation (this is still not clear at this stage), or at least can indicate those patients who are susceptible for poor postoperative bowel function. These patients should be instructed to keep on close follow-up programs or may be advised to receive protracted courses of antibiotics in order to prevent morbidity and mortality known to be associated with Hirschsprung associated enterocolitis (HAEC).

4. Conclusion

Several histopathological features of the examined bowel specimens in HD, other than presence or absence of ganglion cells, are indicative of postoperative functional outcomes. These include the thickness and maturity of nerve bundles, in addition to the presence of histopathological features of acute inflammation.

Funding

None.

Conflict of interest

None.

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