

Variability of histologic pattern and transition zone length in Hirschsprung disease and associated factors

©Riana Pauline Tamba^{*1}, ©Novi Kurnia²

¹Division of Pediatric Surgery, Department of Surgery, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

²Department of Surgery, Cipto Mangunkusumo Hospital, Jakarta, Indonesia

Received: 09/03/2026

Accepted: 19/05/2026

Published: 20/10/2026

Cite this article: Tamba RP, Kurnia N. Variability of histologic pattern and transition zone length in Hirschsprung disease and associated factors. *Surg Child.* 2026;3(4):113-120. doi:10.51271/SOC-0083

*Corresponding Author: Riana Pauline Tamba, rianatamba@gmail.com

ABSTRACT

Aims: Transition zone (TZ) pull-through is a major cause of postoperative obstructive symptoms in Hirschsprung disease (HD). This study aimed to describe TZ histology and its association with disease classification, age, and preoperative stoma.

Methods: A retrospective review of hematoxylin–eosin-stained pull-through specimens was performed. Samples were classified as complete transition zone (CTZ) or incomplete transition zone (ITZ). Nerve fiber diameter, interganglionic interval, and transition-zone length or extent were assessed in the submucosal and intermuscular layers.

Results: CTZ length ranged from 2 to 16 cm. In ITZ specimens, transition-zone features were observed over available specimen lengths of 3 to 33 cm, representing the observed extent rather than the complete anatomical transition-zone length. Overall, nerve fiber diameter, interganglionic interval, and transition-zone measurements were not consistently associated with disease classification, age, or stoma. In the ITZ group, intermuscular nerve fiber diameter was significantly associated with age ($p=0.004$) and stoma ($p=0.001$), while submucosal transition-zone extent was associated with stoma ($p=0.016$). Layer discrepancy and skip areas were also observed.

Conclusion: The TZ in HD shows marked histological and spatial variability, including layer discrepancy and skip areas. These findings support careful histopathological confirmation of the proximal resection margin, but should be interpreted cautiously due to the retrospective design, limited sample size, unequal groups, and incomplete specimen assessment.

Keywords: Hirschsprung disease, Hirschsprung disease/pathology, transition zone

INTRODUCTION

Hirschsprung disease (HD) is a congenital disorder characterized by the absence of parasympathetic ganglion cells in the myenteric and submucosal plexuses of the intestine, beginning at the internal anal sphincter and extending proximally for a variable length. Approximately 80% of cases are classified as short-segment HD involving the rectosigmoid region, 10% as long-segment HD extending to the splenic flexure, and 5-10% as total colonic aganglionosis.¹⁻⁴

The estimated global incidence of HD is 1 in 5,000 live births, with a male-to-female ratio of approximately 4:1.^{1,3,5,6} Data from the Division of Pediatric Surgery Cipto Mangunkusumo Hospital (RSCM) show that 40-50 new patients with HD are referred annually over the past five years.⁷ Clinical manifestations vary depending on patient age and the length of the aganglionic segment. The diagnosis is established by rectal biopsy, and definitive management consists of resection of the entire aganglionic segment along with the transition zone (TZ), followed by pull-through of ganglionated bowel to the anus.¹⁻³

Despite advances in surgical techniques, postoperative obstruction presenting as constipation or Hirschsprung-associated enterocolitis remains a frequent morbidity, reported in 9-40% of cases.^{8,9} Inadequate resection with pull-through performed within the TZ is recognized as a major cause of postoperative obstructive symptoms.^{5,9-14} A meta-analysis by Friedmacher and Puri¹⁵ demonstrated that 34.8% of patients requiring redo pull-through surgery showed abnormal rectal biopsy findings consistent with TZ pull-through. These findings highlight the clinical importance of accurately identifying the TZ during surgery.

Determining the precise length of the TZ intraoperatively is crucial for establishing an adequate resection margin. Kapur¹⁶ reported that in short-segment HD, the TZ generally does not exceed 5 cm, recommending resection at least 5 cm proximal to the first identified ganglionated segment. However, Coyle et al.¹⁷ found that 23% of cases had a TZ longer than 5 cm. Moreover, histopathologic



criteria defining the TZ remain controversial. Hypertrophic nerve fibers and hypoganglionosis are commonly used indicators, yet there is no consensus regarding threshold values for nerve hypertrophy or standardized criteria for hypoganglionosis.¹⁷⁻¹⁹

Factors influencing TZ length are not clearly understood. Some literature suggests that the TZ is longer in long-segment HD compared with short-segment HD.^{17,20} Other studies indicate that HD classification, age, and the presence of stoma may affect nerve fiber diameter within the TZ.^{18,19,21,22} Currently, contrast enema findings and macroscopic bowel appearance remain common guides for identifying the TZ.

Further characterization of the histologic features and length of the TZ is needed. Therefore, this study aimed to describe the histologic characteristics and length of the TZ in children with HD at RSCM and to analyze their association with disease classification, age, and the presence of stoma. The findings are expected to provide a scientific basis for determining an adequate resection margin and reducing postoperative morbidity related to TZ pull-through.

METHODS

Ethics

Ethical approval was obtained from the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (Date: 05.04.2021, Decision No: KET-316/UN2.F1/ETIK/PPM.00.02/2021). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Design

This study was an observational descriptive-analytic study with retrospective histopathological slide review. The study was conducted at Cipto Mangunkusumo National Hospital (RSCM), Jakarta. Data were extracted from archived records of patients diagnosed with HD who underwent pull-through surgery between 2015 and 2020. Histopathological re-evaluation was performed at the Department of Anatomical Pathology, RSCM.

Study Population and Sample Size

The target population consisted of all pediatric patients diagnosed with HD. The accessible population included pediatric patients with HD who underwent pull-through surgery at the Pediatric Surgery Division, RSCM. Total sampling was applied. All eligible patients treated between 2015 and 2020 who met the inclusion and exclusion criteria were included in the study. Sample size calculation was performed using a formula for comparison of two means:

$$n = 2 \left(\frac{(Z\alpha + Z\beta)S}{x_1 - x_2} \right)^2$$

With the following parameters:

$\alpha=5\%$ ($Z\alpha=1.96$)

$\beta=20\%$ (power 80%; $Z\beta=0.84$)

Clinically meaningful difference (x_1-x_2)=10

Standard deviation (S)=15

The minimum required sample size was 35 subjects. However, total sampling was performed, and all eligible patients within the study period were included. Although the final sample exceeded the minimum calculated sample size for the overall analysis, the distribution between CTZ and ITZ groups was unequal. The relatively small CTZ group limited the statistical power for subgroup comparisons. Therefore, subgroup findings should be interpreted as exploratory.

Inclusion and Exclusion Criteria

Patients were included if they were aged ≤ 18 years and had a confirmed diagnosis of HD based on histopathological examination. Only patients who underwent definitive pull-through surgery at RSCM during the study period were considered eligible.

Patients were excluded if they were diagnosed with total colonic aganglionosis, had undergone previous bowel resection prior to definitive pull-through (including Hartmann procedure or prior pull-through), had incomplete resection specimens sent for pathological examination, had specimens that did not include the TZ, or had incomplete medical records or pathology archives.

Variables

The independent variables included HD classification (short-segment or long-segment), age at definitive surgery (<3 months, 3–12 months, >12 months), and presence of preoperative stoma (yes or no). The dependent variables were nerve fiber diameter within the TZ (μm), interganglionic interval within the TZ (μm), and TZ length (cm).

Sample Collection and Analysis

Resected colon specimens obtained during pull-through surgery were reviewed. Based on specimen completeness, samples were categorized into complete transition zone (CTZ) (specimens containing aganglionic, transition, and ganglionic zones) and incomplete transition zone (ITZ) (specimens lacking one or more zones but containing at least the TZ).

CTZ was defined as a resected specimen containing all three histological zones: aganglionic, transition, and ganglionated zones. In this group, both distal and proximal boundaries of the TZ could be identified, allowing complete measurement of TZ length.

ITZ was defined as a specimen that did not contain all three histological zones but still showed histological features of the TZ. This included specimens containing aganglionic and TZs, transition and ganglionated zones, or specimens in which the submitted tissue predominantly showed transition-zone features. Therefore, TZ length in the ITZ group represents the observed length of transition-zone features within the available specimen and should not be interpreted as the definitive full anatomical TZ length.

Hematoxylin-eosin-stained slides were re-evaluated. Photographs were obtained from the proximal and distal luminal ends and at every 1-cm interval along the longitudinal specimen. Images were captured at the submucosal and intermuscular layers using a high-definition Indomicon

camera mounted on an Olympus BX50 light microscope at 20× magnification. Each image measured 1920×1080 pixels (approximately 860×480 μm).

Histopathological evaluation was performed by the principal investigator in consultation with one board-certified anatomical pathologist. The presence of ganglion cells and nerve fibers was assessed in each image. Nerve fiber diameter and interganglionic interval were measured using ImageJ open-source software. Formal interobserver agreement analysis was not performed because the final histopathological interpretation was made by one anatomical pathologist.

TZ identification was based on established histopathological features, including the presence of hypertrophic nerve fibers and reduced ganglion cell density (hypoganglionosis), as described in previous studies. No strict numerical cutoff was applied for nerve fiber diameter, as variability has been reported in the literature; instead, interpretation was based on relative comparison across segments within the same specimen. For each sampled section, multiple representative fields were analyzed. Nerve fiber diameter was measured as the mean of selected representative fibers within each image rather than the maximum value, to reduce measurement bias.

Statistical Analysis

Categorical variables were presented as percentages, whereas continuous variables were expressed as mean±standard deviation if normally distributed or as median if not normally distributed. Data analysis was performed using SPSS version 20.0 for Windows. Descriptive analysis was conducted to assess patient characteristics. Inferential analysis was performed to evaluate the association between independent categorical variables (HD classification, age group, and stoma status) and continuous dependent variables (nerve fiber diameter, interganglionic interval, and TZ length). Shapiro-Wilk was used for normality testing. For comparisons between two groups, the independent t-test was used for normally distributed data, and the Mann-Whitney test was applied for non-normally distributed data. For age categories involving more than two groups, one-way ANOVA was used for normally distributed data, and the Kruskal-Wallis test was used when normality assumptions were not met. A p-value <0.05 was considered statistically significant.

RESULTS

A total of 119 definitive pull-through procedures for HD were performed at the Pediatric Surgery Division of Cipto Mangunkusumo National Hospital between January 2015 and December 2020. Following identification of these cases, histopathological reports were retrieved from the department of anatomical pathology archives, and corresponding medical records were reviewed for eligibility.

The types of definitive procedures included Soave-Boley, transanal endorectal pull-through, Duhamel Adang modification, Duhamel Martin modification, and laparoscopic-assisted transanal endorectal pull-through. Their distribution is presented in **Figure 1**.

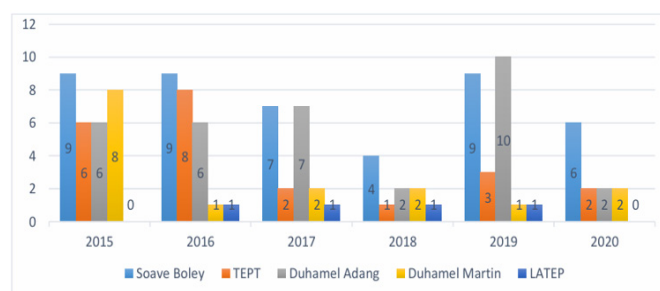


Figure 1. Definitive surgical treatment performed by pediatric surgery division RSCM 2015-2020

RSCM: Cipto Mangunkusumo National Hospital, TEPT: Transanal endorectal pull-through

Of 119 patients who underwent definitive pull-through surgery, 47 met the eligibility criteria and were included in the final analysis. The main reasons for exclusion were total colonic aganglionosis, previous bowel resection or redo pull-through, incomplete specimen submission, absence of TZ in the specimen, incomplete slide sampling, and unretrievable pathology slides. The final sample recruitment flow is summarized in **Figure 2**.

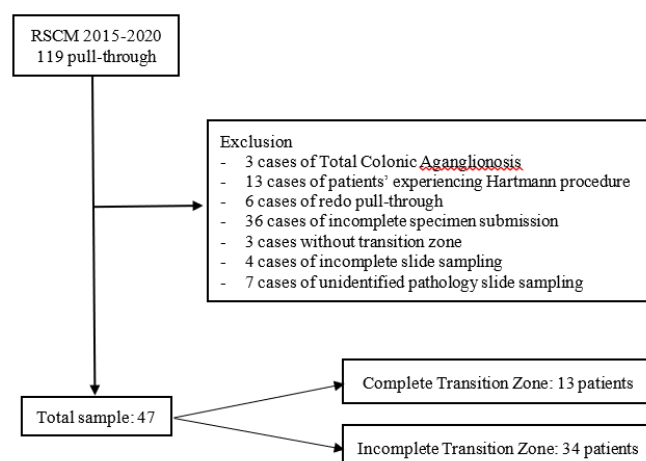


Figure 2. Sample recruitment flow RSCM: Cipto Mangunkusumo National Hospital

A total of 47 patients were included in the final analysis and were divided into two groups based on specimen completeness. The first group consisted of patients with a CTZ, defined as specimens containing aganglionic, transition, and ganglionated zones. The second group consisted of patients with an ITZ, defined as specimens that did not contain all three zones but included at least the TZ. Within the ITZ group, 15 specimens contained aganglionic and TZs, 8 contained transition and ganglionated zones, and 11 showed transition-zone features throughout the submitted tissue. Clinical characteristics of both groups are presented in **Table 1** (CTZ) and **Table 2** (ITZ).

In the CTZ group (n=13), 69.3% were male. The majority were classified as short-segment HD (92.3%), with only one long-segment HD (7.7%). The median age at definitive surgery was 5 months (range 1-17 months). Most patients underwent surgery before 3 months of age (38.5%) or between 3-12 months (38.5%). Preoperative stoma was present in 30.7% of patients. Transanal endorectal pull-through (TEPT) was the most commonly performed definitive procedure.

Table 1. Characteristics of complete transition zone group

Characteristic	Total patients	Median (min-max)
Gender		
Male (%)	9 (69.3%)	
Female (%)	4 (30.7%)	
Classification		
Short segment	12 (92.3%)	
Long segment	1 (7.7%)	
Age at definitive procedure (months)		
<3 months	5 (38.5%)	5 (1-17)
3-12 months	5 (38.5%)	
>12 months	3 (23%)	
Pre-operative stoma	4 (30.7%)	
Definitive procedure		
Soave-Boley	3 (23%)	
TEPT	5 (38.5%)	
Duhamel Adang	1 (7.7%)	
Duhamel Martin	2 (15.4%)	
LATEP	2 (15.4%)	

Categorical variables are presented as n (%). Age is presented as median (min-max) due to non-normal distribution. Min: Minimum, Max: Maximum, TEPT: Transanal endorectal pull-through

Table 2. Characteristics of incomplete transition zone group

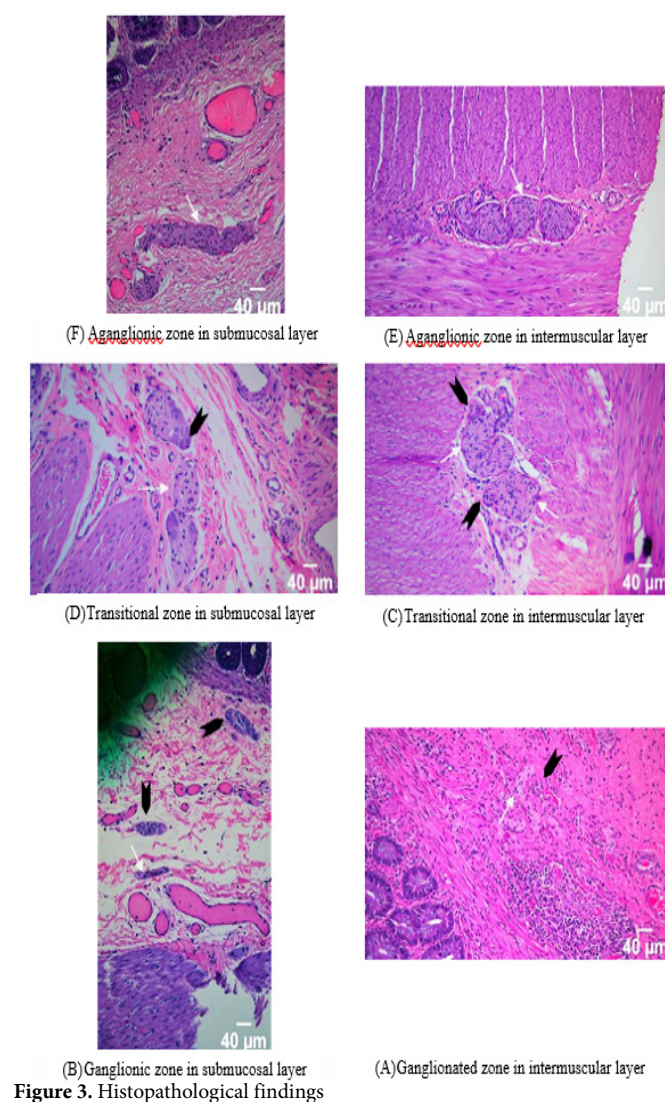
Characteristic	Total patients	Median (min-max)
Gender		
Male (%)	24 (70.6%)	
Female (%)	10 (29.4%)	
Classification		
Short segment	31 (91.1%)	
Long segment	3 (8.9%)	
Age at definitive procedure (months)		
<3 months	8 (23.5%)	8 (1-186)
3-12 months	11 (32.4%)	
>12 months	15 (44.1%)	
Pre-operative stoma	19 (55.9%)	
Definitive procedure		
Soave-Boley	12 (35.3%)	
TEPT	12 (35.3%)	
Duhamel Adang	5 (14.7%)	
Duhamel Martin	4 (11.8%)	
LATEP	1 (2.9%)	

Categorical variables are presented as n (%). Age is presented as median (min-max) due to non-normal distribution. Min: Minimum, Max: Maximum, TEPT: Transanal endorectal pull-through

In the ITZ group (n=34), 70.6% were male. Short-segment HD accounted for 91.1% of cases, and long-segment HD for 8.9%. The median age at definitive surgery was 8 months (range 1-186 months). The majority of patients underwent surgery after 12 months of age (44.1%). Preoperative stoma was present in 55.9% of patients. TEPT and Soave-Boley were the most frequently performed procedures.

Representative histopathological features of the aganglionic zone, TZ, and ganglionated zone in both submucosal and intermuscular layers are shown in **Figure 3**. Evaluation was

performed using hematoxylin-eosin (HE) staining at 20× magnification. In HE staining, ganglion cells and nerve fibers may appear similar in color, making morphological identification essential. Nerve fiber thickening was relatively easy to identify, particularly in the intermuscular layer. Ganglion cells were more difficult to recognize in HE-stained sections alone, especially in the TZ where ganglia and nerve fibers may appear closely approximated. In contrast, in the ganglionated zone, ganglion cells were more readily identified, while nerve fibers appeared thinner and less prominent.

**Figure 3.** Histopathological findings

In the CTZ group, the aganglionic segment length ranged from 2-11 cm, the TZ length ranged from 2-16 cm, and the ganglionated segment length ranged from 1-7 cm. In the ITZ group, transition-zone features were observed within available specimen lengths ranging from 3 to 33 cm. Because these specimens were incomplete, this range should be interpreted as the observed extent of transition-zone features rather than the complete anatomical TZ length. Overall, nerve fiber diameter was greater in the aganglionic zone compared with the TZ. The interganglionic interval was greater in the TZ compared with the ganglionated zone. The TZ defined by nerve hypertrophy in the submucosal and intermuscular layers generally overlapped within the same section; however, several differences were observed.

In the CTZ group, four patients showed the most distal ganglion in the submucosal layer located 2-3 cm distal to the most distal ganglion in the intermuscular layer. Conversely, only one patient showed the opposite finding, in which the most distal ganglion in the submucosal layer was located 2 cm proximal to the most distal ganglion in the intermuscular layer. In the ITZ group, the difference between the two layers ranged from 1-6 cm, with a median of 2 cm. The TZ length in the submucosal and intermuscular layers was also not symmetrical. In some specimens, the TZ in the submucosal layer was longer, whereas in others the intermuscular TZ was longer.

Areas with normal nerve fiber diameter were observed between regions of nerve hypertrophy, particularly in the submucosal layer, indicating that nerve hypertrophy within the TZ was not uniformly distributed. In the CTZ group, these “skip areas” ranged from 1-2 cm, whereas in the ITZ group they could reach up to 6 cm. The mean nerve fiber

diameter, interganglionic interval, and TZ length in each layer and their associations with HD classification, age, and presence of stoma in the CTZ group are presented in Table 3, while the results for the ITZ group are presented in Table 4.

In the CTZ group, nerve fiber diameter in the submucosal layer of long-segment HD was smaller than that observed in short-segment. In contrast, in the intermuscular layer, the opposite pattern was observed. The TZ length in short-segment HD was also shorter than that in long-segment. In the ITZ group, no difference in nerve fiber diameter was observed between short-segment and long-segment HD, either in the submucosal or intermuscular layers.

In both CTZ and ITZ groups, nerve fiber diameter tended to increase with age; however, a statistically significant association was only found in the intermuscular layer of the ITZ group. When comparing patients with and without preoperative stoma, nerve fiber diameter is tended to be greater

Table 3. Histological characteristics of complete transition zone

Variables	Submucosal layer						Intermuscular layer						Total	
	Ø µm	p	s µm	p	ℓ cm	p	Ø µm	p	s µm	p	ℓ cm	p	ℓ cm	p
Classification														
Short segment	49.7±6	0.023*	246.4±65	0.71*	3.5(1-15)	0.502*	51.3 (0-89)	0.348*	212.5 (60-269.8)	1.0#	3.5(0-13)	0.416#	6.5±3	0.89*
Long segment	32.4		272.6		6		60		207.8		6		6	
Age														
< 3 months	51.4±3	0.576\$	278.5±79	0.371\$	4±3	0.411\$	49.7±32	0.676\$	151.7±100	0.321\$	3±2	0.321\$	5±2	0.447\$
3-12 months	46.5±5		219.5±44		4.2±1		48.3(45-62)		220.8±52		5.4±3		6.8±2	
>12 months	46.4±15		246.2±55		7.6±6		56.4±3		208.8±12		7±5		8.6±6	
Stoma														
Yes	47.1±12	0.716*	234.2±51	0.616*	6.5±5	0.351*	57.9±3	0.446*	224±32	0.306*	6.2±4	0.378*	5(4-16)	0.938*
No	48.9±5		254.6±70		4.2±2		48.6±22		177±83		4.2±3		6.1±2	
Ø: Nerve fiber diameter (µm), s: Interganglionic distance (µm), ℓ: Length (cm). Data are presented as mean±standard deviation (SD) for normally distributed variables and median (min–max) for non-normally distributed variables. *Independent t-test, #Mann–Whitney test, \$ANOVA, & Kruskal–Wallis test. Min: Minimum, Max: Maximum														

Ø: Nerve fiber diameter (µm), s: Interganglionic distance (µm), ℓ: Length (cm). Data are presented as mean±standard deviation (SD) for normally distributed variables and median (min-max) for non-normally distributed variables. *Independent t-test, [#]Mann-Whitney test, ^sANOVA, & Kruskal-Wallis test. Min: Minimum, Max: Maximum

Table 4. Histological characteristics of incomplete transition zone

Variables	Submucosal layer						Intermuscular layer						Total	
	Ø µm	p	s µm	p	ℓ cm	p	Ø µm	p	s µm	p	ℓ cm	p	ℓ cm	p
Classification														
Short segment	53.9±11	0.962*	224.8±37	0.136*	10.1±6	0.073*	92.8±21	0.921*	232.9±53	0.412*	10.8±5	0.514*	11.9±5	0.386*
Long segment	54.2±7		190.9±21		17.3±10		91.5±19		206.7±18		17.6±15		19.6±12	
Age														
<3 months	55.9±14	0.792 ^s	216.7±29	0.235 ^s	8.1±4	0.082 ^s	78.5±8	0.004 ^s	222.6±66	0.468 ^s	9.3±3	0.186 ^s	10.3±4	0.308 ^s
3-12 months	54.3±13		209.2±30		8.6±6		86.1±21		219.4±62		9.6±5		11.3±5	
>12 months	52.5±7		233.8±43		12(6-29)		105.1±19		243.2±32		13.8±8		13(6-33)	
Stoma														
Yes	54.2±7	0.889*	229.2±40	0.201*	12(6-29)	0.016 [#]	104±20	0.001*	232.8±63	0.832*	12.8±8	0.165*	13(4-33)	0.143 [#]
No	53.6±14		212.5±31		7.9±5		78.3±9		228.9±43		9.6±4		6.1±2	
Ø: Nerve fiber diameter (µm), s: Interganglionic distance (µm), ℓ: Length (cm). Data are presented as mean±standard deviation (SD) for normally distributed variables and median (min–max) for non-normally distributed variables. *Independent t-test, #Mann–Whitney test, \$ANOVA, & Kruskal–Wallis test. Min: Minimum, Max: Maximum														

Ø: Nerve fiber diameter (µm), s: Interganglionic distance (µm), ℓ: Length (cm). Data are presented as mean±standard deviation (SD) for normally distributed variables and median (min-max) for non-normally distributed variables. *Independent t-test, [#]Mann-Whitney test, ^sANOVA, & Kruskal-Wallis test. Min: Minimum, Max: Maximum



in patients with stoma, particularly in the intermuscular layer but not consistently statistically significant. Regarding the interganglionic interval, no significant differences were observed according to HD classification, age group, or stoma status in either CTZ or ITZ groups. The interganglionic interval in the ganglionated zone is smaller than that in the TZ; however, the difference was minimal and not statistically significant. For transition-zone measurements, both CTZ and ITZ groups showed numerically greater values in long-segment HD, older patients, and patients with preoperative stoma; however, these findings were not consistently statistically significant and should be interpreted cautiously.

A post-hoc power consideration suggests that the study is underpowered for subgroup analyses, particularly for comparisons involving the CTZ group and long-segment HD. Therefore, non-significant findings should be interpreted cautiously, as they may reflect type II error.

DISCUSSION

The main finding of this study is that the TZ in HD shows marked histological and spatial variability. In the CTZ group, transition-zone length ranged from 2 to 16 cm, while in ITZ specimens, transition-zone features were observed over available specimen lengths of 3 to 33 cm.

A substantial number of patients were excluded due to incomplete specimens submitted for pathological examination. During pull-through procedures, particularly in patients with previous stoma creation, bowel resection may be performed in multiple segments. In many cases, only selected portions of the resected bowel are submitted for pathological confirmation of ganglion cells. This practice may result in reports indicating that the proximal margin still corresponds to the TZ and may contribute to incomplete resection.

Preoperative stoma was analyzed because it may represent staged management, delayed definitive surgery, previous bowel dilatation, and longer exposure to functional obstruction. However, stoma status should not be interpreted as a direct causal factor for transition-zone length.

Evaluation of pathology reports also demonstrated variability in intraoperative frozen-section biopsy techniques and specimen submission.²³ Frozen-section biopsy is essential for confirming the presence of ganglion cells before completing the pull-through procedure; however, the biopsy location and distance between biopsy sites varied among surgeons. Inconsistent sampling may lead to inaccurate identification of the TZ, which remains one of the major causes of postoperative obstructive symptoms.⁹

The prevalence of TZ pull-through observed in this study was 25.2%. This finding is comparable with the systematic review by Friedmacher and Puri,¹⁵ which reported a rate of 25.4% of TZ pull-through among patients requiring redo surgery. Similarly, Ghosh et al.²⁴ reported a prevalence of approximately 16% in a tertiary referral center. These findings suggest that incomplete resection of the TZ remains a persistent challenge in the surgical management of HD.

Several studies have suggested that evaluation of the proximal doughnut specimen during pull-through surgery may reduce the risk of TZ pull-through. Das et al.²⁵ demonstrated that examination of the proximal doughnut specimen can identify residual TZ tissue that may not be detected through localized biopsy. In addition, Murthi et al.²⁶ showed that routine circumferential doughnut biopsy during surgery prevented TZ pull-through in a substantial proportion of patients initially considered to have ganglionic bowel.

The demographic characteristics of the study population were consistent with previously reported epidemiological data. HD occurs predominantly in males, with a male-to-female ratio approaching 4:1, and short-segment HD represents the majority of cases.⁷ These findings are consistent with the distribution observed in the present study.

Histopathological evaluation showed that hypertrophic nerve fibers remain a useful criterion for identifying the TZ. However, interpretation of nerve hypertrophy should consider patient age. Kapur¹⁹ reported that nerve trunks larger than 40 μm may also be observed in normal rectal tissue, particularly in children older than one year. The 40 μm threshold was originally proposed by Monforte-Muñoz et al.¹⁸ as a diagnostic criterion for hypertrophic nerve trunks in HD.

An additional observation was the presence of skip areas in which histologically normal segments were identified within the TZ. These areas ranged from 1 to 6 cm and were most commonly observed in the submucosal layer. The discrepancy between the submucosal and intermuscular layers suggests that the TZ does not progress uniformly across bowel wall layers. In some cases, the submucosal layer appeared to extend more distally, whereas the opposite pattern was observed in others. This finding indicates that limited focal assessment may underestimate the true extent of the TZ. This finding further supports the importance of careful intraoperative evaluation and comprehensive pathological examination to avoid incomplete resection of the TZ.²⁷

The presence of histologically normal-appearing skip areas within the TZ has important clinical implications. These areas may create false reassurance during intraoperative frozen-section assessment if only limited focal biopsies are taken. Therefore, careful proximal margin assessment and consideration of circumferential or doughnut evaluation may help reduce the risk of transition-zone pull-through.²⁷

In long-segment HD, nerve hypertrophy may be less prominent or present only focally. Studies by Narayanan et al.²¹ and Alnajar et al.²² demonstrated that hypertrophic nerve fibers may not always be observed in long-segment HD. Nevertheless, hypertrophic nerve fibers were still identified in several long-segment HD in this study, suggesting that this feature can still support identification of the TZ when interpreted alongside other histological findings.

The distance between ganglion cells was also evaluated to assess hypoganglionosis. According to Kapur and Kennedy,²⁸ hypoganglionosis is characterized by reduced ganglion cell density and smaller ganglion size. Coyle et al.¹⁷ proposed



that ganglion spacing greater than 730 μm may indicate hypoganglionosis within the TZ. However, in the present study, ganglion spacing did not differ significantly between groups, suggesting that this parameter alone may not reliably distinguish the TZ.

The median TZ length in this study was comparable with findings reported by Coyle et al.,¹⁷ who described a median length of approximately 6 cm. In the ITZ group, transition-zone features were observed over longer available specimen lengths. However, because these specimens were incomplete, this finding should be interpreted as the observed extent of transition-zone features rather than the complete anatomical length of the TZ.

Multivariable analysis was not performed due to limited sample size and risk of model overfitting. Therefore, potential confounding between variables such as age, stoma status, and disease severity cannot be excluded.

Limitations

This study has several limitations. First, the retrospective design and the exclusion of a large proportion of the initial cohort may have introduced selection bias, particularly because several patients were excluded due to incomplete specimen submission, missing pathology records, incomplete slide sampling, or unretrievable slides. Second, the relatively small CTZ group and the unequal distribution between CTZ and ITZ groups limited the statistical power for subgroup analyses. Given the small CTZ group ($n=13$) and the very limited number of long-segment cases, the study is likely underpowered to detect moderate differences, increasing the risk of type II error. Third, the ITZ group was heterogeneous because the available specimens did not always contain all three histological zones; therefore, measurements in this group represent only the observed extent of transition-zone features within the submitted specimens rather than the complete anatomical transition-zone length. Fourth, histopathological evaluation was performed using slides prepared for routine clinical service, which included longitudinal sampling from only one side of the bowel, thereby the circumferential variability of the TZ could not be assessed. This may result in underestimation or overestimation of the true TZ length, particularly in the presence of skip areas. Finally, formal interobserver agreement analysis was not performed.

CONCLUSION

This study demonstrates substantial variability in the histological appearance and observed extent of the TZ in HD. The TZ may differ between the submucosal and intermuscular layers and may contain histologically normal-appearing skip areas, which has important implications for intraoperative margin assessment. These findings support careful histopathological confirmation of the proximal resection margin to reduce the risk of transition-zone pull-through. However, the results should be interpreted cautiously because of the retrospective design, limited sample size, unequal CTZ and ITZ groups, and incomplete circumferential assessment.

ETHICAL DECLARATIONS

Ethics Committee Approval

Ethical approval was obtained from the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (Date: 05.04.2021, Decision No: KET-316/UN2.F1/ETIK/PPM.00.02/2021).

Informed Consent

This retrospective study used pre-existing anonymized patient data. No additional intervention was performed, and there was no direct patient contact. The study was approved by the Ethics Committee, and the requirement for written informed consent was waived by the ethics committee.

Peer Review Process

This manuscript was subject to external peer review.

Conflict of Interest

The authors declare no conflicts of interest related to this study.

Financial Disclosure

The authors received no financial support for the conduct or publication of this research.

Author Contributions

Concept: RPT; Design: RPT; Control: RPT, NK; Resources: RPT, NK; Materials: RPT, NK; Data Collection and/or Processing: RPT, NK; Analysis and/or Interpretation: RPT, NK; Literature Review: NK; Writing the Article: RPT, NK; Critical Review: RPT, NK.

Acknowledgements

The authors would like to thank the Pediatric Surgery Division and the Department of Anatomical Pathology of Cipto Mangunkusumo National Hospital for their support in providing access to clinical records and histopathological specimens used in this study. We also acknowledge the Faculty of Medicine, Universitas Indonesia, for facilitating the conduct of this research. The authors are grateful to all staff members who contributed to data collection and technical assistance during the histopathological evaluation and manuscript preparation.

REFERENCES

1. Kartono D. Penyakit Hirschsprung. Jakarta: Sagung Seto; 2004.
2. Langer JC. Hirschsprung disease. In: Coran AG, Adzick NS, Krummel TM, Laberge J-M, Shamberger RC, Caldamone AA, editors. Pediatric Surgery. 7th ed. Saunders; 2012.
3. Langer JC. Hirschsprung disease. In: Holcomb GW, Murphy JP, St. Peter SD, editors. Holcomb and Ashcraft's Pediatric Surgery. Elsevier Inc.; 2020.
4. Menteri Kesehatan Republik Indonesia. Keputusan Menteri Kesehatan Republik Indonesia Nomor HK.01.07/MENKES/474/2017 tentang Pedoman Nasional Pelayanan Kedokteran Tata Laksana Penyakit Hirschsprung. 2017.
5. Taguchi T, Matsufuji H, Ieiri S. Hirschsprung's Disease and the Allied Disorders. Singapore: Springer; 2019.
6. Bradnock TJ, Knight M, Kenny S, Nair M, Walker GM. Hirschsprung's disease in the UK and Ireland: incidence and anomalies. *Arch Dis Child*. 2017;102(8):722-727. doi:10.1136/archdischild-2016-311872
7. Wiradeni A. Pengaruh panjang usus aganglionik terhadap morbiditas penderita penyakit Hirschsprung pasca operasi definitif di Indonesia [thesis]. Universitas Indonesia; 2020.



8. Kapur RP, Smith C, Ambartsumyan L. Postoperative pullthrough obstruction in Hirschsprung disease: etiologies and diagnosis. *Pediatr Dev Pathol.* 2020;23(1):40-59. doi:10.1177/1093526619890735
9. Langer JC, Rollins MD, Levitt M, et al. Guidelines for the management of postoperative obstructive symptoms in children with Hirschsprung disease. *Pediatr Surg Int.* 2017;33(5):523-526. doi:10.1007/s00383-017-4066-7
10. Puri P. Hirschsprung's Disease and Allied Disorders. 4th ed. 2019.
11. Ralls MW, Coran AG, Teitelbaum DH. Reoperative surgery for Hirschsprung disease. *Semin Pediatr Surg.* 2012;21(4):354-363. doi:10.1053/j.sempedsurg.2012.07.011
12. Ghose SI, Squire BR, Stringer MD, Batcup G, Crabbe DCG. Hirschsprung's disease: problems with transition-zone pull-through. *J Pediatr Surg.* 2000;35(12):1805-1809. doi:10.1053/jpsu.2000.19263
13. Soh HJ, Nataraja RM, Pacilli M. Prevention and management of recurrent postoperative Hirschsprung's disease obstructive symptoms and enterocolitis: systematic review and meta-analysis. *J Pediatr Surg.* 2018;53(12):2423-2429. doi:10.1016/j.jpedsurg.2018.08.024
14. Saadai P, Trappey AF, Goldstein AM, et al. Guidelines for the management of postoperative soiling in children with Hirschsprung disease. *Pediatr Surg Int.* 2019;35(8):829-834. doi:10.1007/s00383-019-04497-y
15. Friedmacher F, Puri P. Residual aganglionosis after pull-through operation for Hirschsprung's disease: a systematic review and meta-analysis. *Pediatr Surg Int.* 2011;27(10):1053-1057. doi:10.1007/s00383-011-2958-5
16. Kapur RP. Histology of the transition zone in Hirschsprung disease. *Am J Surg Pathol.* 2016;40(12):1637-1646. doi:10.1097/PAS.0000000000000711
17. Coyle D, O'Donnell AM, Tomuschat C, Gillick J, Puri P. The extent of the transition zone in Hirschsprung disease. *J Pediatr Surg.* 2019;54(11):2318-2324. doi:10.1016/j.jpedsurg.2019.04.017
18. Monforte-Muñoz H, Gonzalez-Gomez I, Rowland JM, Landing BH. Increased submucosal nerve trunk caliber in aganglionosis: a positive and objective finding in suction biopsies and segmental resections in Hirschsprung's disease. *Arch Pathol Lab Med.* 1998;122(8):721-725.
19. Kapur RP. Submucosal nerve diameter greater than 40 µm is not a valid diagnostic index of transition zone pull-through. *J Pediatr Surg.* 2016;51(10):1585-1591. doi:10.1016/j.jpedsurg.2016.06.007
20. Thakkar HS, Blackburn S, Curry J, et al. Variability of the transition zone length in Hirschsprung disease. *J Pediatr Surg.* 2020;55(1):63-66. doi:10.1016/j.jpedsurg.2019.09.056
21. Narayanan SK, Soundappan SS, Kwan E, Cohen RC, Charlton A, Cass DT. Aganglionosis with the absence of hypertrophied nerve fibres predicts disease proximal to rectosigmoid colon. *Pediatr Surg Int.* 2016;32(3):221-226. doi:10.1007/s00383-015-3835-4
22. Alnajar H, Murro D, Alsadi A, Jakate S. Spectrum of clinicopathological deviations in long-segment Hirschsprung disease compared with short-segment Hirschsprung disease: a single-institution study. *Int J Surg Pathol.* 2017;25(3):216-221. doi:10.1177/1066896916675729
23. Moore SW. Hirschsprung disease: current perspectives. *Open Access Surg.* 2016;9:39-50. doi:10.2147/OAS.S81552
24. Ghosh DN, Liu Y, Cass DT, Soundappan SSV. Transition zone pull-through in Hirschsprung's disease: a tertiary hospital experience. *ANZ J Surg.* 2017;87(10):780-783. doi:10.1111/ans.13745
25. Das K, Kini U, Babu MK, Mohanty S, D'Cruz AJ. The distal level of normally innervated bowel in long segment colonic Hirschsprung's disease. *Pediatr Surg Int.* 2010;26(6):593-599. doi:10.1007/s00383-010-2603-8
26. Murthi G, Townley O, Lindley R, Cohen M. Routine use of circumferential 'Doughnut' biopsy in pull through surgery for Hirschsprung's disease: advantages and limitations. *J Indian Assoc Pediatr Surg.* 2021;26(2):111. doi:10.4103/jiaps.JIAPS_43_20
27. De La Torre L, Wehrli LA. Error traps and culture of safety in Hirschsprung disease. *Semin Pediatr Surg.* 2019;28(3):151-159. doi:10.1053/j.sempedsurg.2019.04.013
28. Kapur RP, Kennedy AJ. Histopathologic delineation of the transition zone in short segment Hirschsprung disease. *Pediatr Dev Pathol.* 2013;16(4):252-266. doi:10.2350/12-12-1282-OA.1