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Original Article PEDIATRIC SURGERY

Full-thickness rectal biopsies in diagnosis of Hirschsprung disease: 13 years of experience of a single center

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ABSTRACT

OBJECTIVE: An experience regarding full-thickness rectal biopsies (FTRB) indications and results is presented.

METHODS: The records of patients who underwent FTRB between January 2010 and January 2022 were retrospectively reviewed.

RESULTS: 107 patients were included. There were 66 men (61.6%) and 41 women (38.3%). The median age at biopsy was 15 (1–196) months. FTRB was performed in 81 patients who were unable to pass meconium in the first 48 hours or had intractable constipation. A stoma was performed in 26 patients before the rectal biopsy. Contrast colon radiography was conducted in 61 patients and/or anorectal manometry in 32 patients. Of the biopsies, 74 were full-thickness while 33 were not. Biopsies were repeated in 12 patients. Of the 49 (45%) patients with aganglionic specimens, data about nerve hypertrophy was reported in 37. Among these, 33 had nerve hypertrophy and 4 did not. A definitive surgery was performed in 44 of the 49 patients diagnosed with Hirschsprung disease (HD). Complications were observed in 7 (6.5%) of the patients.

CONCLUSION: HD was histopathologically diagnosed. Biopsies that are not full-thickness can be of value when using immunohistochemistry stains.

Keywords: Children; Hirschsprung disease; immunohistochemical examination; intractable constipation; rectal biopsy.

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Hirschsprung disease (HD) is a developmental disease of the enteric nervous system characterized by absence of ganglia in the submucosal and myenteric plexuses of the distal bowel [1]. The incidence of the disease is 1 in 5000 live births, and it occurs 4 times more frequently in boys compared to girls [2]. Eighty to ninety percent of HD patients are diagnosed in the neonatal period. A subgroup of HD patients is composed of older patients who present with severe constipation later in life [1]. Although various diagnostic tests such as contrast enema radiographies of the colon and anorectal manometry are used in the diagnosis of HD, a definite diagnosis is only possible with histopathological evaluation.

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Diagnostic rectal biopsies include superficial and deep submucosal biopsy and full-thickness biopsies involving muscularis propria and myenteric plexus. In the diagnosis of Hirschsprung's disease (HD), the evaluation of submucosal samples has been emphasized in various studies as a sufficient alternative to full-thickness biopsy. While the presence or absence of ganglion cells is examined using Hematoxylin-Eosin (H&E) staining, calretinin immunohistochemical staining provides high diagnostic accuracy in supporting the diagnosis [3]. According to a study published by Zemheri et al. in 2021 [4], it has been noted that in some controversial cases, HD can be diagnosed without the need for a full-thickness biopsy. Negative calretinin staining indicates the aganglionic region and strongly supports the diagnosis.

In this study, we aimed to evaluate the diagnostic efficiency of rectal biopsies performed because of suspected HD.

MATERIALS AND METHODS

After ethics committee approval was obtained (Istanbul Medeniyet University Goztepe Training and Research Hospital, date: 13.04.2022, number: 2022/0229), epicrisis and pathology records of the patients, who underwent rectal biopsy because of suspected HD in a 13-year period between January 1, 2010, and January 1, 2022, were examined retrospectively. The study was conducted in accordance with the Declaration of Helsinki. The full-thickness biopsies were obtained surgically from the posterior rectal wall under general anesthesia in the dorsal lithotomy position. Biopsy samples were collected 1–3 cm above the dentate line, depending on the age of the patient. The antibody used for calretinin staining was prepared at a dilution ratio of 1:100, and the incubation time was set to 1 hour at 37 °C. All samples were fixed in formalin, embedded in paraffin blocks, and sectioned using a microtome.

Statistical Analysis

Statistical analysis was performed using SPSS 22.0 (SPSS Inc., Chicago, IL) package program. Comparisons between groups were performed using the Mann-Whitney U test. Statistical significance level was determined as $p<0.05$.

RESULTS

This study analyzed 119 rectal biopsies obtained from 107 patients with suspected HD, emphasizing the importance of histopathological and immunohistochemical techniques in achieving accurate diagnoses.

Highlight key points

- Rectal biopsy remains the gold standard for the diagnosis of Hirschsprung disease.
- In cases where the rectal biopsy is not full-thickness, demonstrating the absence of ganglion cells with the addition of Calretinin and S-100 staining methods in addition to the classical H&E staining method is diagnostic of Hirschsprung disease.
- Immunohistochemical evaluation together with H&E is sufficient to make the diagnosis, regardless of the patient's age and biopsy amount.

The study population included 66 males (61.6%) and 41 females (38.3%), with a median age at biopsy of 15 months (range: 1–196 months). Standing abdominal radiography was performed in all patients, while contrast enema radiography and anorectal manometry were conducted in 59.8% and 31% of cases, respectively. Both tests were performed together in 28.2% of these patients. Surgical stomas were present in 26 patients before biopsy, primarily due to intestinal perforation (62.5%) or neonatal intestinal obstruction (45.8%). For 81 patients (75%), biopsy was performed to investigate intractable constipation, with a median age of 21 months in this group ($p=0.026$).

Histopathological examination with H&E staining identified mature ganglion cells in 53.2% (57/107) of cases (Table 1). Full-thickness biopsies involving all layers of the rectum were obtained in 74 samples (69%), while the remaining 33 (31%) samples involved only the mucosa and submucosa. Nerve hypertrophy was noted in 80% (86/119) of biopsy samples, further supporting HD diagnoses.

Calretinin staining was performed in 84 samples (78.5%), increasing the detection of ganglion cells to 73.7% (42/57) compared to H&E alone (Fig. 1). Among 50 patients in whom ganglion cells were not detected via H&E staining, calretinin staining was negative in 40 (80%), supporting the diagnosis of HD. Calretinin staining was not performed in 15 patients, and S100 staining was conducted in 31 (28.9%) samples (Fig. 2, 3). Positive S100 staining identified nerve cells in 12 of the 39 cases where ganglion cells were detected with calretinin (Fig. 4, Table 2).

Among patients without ganglion cells in initial biopsies, HD was diagnosed in 82% (41/50) based on clinical, radiological, and manometric findings. Biopsy was repeated in nine patients from this group, confirming HD in five cases. Of the 12 patients who underwent repeat biopsies overall, 37.6% had initial superficial biopsies, highlighting the importance of obtaining full-thickness samples.

Table 1. Pathological features of first biopsy materials with Haematoxylin and Eosin stain

Mature ganglion cells	Nerve hypertrophy	FTRB n=74 (69.1%)	Non FTRB n=33 (30.9%)	Total n=107 (100%)
Present (n=57)				
	Present	18.9	9.1	17 (15.9)
	Absent	18.9	27.2	23 (21.5)
	Not reported	18.9	9.1	17 (15.9)
Absent (n=50)	Present	37.9	42.4	42 (39.3)
	Absent	2.7	6.1	4 (3.7)
	Not reported	2.7	6.1	4 (3.7)
Total		**74 (100)**	**33 (100)**	**107 (100)**

FTRB: Full-thickness rectal biopsy.

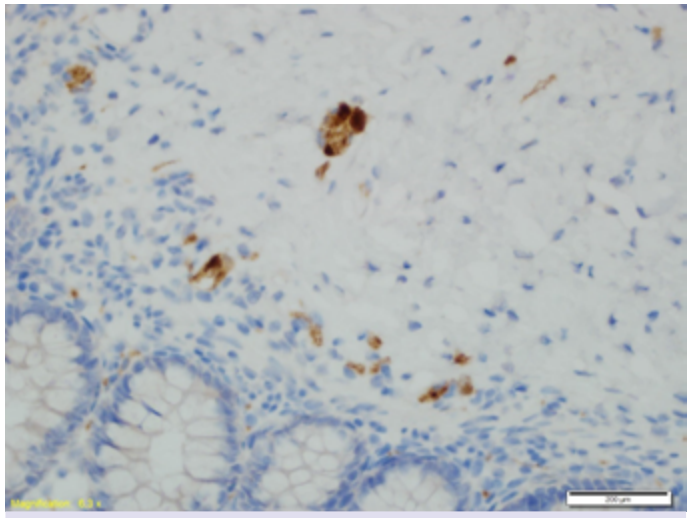

Figure 1. Mature ganglion cells were detected with H/E staining, ganglion cells were demonstrated with calretinin staining.

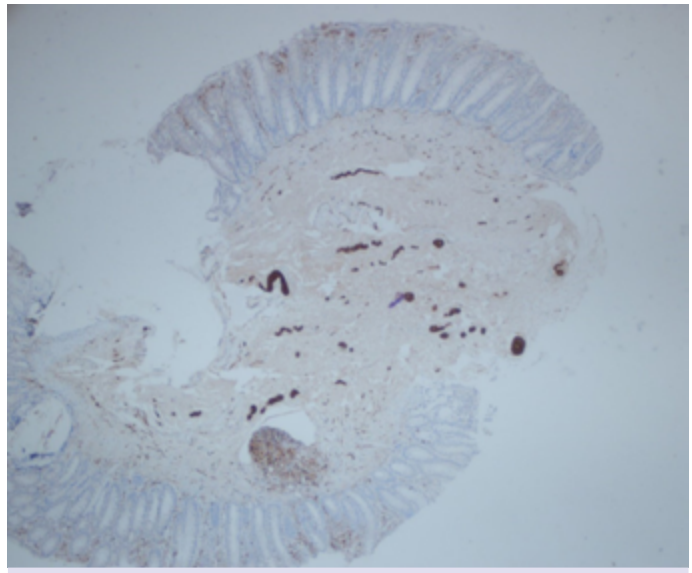

Figure 2. Ganglion cells and nerve cells were demonstrated with S100 staining.

In three patients with ganglion cells detected on both H&E and calretinin staining, persistent clinical suspicion for HD prompted repeated biopsies. These confirmed ganglion cells and led to a diagnosis of internal sphincter achalasia in one patient, who was successfully treated with botulinum toxin injections.

Radiological and manometric studies demonstrated high diagnostic concordance with biopsy results. A transition zone consistent with HD was identified in 37 patients (57.8%) on contrast enema radiography, and HD was confirmed in 92% of these cases. Anorectal manometry showed a negative rectoanal inhibitory reflex (RAIR) in 57.5% (19/33) of patients, and HD was confirmed in 42% of these cases. Positive RAIR findings excluded HD in most cases, although exceptions were noted.

Surgical outcomes validated the diagnostic process, with definitive surgery performed in 42 patients (91%) diagnosed with HD. Stoma closures were achieved in nine cases, including one patient without HD. Five patients (4.7%) were lost to follow-up, including four with HD. Procedure-related complications occurred in 5.8% (7/119) of biopsies, including six cases of benign rectal stricture resolved by dilatation and one instance of rectal bleeding managed conservatively.

TABLE 2. H&E staining with Calretinin staining and S-100 staining

H&E staining	Calretinin staining			S-100 staining		
	+	–	Non reported	+	–	Non reported
Ganglion cell (+) Nerve hypertrophy (+) n=17	14	–	3	7	–	10
Ganglion cell (+) Nerve hypertrophy (-) n=23	2	–	1	7	–	16
Ganglion cell (+) Nerve hypertrophy (non reported) n=17	6	–	11	–	–	17
Ganglion cell (-) Nerve hypertrophy (-) n=4	–	2	2	–	–	4
Ganglion cell (-) Nerve hypertrophy (+) n=42	2 weak	37	3	17	–	25
Ganglion cell (-) Nerve hypertrophy (non reported) n=4	–	1	3	–	–	4
Total: 107 (%)	44 (41.2)	40 (47.3)	23 (21.5)	31 (29)	–	76 (71)

H&E: Hematoxylin-Eosin.

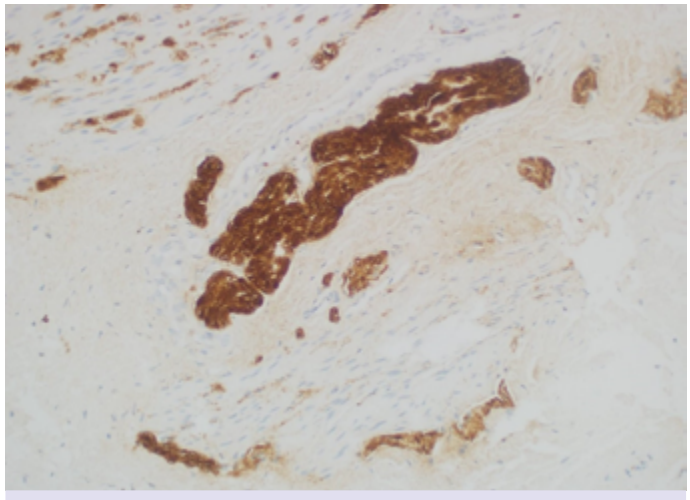

FIGURE 3. Ganglion cells and nerve cells were demonstrated with S100 staining.

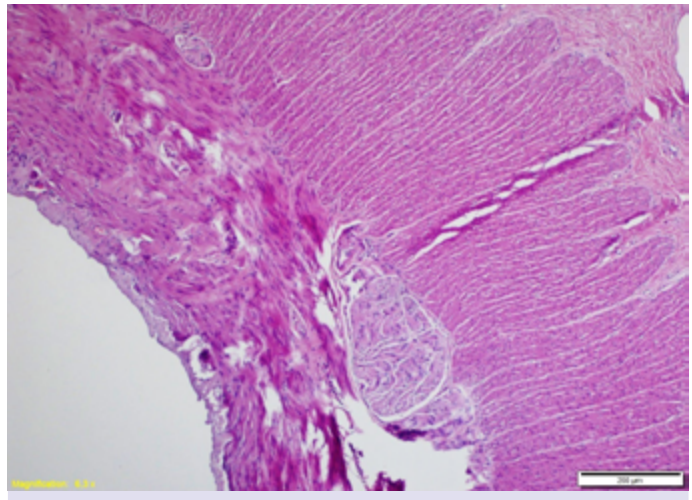

FIGURE 4. Mature ganglion cells and nerve cells were not detected with S100 staining.

In conclusion, 46 patients (43%) were diagnosed with HD in this series. Nerve hypertrophy was identified in 87% of HD cases, underscoring its diagnostic significance.

DISCUSSION

The diagnosis of HD typically begins with a careful evaluation of clinical findings and radiological imaging

[5]. A contrast enema showing a transition zone and a narrowed distal segment is an important diagnostic indicator. However, in neonates, the absence of a significant diameter difference between the aganglionic segment and the normal colon can complicate diagnosis [5]. In this study, a transition zone was identified in 92% of HD cases using contrast enema, yet histopathological evaluation remains essential for confirmation.

Anorectal manometry is a valuable, non-invasive diagnostic tool that demonstrates the absence of a normal rectoanal inhibitory reflex (RAIR) in HD [6]. While RAIR absence was observed in 64% of HD patients in a prior study, this rate was 42% in our series. Despite its usefulness, rectal biopsy remains the gold standard for HD diagnosis, particularly in cases of strong clinical suspicion or inconclusive manometry results.

HD must be excluded in neonates presenting with delayed meconium passage, abdominal distension, or failure to defecate [2]. Most patients are diagnosed during the neonatal period, with 62% of cases in our study presenting early due to complications such as intestinal stricture or perforation. Rectal washouts are used to stabilize neonates clinically suspected of HD, and biopsies are either performed during surgery or delayed until the patient becomes more stable.

Older children with intractable constipation also represent a significant diagnostic challenge. These patients often undergo multiple therapies before HD is considered, leading to delayed diagnosis [7]. In this study, the age at diagnosis was significantly higher among patients undergoing biopsies for intractable constipation, with a 34% HD diagnosis rate. This reflects delays in referral or diagnostic biopsies in lower-tier facilities before reaching our tertiary center.

Biopsy location and tissue adequacy are critical for diagnosis. Ideally, biopsies are taken 2 cm proximal to the dentate line to avoid sampling from the physiologically hypoganglionic zone near the anus or missing ganglion cell absence in short-segment HD [3]. Repeat biopsies were necessary in two cases in this study, confirming ganglion cells in one patient and excluding HD.

Suction biopsies are widely used for HD diagnosis but are often limited by insufficient submucosal tissue. [6] Full-thickness biopsies are superior in such cases. The rate of inadequate biopsies due to insufficient submucosa in suction biopsies has been reported at 10–30% [8]. In our study, the overall rate of inadequate biopsies was 11.2%, which we attributed to technical factors despite involving experienced surgeons.

Histopathological evaluation of biopsies typically begins with H&E staining to determine ganglion cell presence. However, in cases where ganglion cells are absent, additional immunohistochemical staining, such as calretinin, provides critical diagnostic support. Negative calretinin staining strongly supports HD, regardless of tissue depth or amount [4]. In this study, 11 patients with non-full-thickness biopsies showing no ganglion cells were diagnosed with HD without requiring repeat biopsies. Immunohistochemical (IHC) analysis was performed in 10 of these cases, all showing negative calretinin staining, while hypertrophic nerve fibers were demonstrated with S100 staining in seven cases. Nerve hypertrophy is another important histopathological finding in HD diagnosis. Submucosal nerve fibers exceeding 40 μm are a significant marker of HD, with diagnostic accuracy further enhanced by S100 staining [3]. In our series, nerve hypertrophy was reported in 87% of histopathology-confirmed HD cases. Therefore, confirming the presence of nerve hypertrophy with S100 staining is extremely important to confirm the diagnosis [4].

In five patients, ganglion cells were absent and calretinin staining was negative in the initial biopsy but positive in the repeat biopsy. This variability highlights the importance of careful biopsy sampling and adequate tissue preparation. Evaluation of non-full-thickness biopsy samples with a combination of H&E and calretinin may be sufficient for diagnosis.

Although major complications are not expected following full-thickness rectal biopsy, the complication rate has been reported to range between 0% and 15% in the literature [9]. In our study series, this rate was found to be 5.8%. One patient who had rectal bleeding that did not require transfusion and one of the patients who developed a dilatation-responsive stricture recovered uneventfully.

Due to the retrospective nature of the study, calretinin staining was not performed in some patients and this information was not included in the pathology reports, which is a limitation of the study.

Conclusion

In conclusion, while full-thickness biopsies are traditionally considered the gold standard, submucosal biopsies combined with immunohistochemical methods, such as calretinin and S100 staining, can provide accurate diagnoses in most cases. This method is considered a significant alternative due to its reduced invasiveness and ability to facilitate a faster diagnosis. However, it should always be interpreted in conjunction with the clinical context and other histological findings.

Ethics Committee Approval: The Istanbul Medeniyet University Goztepe Training and Research Hospital Clinical Research Ethics Committee granted approval for this study (date: 13.04.2022, number: 2022/0229).

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