OPEN ACCESS https://doi.org/10.59736/IJP.24.01.1053

ORIGINAL ARTICLE

Diagnostic significance of calretinin in Hirschsprung's Disease

Shah Syed Salah Ud Din[1], Khan Abdul Hammed[1], Shah, Zahir[1], Abdullah, Farooq[1], Khan, Ubaidullah[1], Saleem Mahmood[1], Khan Naseem[1]

[1]Department of Surgery, Paediatric surgery division, Lady Reading Hospital Peshawar, KPK, Pakistan

ABSTRACT

Background: Rectal biopsy is considered to be the gold standard for diagnosing Hirschsprung's disease (HD). At our center, both hematoxylin and eosin (H&E) staining and Calretinin immunohistochemistry are routinely employed to enhance diagnostic accuracy. This study aimed to evaluate the diagnostic significance of Calretinin staining compared to traditional H&E staining in children with suspected HD.

Methods: A cross-sectional study was conducted at the Department of Pediatric Surgery, from October, 2021, to May, 2023. A total of 166 patients with clinical and radiological suspicion of HD underwent rectal or colonic biopsies. Patients were categorized into five histological groups based on H&E findings. Data were analyzed using SPSS v22, with sensitivity, specificity, and predictive values calculated using 2×2 contingency tables. Statistical significance was set at $p \leq 0.05$.

Results: Among the 213 patients analyzed for Calretinin staining, 165 cases (77.4%) as HD (Calretinin-negative) were identified and excluded HD in 48 cases (22.6%). out of 166, 128 (77.1%) were males and 38 (22.9%) females. Age distribution showed that 93 patients (56%) were between 0–7 years, while 73 patients (44%) were between 8–14 years. In comparison, H&E staining diagnosed 160 cases (75.1%) as HD. Calretinin demonstrated a sensitivity of 100%, specificity of 100%, positive predictive value (PPV) of 100%, negative predictive value (NPV) of 100%, and overall diagnostic accuracy of 100%. Notably, in histologically ambiguous cases (Group C), Calretinin staining helped reclassify 5 of 12 biopsies as a ganglionic, significantly impacting diagnosis and treatment planning.

Conclusion: Calretinin immunohistochemistry is a reliable and valuable adjunct in the diagnosis of Hirschsprung's disease. With high sensitivity, specificity, and diagnostic accuracy, Calretinin proves particularly useful in ambiguous cases and should be considered a standard part of histopathological evaluation for HD.

Keywords: Calretinin, H&E staining, Hirschsprung's disease, Immunohistochemistry

This article may be cited as: khan UU, Shah SS uddin, Khan AH, Shah Z, Abdullah F, Khan N. Diagnostic significance of calretinin in Hirschsprung's Disease. Int J Pathol;24(1):17-22. https://doi.org/10.59736/IJP.24.01.1053

Introduction

Hirschsprung's disease (HD) is a congenital disorder characterized by the absence of ganglion cells in the enteric nervous system, resulting in functional bowel obstruction. It occurs in approximately 1 in 5,500 live births (1). There is marked male predominance at a male-to-female ratio of 4:1. The disease most commonly affects the rectosigmoid region (approximately 80% of cases), although longer segments or total colonic aganglionosis can also occur (2).

CORRESPONDING AUTHOR:
Ubaidullah Khan
Asst Professor, Farooq hospital Islamabad
ex-head of paediatric surgery LRH,
Peshawar. Email: ubaidafr@gmail.com

Clinically, neonates typically present with delayed passage of meconium, abdominal distension, and feeding intolerance. Older children may exhibit chronic constipation, abdominal pain, and failure to thrive. Early diagnosis is essential to prevent complications such as enterocolitis, bowel perforation, and malnutrition (3).

The gold standard for diagnosis remains the histopathological examination of rectal biopsy specimens, typically stained with hematoxylin and eosin (H&E) (4). The hallmark findings include the absence of ganglion cells and the presence of hypertrophic nerve trunks in the submucosal and myenteric plexuses. However, in some cases, these features may be equivocal ganglion cells may be scant or difficult to identify, and nerve hypertrophy may be absent, particularly in proximal disease or in early life. Moreover, hypertrophic nerves may also be present in cases of chronic functional constipation, further complicating the interpretation.

To enhance diagnostic reliability, immunohistochemical (IHC) staining techniques have been explored. While acetylcholinesterase (AChE) staining was initially adopted widely, it suffers from technical limitations and significant inter-observer variability (5). In contrast, calretinin, a calcium-bindingprotein involved in neuronal function, has emerged as a promising marker. Calretinin immunostaining typically highlights nerve fibers and ganglion cells in ganglionic segments but is absent in a ganglionic bowel, offering a more objective diagnostic tool (6).

Initial work by Bar shack et al. and subsequent studies by Guinard-Samuel and Kapur have validated the utility of calretinin staining in differentiating aganglionic from ganglionic bowel segments (7,8). Despite its growing use, literature on the practical challenges and diagnostic reliability of calretinin in varied clinical settings remains limited (9).

This study aims to evaluate the diagnostic accuracy of calretinin immunohistochemistry compared to routine H&E staining in children suspected of having Hirschsprung's disease. Specifically, we aim to highlight calretinin's role in resolving ambiguous cases and to assess its diagnostic value across various biopsy depths and anatomical sites.

Results

A total of 213 intestinal biopsies were collected from 166 pediatric patients with clinical suspicion of Hirschsprung's disease (HD). Of these, 128 patients (77.1%) were male and 38 (22.9%) were female, yielding a male-to-female ratio of approximately 3.4:1. The mean age was 7.31 ± 3.91 years, ranging from 5 days to 14 years. Age distribution showed that 93 patients (56%) were between 0–7 years, while 73 patients (44%) were between 8–14 years.

Biopsies were taken from various anatomical locations based on contrast studies and intraoperative findings:

- Rectal biopsies: 179
- Rectosigmoid: 12
- Splenic flexure: 8
- Transverse colon: 6
- Ascending colon: 4
- Ileum: 4

Additionally, 13 rectal biopsies were repeated due to specimen inadequacy or loss. Biopsies were categorized by depth:

- Full-thickness biopsies: 176
- Submucosal biopsies: 37

Biopsy specimens were categorized into five groups based on H&E staining (Table 1).

Table 1: H&E Diagnostic Groups vs. Calretinin Findings

Group	H&E Interpretation	N	Calretinin Negative (HD)	Calretinin Positive (No HD)
A	No ganglion cells, nerve hypertrophy	137	137	0
B	No ganglion cells, no nerve hypertrophy	23	23	0
C	Scanty ganglion cells, no nerve hypertrophy	12	5	7
D	Ganglion cells present, no nerve hypertrophy	28	0	28
E	Ganglion cells present, nerve hypertrophy	13	0	13
Total		213	165	48

Group C is diagnostically significant – Calretinin clarified 5 ambiguous cases as HD.

Significance in Ambiguous Cases (Group C)

Among 12 biopsies in Group C (scanty ganglion cells on H&E), Calretinin staining identified 5 cases as negative (suggesting aganglionosis). These results had a direct impact on diagnosis and management, highlighting Calretinin's diagnostic value in equivocal or borderline cases. Fig 1 and 2 demostrate calretinin staining and its absence respectively.

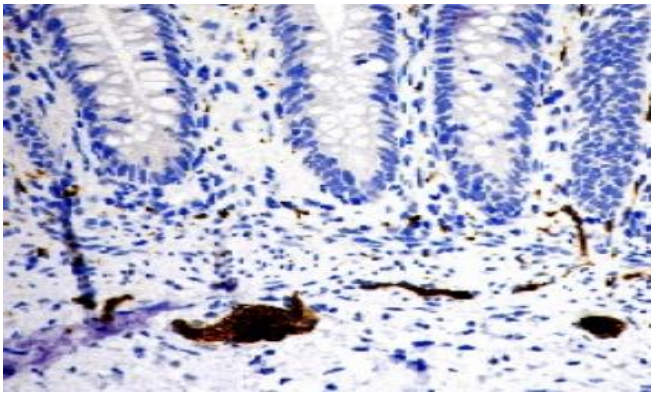

Fig 1. Calretinin staining (dark areas) confirming the presence of ganglion cells

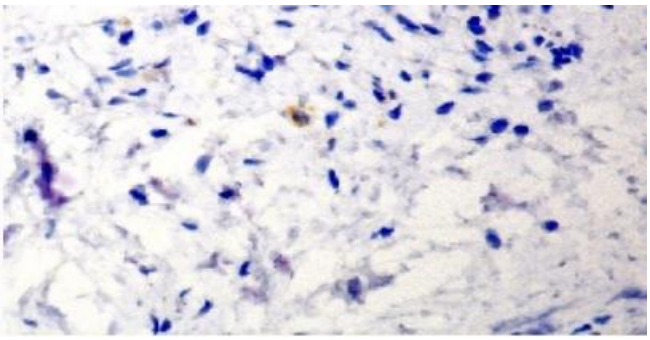

Fig 2. The absence of calretinin staining indicates the absence of ganglion cells, indicative of probable Hirschsprung's disease

Diagnostic Performance of Calretinin (Table 2)

Table2: Comparison of H&E and Calretinin Staining in Diagnosis of Hirschsprung's Disease

Parameter	H&E Staining	Calretinin Staining
Total Biopsies Analyzed	213	213
Positive for HD (Aganglionosis)	160 (75.1%)	165 (77.4%)
Negative for HD (Ganglionosis)	53 (24.9%)	48 (22.6%)
Sensitivity	96.9%	100%
Specificity	89.5%	100%
Positive Predictive Value (PPV)	96.96%	100%
Negative Predictive Value (NPV)	89.58%	100%
Diagnostic Accuracy	95.3%	100%

Table 3: Based on comparison with H&E as the reference standard:

Parameter	H &E	Calretinin
Sensitivity	96.9%	100%
Specificity	89.5%	100%
Positive Predictive Value	96.96%	100%
Negative Predictive Value	89.58%	100%
Overall Diagnostic Accuracy	95.3%	100%

Statistical Stratification:

- Age Groups: No significant difference in diagnostic performance of Calretinin between patients aged 0–7 years and 8–14 years ($p = 0.30$).
- Biopsy Site: No statistically significant association between biopsy site and diagnostic accuracy of Calretinin ($p = 0.063$).
- Biopsy Depth: Calretinin staining remained effective regardless of whether submucosal or full-thickness samples were used.

Discussion

Hirschsprung's disease (HD) is a major cause of neonatal intestinal obstruction and chronic constipation in children. Accurate and early diagnosis is critical to prevent complications such as enterocolitis, perforation, and growth failure. The gold standard for diagnosis remains the histopathological examination of rectal biopsies using hematoxylin and eosin (H&E) staining (4). However, this technique can yield inconclusive results in certain scenarios, particularly when ganglion cells are sparse or when nerve hypertrophy is absent. In this context, immunohistochemical markers like calretinin have emerged as valuable adjuncts (7).

our study, demonstrated sensitivity, specificity, positive predictive value, negative predictive value as 100% for Calretinin on immunohistochemistry and an overall diagnostic accuracy of 100. These values are consistent with those reported in the literature, where sensitivity and specificity typically range from 93–100% and 93.8–100%, respectively (4, 18). Our findings support the growing consensus that calretinin staining is a robust and reliable diagnostic tool in the evaluation of suspected HD.

Importantly, Calretinin staining proved especially beneficial in Group C, where 12 biopsies exhibited scanty ganglion cells on H&E. Of these, 5 were classified as aganglionic by calretinin staining. In such borderline presentations, Calretinin immunostaining served as a crucial decision-making tool, potentially altering patient management and surgical planning.

Calretinin's utility is not limited by patient age, biopsy depth, or anatomical site, as demonstrated by the absence of statistically significant associations in our stratified analysis. This makes it a versatile tool that can be applied in various clinical settings, from neonatal biopsies to older children with long-standing symptoms (15, 19).

Comparatively, acetylcholinesterase (AChE) staining, once considered the primary adjunct method, has shown variable performance and is technically challenging. Interobserver variability and interpretive complexity often limit its reliability. In contrast, calretinin staining offers greater consistency, clearer interpretation, and broader reproducibility across pathology laboratories.

Similar studies by Barshack et al., Guinard-Samuel, and Zemheri et al. have supported our findings. The strong correlation between calretinin negativity and absence of ganglion cells in HD-affected tissue underscores its diagnostic value. Furthermore, the absence of false positives in normal ganglionated bowel segments supports its specificity, though caution must still be exercised in interpreting weak or patchy staining (7, 8, 15,).

While calretinin is not a replacement for H&E, it enhances diagnostic confidence Its consistent performance across age groups and biopsy sites further reinforces its clinical utility.

Conclusion

Calretinin immunohistochemistry is a reliable and diagnostically valuable adjunct to traditional H&E staining in the evaluation of Hirschsprung's disease. It demonstrated high sensitivity, specificity, and diagnostic accuracy, particularly in cases where routine histopathology is inconclusive. Calretinin staining proved especially useful in resolving ambiguous findings such as scanty ganglion cells or absent nerve hypertrophy helping to avoid misdiagnosis and ensuring timely and appropriate surgical intervention. Based on our findings, Calretinin may be incorporated into routine diagnostic protocols for suspected HD, especially in challenging or borderline cases.

Limitations:

This study was conducted at a single tertiary care center with a limited sample size, which may restrict the generalizability of the findings. Additionally, interobserver variability in interpreting Calretinin staining was not specifically analyzed, and long-term follow-up of cases with discordant results was not included.

Recommendations:

Future multicenter studies with larger sample sizes and standardized reporting criteria are recommended to validate these findings. Comparative studies involving other immunohistochemical markers, such as S-100 or AChE, may also help establish a comprehensive diagnostic algorithm. Incorporating digital pathology or AI-assisted analysis could further reduce interpretive variability and improve diagnostic precision.

Conflict of Interest: Nil
Source of Funding: Nil

References

1. Ali A, Haider F, Alhindi S. The prevalence and clinical profile of Hirschsprung's disease at a tertiary hospital in Bahrain. *Cureus*. 2021;13(1): e12480.
2. Verkuijl SJ, Meinds RJ, van der Steeg AFW, van Gemert WG, de Blaauw I, Witvliet MJ, et al. Functional Outcomes After Surgery for total Colonic, long-Segment, versus Rectosigmoid Segment Hirschsprung Disease. J Pediatr Gastroenterol Nutr. 2022 Mar;74(3):348–54.
3. Granéli C, Dahlin E, Börjesson A, Arnbjörnsson E, Stenström P. Diagnosis, Symptoms, and Outcomes of Hirschsprung's Disease from the Perspective of Gender. Surg Res Pract. 2017; 2017:9274940.
4. Beltman L, Windster JD, Roelofs JJTH, van der Voorn JP, Derikx JPM, Bakx R. Diagnostic accuracy of calretinin and acetylcholinesterase staining of rectal suction biopsies in Hirschsprung disease examined by unexperienced pathologists. *Virchows Arch*. 2022;481(2):245-252.
5. Hirschsprung disease. *Semin Pediatr Surg*. 2022;31(2):151-6.
6. Matsukuma K, Gui D, Saadai P. Hirschsprung Disease for the Practicing Surgical Pathologist. Am J Clin Pathol. 2023 Mar1;159(3):228–41.
7. Barshack I, Fridman E, Goldberg I, Chowers Y, Kopolovic J. The loss of calretinin expression indicates aganglionosis in Hirschsprung's disease. J Clin Pathol. 2004 Jul;57(7):712–6.
8. Guinard-Samuel V, Bonnard A, De Lagausie P, Philippe-Chomette P, Alberti C, El Ghoneimi A, et al. Calretinin immunohistochemistry: a simple and efficient tool to diagnose Hirschsprung disease. *Mod Pathol*. 2009;22(10):1379-84.

9. Kapur RP,Reed RC, Finn LS, Patterson K, JohansonJ,RutledgeJC.Calretininimmunohistochemistry versus acetylcholinesterase histochemistry in the evaluation of suction rectal biopsies for Hirschsprung Disease. Pediatr Dev Pathol Off J Soc Pediatr Pathol Paediatr Pathol Soc. 2009;12(1):6–15.
10. Aziz ZW, Al Hajjar BK, Al Hajjar ZAA, Almola EMA, Jawher NMT, Muhammad RK. Diagnostic accuracy of haematoxylin-eosin staining in comparison to calretinin and S100 for the assessment of ganglion cells in rectal biopsy. J Pak Med Assoc. 2022;72(6):1123–7.
11. Rakhshani N, Araste M, Imanzade F, Panahi M, Safarnezhad Tameshkel F, Sohrabi MR, et al. Hirschsprung Disease Diagnosis: Calretinin Marker Role in Determining the Presence or Absence of Ganglion Cells. Iran J Pathol. 2016;11(4):409–15.
12. Singh SK, Gupta UK, Aggarwal R, Rahman RA, Gupta NK, Verma V. Diagnostic Role of Calretinin in Suspicious Cases of Hirschsprung's Disease. Cureus [Internet]. 2021;13(2):e13373.
13. Calkins CM. Hirschsprung Disease beyond Infancy. Clin Colon Rectal Surg. 2018 Mar;31(2):51–60.
14. Jeong H, Jung HR, Hwang I, Kwon SY, Choe M, Kang YN, et al.Diagnostic Accuracy of CombinedAcetylcholinesteraseHistochemistry and Calretinin Immunohistochemistry of Rectal Biopsy Specimens in Hirschsprung's Disease.Int J Surg Pathol. 2018 Sep;26(6):507–13.
15. Zemheri E, Engin Zerk P, Ulukaya DurakbasaC.Calretininimmunohistochemical staining in Hirschsprung's disease: An institutional experience. North Clin Istanb. 2021;8(6):601–6.
16. Zhang C, Wang M, Lin S, Xie R. Calretinin-Expressing Synapses Show Improved Synaptic Efficacy with Reduced Asynchronous Release during High-Rate Activity.JNeurosci.2022Mar 30;42(13):2729—42.
17. Pradhan P, Pal BC, Rath G. Role of Calretinin Immunohistochemical Stain in Evaluation of Hirschsprung's Disease. J Clin Diagnostic Res. 2021;15(1):8–11.
18. Kazemi Aghdam M, Khoddami M, Mollasharifi T, Almasi-Hashiani A. Diagnostic Value of Calretinin and S100 Immunohistochemistry in Hirschsprung's Disease. Int J Pediatr. 2019;7(6):9577–89.
19. Musa ZA, Qasim BJ, Ghazi HF, Al Shaikhly AWAK. Diagnostic roles of calretinin in Hirschsprung disease: a comparison to neuron-specific enolase. *Saudi J Gastroenterol*.2017;23(1):60-6

HISTORY	
Date received:	07-02-2026
Date sent for review:	02-03-2026
Date received reviewer's comments:	31-03-2026
Date received revised manuscript:	04-04-2026
Date accepted:	04-04-2026

CONTRIBUTION OF AUTHORS	
Contribution	**Authors**
Conception/Design	SS, UK
Data acquisition, analysis and interpretation	SZ, KA, UK
Manuscript writing and approval	AF, KN, UK

All the authors agree to take responsibility for every facet of the work, making sure that any concerns about its integrity or veracity are thoroughly examined and addressed.