



COMPARATIVE EVALUATION OF HEMATOXYLIN-EOSIN STAINING AND CALRETININ IMMUNOHISTOCHEMISTRY IN RECTAL BIOPSIES FOR THE DIAGNOSIS OF HIRSCHSPRUNG'S DISEASE IN NEONATES AND INFANTS

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ABSTRACT

Background: Hirschsprung's disease is a congenital disorder characterized by the absence of ganglion cells in the distal bowel leading to functional intestinal obstruction. Histopathological examination of rectal biopsies remains the gold standard for diagnosis. However, identification of ganglion cells in neonates and infants can be difficult on routine hematoxylin and eosin staining due to immature morphology. Calretinin immunohistochemistry has emerged as a useful adjunct diagnostic tool. **Aim:** To evaluate the diagnostic utility of calretinin immunohistochemistry compared with routine H&E staining in rectal biopsies for diagnosing Hirschsprung's disease. **Objectives:** To assess the presence of ganglion cells using H&E staining and Calretinin IHC in paraffin embedded rectal biopsy specimens. To compare the diagnostic accuracy of H&E versus Calretinin IHC for diagnosing aganglionosis (HD) and ganglionated (Non-HD) cases. **Materials and Methods:** This cross-sectional observational study was conducted in the Department of Pathology, Niloufer hospital, Hyderabad over a period of two years. A total of 52 rectal and colonic biopsy specimens from neonates and infants clinically suspected of Hirschsprung's disease were evaluated using H&E staining and calretinin immunohistochemistry. **Results:** Among the 52 cases, males constituted 90.4% and females 9.6%. Constipation (36.5%) was the most common presenting complaint. On H&E staining, ganglion cells were absent in 37 cases (71.2%) and present in 15 cases (28.8%). Calretinin immunohistochemistry showed negative staining in 49 cases (94.2%) and positive staining in 3 cases (5.8%). Short-segment Hirschsprung's disease accounted for 84.6% of cases. **Conclusion:** Calretinin immunohistochemistry is a reliable adjunct to routine histopathology and improves diagnostic accuracy in rectal biopsies of Hirschsprung's disease.

KEYWORDS : Hirschsprung's disease, Calretinin, Rectal biopsy, Immunohistochemistry, Ganglion cells

INTRODUCTION

Hirschsprung's disease (HD) is a congenital disorder of the enteric nervous system characterized by the absence of ganglion cells in the distal bowel. This results in failure of coordinated peristalsis and leads to functional intestinal obstruction. The disease commonly presents in neonates and infants with symptoms such as delayed passage of meconium, abdominal distension, and chronic constipation.

Histopathological examination of rectal biopsy specimens demonstrating aganglionosis remains the gold standard for diagnosis. Routine hematoxylin and eosin staining is widely used to identify ganglion cells in the submucosal and myenteric plexuses. However, in neonatal tissue, ganglion cells are often immature and difficult to identify.

Immunohistochemical markers have therefore been introduced to enhance diagnostic accuracy. Calretinin, a calcium-binding protein expressed in enteric neurons and intrinsic nerve fibers, has been shown to be a sensitive marker for detecting ganglion cells.

MATERIALS AND METHODS

This cross-sectional observational study was conducted in the Department of Pathology, Osmania general hospital, Hyderabad in collaboration with Niloufer Hospital. The study period extended from March 2024 to March 2026.

A total of 52 rectal and colonic biopsy specimens obtained from neonates and infants clinically suspected of Hirschsprung's disease were included in the study.

Inclusion Criteria:

- Neonates and infants below one year of age with clinical suspicion of Hirschsprung's disease
- Patients undergoing rectal or colonic biopsy

- Exclusion Criteria:
- Inadequate biopsy specimens
- Poorly preserved tissues
- Lack of parental consent

Biopsy specimens were fixed in 10% neutral buffered formalin and processed using routine histopathological techniques. Routine H&E staining was performed for evaluation of ganglion cells and hypertrophic nerve bundles. Calretinin immunohistochemistry was performed using monoclonal anti-calretinin antibody.

RESULTS

Table 1. H&E evaluation of ganglion cell presence (n = 52)

H&E Ganglion Cells	Frequency	Percent (%)
Absent	37	71.2
Present	15	28.8
Total	52	100.0

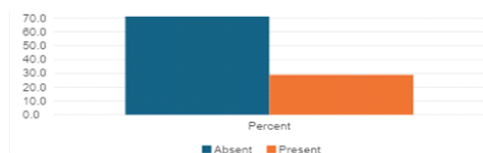


Figure 1. Distribution of ganglion cell presence on H&E staining.

Calretinin Immunohistochemical Findings

Calretinin immunohistochemistry showed negative staining in 49 cases (94.2%) and positive staining in 3 cases (5.8%). Therefore, the vast majority of biopsy specimens lacked calretinin expression, with only a small subset demonstrating immunoreactivity. All 52 specimens were successfully evaluated.

Table 2. Calretinin immunohistochemical findings (n = 52)

Calretinin IHC	Frequency	Percent (%)
Negative	49	94.2
Positive	3	5.8
Total	52	100.0

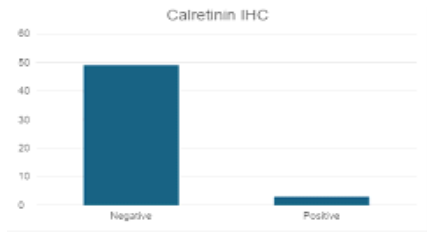
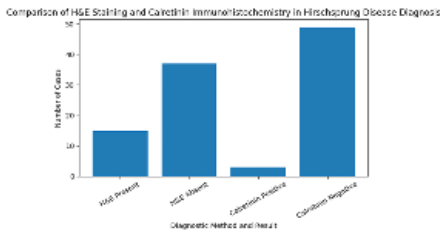


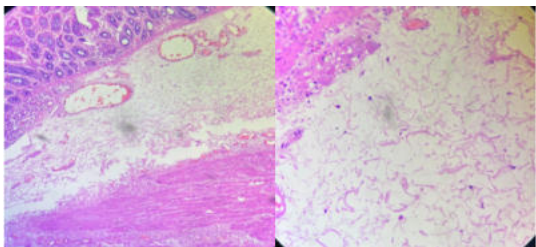
Figure 2. Distribution of calretinin immunohistochemical staining pattern showing absence of ganglion cells

Table: 2 Comparison Between H&E Staining and Calretinin Immunohistochemistry

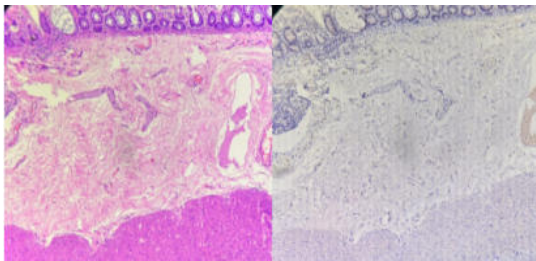
Diagnostic method	Positive or Present (Ganglion cells)	Negative/Absent (Aganglionosis)	Total cases
H&E Staining	15	37	52
Calretinin	3	49	52



Histopathological Findings:



H&E section of rectal biopsy shows presence of ganglion cells in normal segment



H&E SECTION SHOWS HYPERTROPHIC NERVE BUNDLES
CALRETININ SHOWS ABSENCE OF GANGLION CELLS

DISCUSSION

Hirschsprung's disease is a common cause of neonatal intestinal obstruction. Early diagnosis is essential to prevent complications such as enterocolitis and intestinal perforation. In the present study, a strong male predominance was observed, which is consistent with previous reports indicating a higher incidence in males.

for identifying ganglion cells. However, the identification of ganglion cells may be difficult in neonatal biopsies because of immature morphology.

Calretinin immunohistochemistry has been shown to be a useful adjunct in diagnosing Hirschsprung's disease. Studies have demonstrated that calretinin staining highlights ganglion cells and intrinsic nerve fibers in normally innervated bowel but is absent in aganglionic segments.

Previous studies have reported high sensitivity and specificity of calretinin staining in diagnosing Hirschsprung's disease. The findings of the present study are consistent with these observations.

CONCLUSION

Calretinin immunohistochemistry is a valuable adjunct to routine histopathology in the diagnosis of Hirschsprung's disease. It improves diagnostic accuracy and is particularly useful in neonatal rectal biopsies where ganglion cells are difficult to identify using routine staining.

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