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Review Article

# Diagnostic Value of Calretinin Immunohistochemistry in Hirschsprung Disease - A Retrospective Archive Review

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## ABSTRACT

**Background**: Hirschsprung disease is a congenital genetic disease causing functional intestinal obstruction due to the absence of ganglion cells. Presence of ganglion cells can be diagnosed by histopathological examination. Because of subjective variation in diagnosis and the presence of immature ganglion cells, further confirmation can be done with immunohistochemistry. Calretinin has emerged as the effective marker for confirming the diagnosis of Hirschsprung disease.
**Aims And Objectives:** To assess the diagnostic role of calretenin immuno staining in Hirschsprung disease and to confirm the histopathological diagnosis retrospectively from the archives of Department of Pathology, Tirunelveli.
**Methods**: This is a Retrospective cohort study conducted on 10 cases of Hirschsprung disease reported in the Department of Pathology, Tirunelveli medical college. The details like age, sex, clinical diagnosis, specimen gross features, microscopic features and HPE impression are taken. Calretinin is the IHC marker used in this study.
**Results**: All the 10 cases are male patients. Age ranged from 3 days to 5 years. Study of IHC expression of Calretinin shows negativity in the aganglionic segments and positive staining in ganglionic segments.
**Conclusion**: Calretinin is considered as an effective marker in diagnosis of Hirschsprung disease. It helps in overcoming difficulties encountered in identifying ganglion cells in hematoxylin and eosin staining.

***Keywords:*** Hirschsprung disease, Calretinin, Ganglion cells, Histopathology.

## INTRODUCTION

Hirschsprung disease also known as congenital aganglionic megacolon is a genetic disorder of enteric nervous system. Hirschsprung disease is often associated with other genetic disorders like Down syndrome and Wardenberg syndrome.[1] The disease is characterized by absence of ganglion cells in Meissner submucosal plexus and the Auerbach myenteric plexus. It happens when the normal migration of neural crest cells from cecum to rectum is disturbed. This accounts for 1 in 5000 live births which most commonly affects males but tends to be severe in females.[2,3] Meconium not passed in immediate postpartum period is the usual sign which hints towards underlying pathology. The most common symptom of children is constipation. Hirschsprung disease can be diagnosed histopathologically by visualizing absence of ganglion cells in the affected segment of intestine. Histopathological examination can be correlated with further examination with Immunohistochemistry on calretinin.

## REVIEW OF LITERATURE:

Hirschsprung disease is a functional intestinal obstruction due to congenital deficiency of the normal myenteric plexus parasympathetic ganglion cells in the distal portion of the large intestine. This disease mostly presents at birth and also in children. Male Female ratio is 4:1 worldwide[4] Hirschsprung disease occurs due to disruption of the migration and differentiation of neural crest cells at the level of the enteric nervous system. This system is under the control of the RET gene and its ligands. Disruption of this process causes total absence of ganglion cells in the nerve plexuses which leads to overactivity of the intestine with the persistent release of acetylcholine. This is followed by continuous contraction of the narrowed colonic segment and progressive secondary dilatation of the healthy proximal colon.[5,6]

Hirschsprung disease is diagnosed based on a combination of clinical features, radiological findings, and histopathological examination of the sample. Infants usually presents with abdominal distension, inability to pass meconium or stools, bilious vomiting. Radiological examination like ultrasound and CT abdomen can be done to rule out colonic obstruction. Yet another special investigation is Anorectal manometry which shows absence of anorectal reflex in Hirschsprung disease. Treatment modalities for Hirschsprung disease includes surgical treatment with resection of the aganglionic segment and reconstitution of the intestinal passage after the first year of life, following bridging therapy with colostomy. In histopathology examination of the biopsy specimen confirms the diagnosis by the absence of ganglion cells in submucosal and myenteric plexus with hypertrophy of nerve fibers in the aganglionic segment. Ganglion cells are polygonal cells with abundant fibrillar eosinophilic cytoplasm, eccentric nucleus, and a large nucleolus. The presence of ganglion cells can be further confirmed with the immunohistochemical marker Calretinin. Immunohistochemistry (IHC) is a useful method to identify ganglion cells and nerve hypertrophy.[7,8]

Calretinin is a 29 kilodalton calcium binding protein of the calmodulin superfamily. It shows both nuclear and cytoplasmic staining. Absence of calretinin causes the accumulation of intra-cytoplasmic calcium with consequent hyperexcitability and cell degeneration. Its expression is present physiologically in all human tissues but to be particular in central and peripheral nervous system. Calretinin is mainly used in the diagnosis of Hirschsprung disease but also helps in ruling out sex cord stromal or mesothelial lineage. It is also positive in adrenal cortical lesions, mesonephric carcinoma, female adnexal tumor of probable Wolffian origin.[9,10]

## AIMS AND OBJECTIVES:

To assess the diagnostic role of calretenin immuno staining in Hirschsprung disease and to confirm the histopathological diagnosis retrospectively from the archives of Department of Pathology, Tirunelveli.

## MATERIALS AND METHODS:

This study was conducted on 10 cases of suspected Hirschsprung disease which are reported in Tirunelveli Medical college in the histopathology lab on tissue blocks.

**Duration of study:** 2 months

**Type of study:** Retrospective cohort study

In this retrospective cohort study, paraffin blocks and slides from 10 patients suspected to have Hirschsprung disease during the period of 2 months were retrieved from the archive of Department of Pathology, Tirunelveli medical college, Tirunelveli. This study has been approved by the Ethics Committee. Initially paraffin blocks were examined for histopathological examination using Hematoxylin and Eosin stain.

Diagnostic criteria for HD in rectal mucosal biopsy are absent ganglion cells and presence of hypertrophic nerve bundles in sub mucosa. Diagnostic criteria for non-HD in rectal mucosal biopsy is at least one ganglion cell is identified in one or more tissue sections.

H and E stained sections were reported initially followed by immunohistochemistry on calretinin was done. Results were recorded.

| The following results are observed with the application of IHC | | | | | |
|---|---|---|---|---|---|
| Case No. | Age/Sex | Biopsy Specimen | | Ganglion cells on H & E | Calretinin expression |
| 1. | 5 years/Male | Bowel resection | Proximal segment | Present | Positive |
| | | | Distal Segment | Present | Positive |
| | | | Transition zone | Absent | Negative |
| 2. | 2 years/Male | Rectal biopsy | | Absent | Negative |
| 3. | 6 days/Male | Bowel resection | Proximal segment | Present | Positive |
| | | | Distal Segment | Present | Positive |
| | | | Transition zone | Suspicious | Negative |
| 4. | 3 Days/Male | Rectal biopsy | | Absent | Negative |
| 5. | 1 Year/Male | Rectal biopsy | | Absent | Negative |
| 6. | 15 Days/Male | Rectal biopsy | | Absent | Negative |
| 7. | 10 Days/Male | Rectal biopsy | | Suspicious | Negative |
| 8. | 10 Months/Male | Rectal biopsy | | Absent | Negative |
| 9. | 6 Days/Male | Rectal biopsy | | Absent | Negative |
| 10. | 3 Months/Male | Rectal biopsy | | Absent | Negative |

**OBSERVATION AND RESULTS:**

All the 10 cases studied were Male patients. Age group of presentation ranged between 3 days to 5 years. A confirmatory histopathological diagnosis of Hirschsprung disease was made in 8 cases by Hematoxylin and Eosin stain. The other 2 cases were suspicious for ganglion cells. Immunohistochemistry on calretinin was done on all 10 cases to confirm diagnosis. The segments with absence of ganglion cells showed calretinin negativity and the segments with ganglion cells showed positive staining. Those that were suspicious for ganglion cells also showed negative calretinin staining and confirmed the diagnosis.

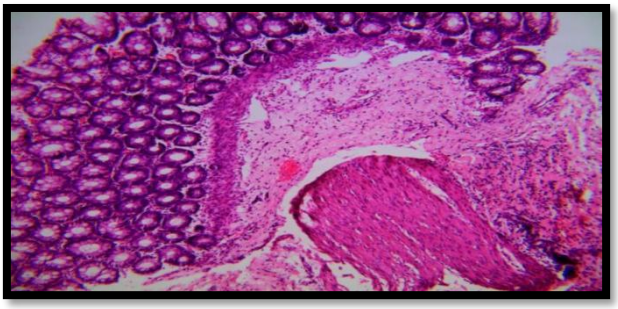

**Figure 1: Colonic segment- Ganglion cells absent ( H&E)**

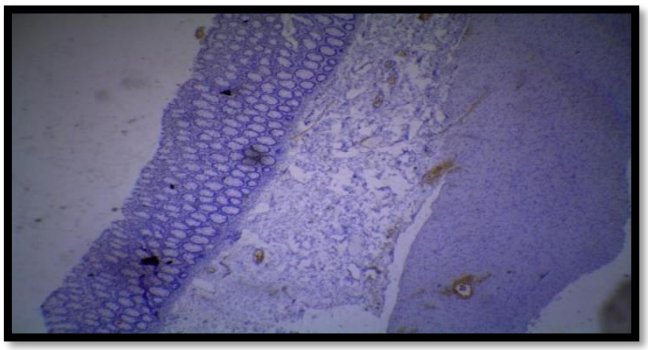

**Figure 2: Colonic segment showing calretinin negativity**

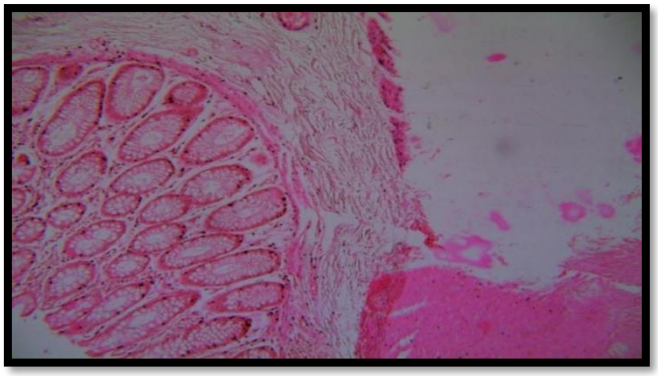

**Figure 3:Bowel segment – Ganglion cells – Absent(H &E)**

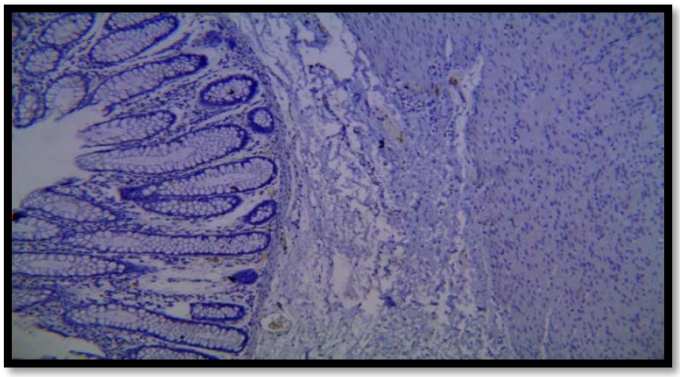

**Figure 4: Bowel segment – calretinin shows negativity**

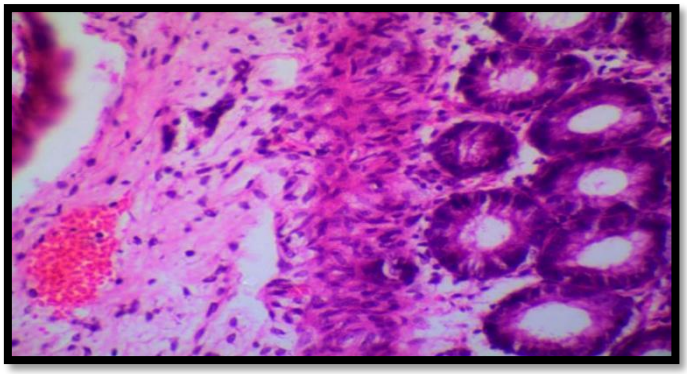

**Figure 5: Bowel segment – Ganglion cells – Absent(H&E)**

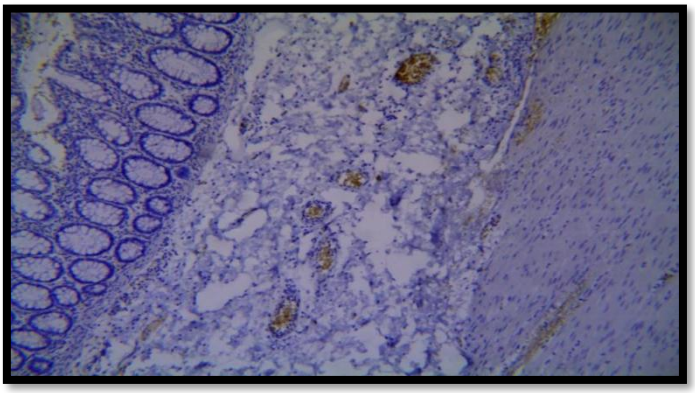

**Figure 6: Calretinin negativity**

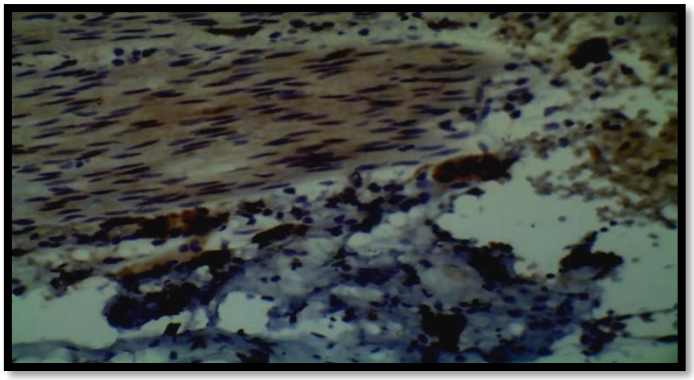

**Figure 7: Proximal Segment of colon showing calretinin positivity for ganglion cells.**

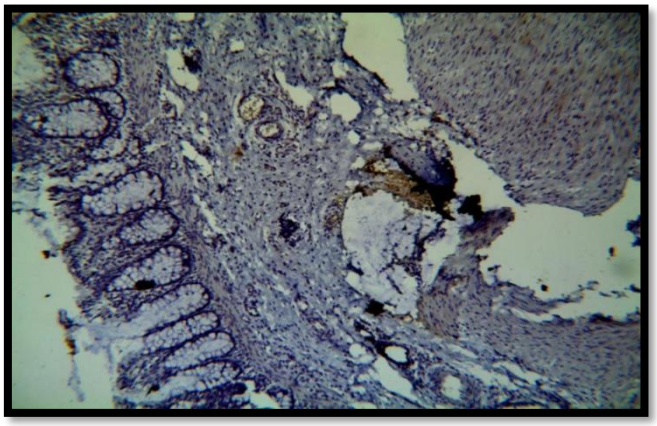

**Figure 8**: Aganglionic segment of bowel showing calretinin negativity

**DISCUSSION:**

Hirschsprung disease is a complex genetic disorder which describes the absence of enteric neurons along variable length of intestine. It is a congenital malformation of the hindgut with absence of parasympathetic intrinsic ganglion cells in the submucosal and myenteric plexuses. It is most common among males than females. This disease usually occurs in infants and in young children. They typically presents with abdominal distension, chronic constipation and bilious vomiting.[11,12] In our study, all 10 cases were male population with age group between 3 days to 5 years. Our study population also presented with complaints of inability to pass meconium, constipation and abdominal distension.

In a study by Barshack et al., showed that aganglionic segments revealed absence of calretinin expression in ganglion cells and in the nerve fiber in HD, and conversely calretinin expression was positive in both ganglion cells and nerve fibers in ganglionic areas of HD.[14] A study by Naseer et al., shows that calretinin was 96% specific and 78% sensitive to rule out ganglion cells.[16] In a study by Musa et al,. shows that sensitivity and specificity of calretinin is 100% in confirming the diagnosis of Hirschsprung disease.[15]

Our study shows that, among 10 cases suspected as Hirschsprung disease clinically, 8 cases were negative for ganglion cells and 2 cases were suspicious for ganglion cells in Hematoxylin and Eosin stain. This was further followed by immunohistochemistry on calretinin. Calretinin showed negative expression for those 8 cases and remaining 2 cases which was suspicious also turned out to be negative which confirms the diagnosis of Hirschsprung disease. For those 2 cases which were suspicious for ganglion cells was in age group less than 5 months which indicates that the incomplete maturation of ganglion cells made the histopathological examination difficult to diagnose. Hence, Calretinin has been considered to be highly specific and sensitive marker for diagnosing Hirschsprung disease.[13,14]

**CONCLUSION:**

Hirschsprung disease can be diagnosed in histopathological examination by the presence or absence of ganglion cells. But this is a highly subjective method which needs further confirmation by sophisticated histochemistry technique like Acetylcholinesterase.[15] In recent times, IHC with calretinin which is a cost effective alternative is used nowadays for confirmation of diagnosis. In adjunct to histopathological confirmation of absence of ganglion cells, additionally IHC using calretinin staining can be routinely done for arriving at a confirm diagnosis.

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