

Original Research

Assessment Of The Role Of Calretinin In Evaluation Of Hirschsprung's Disease By IHC At MGMMC Indore.

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Abstract-

Introduction- Hirschsprung's disease is a congenital disorder characterised by aganglionosis involving a segment of rectum and proximal bowel. It is caused by congenital absence of the Meissner's and Auerbach's autonomic plexus (aganglionosis) in the intestinal wall. In 75% of instances, the disease is confined to the distal colon, but it can also affect the entire colon or both the large and small bowels; the denervated area is invariably contiguous. The failure of neuroblasts to migrate from the neural crest is hypothesized to be the cause of aganglionosis.

Materials And Methods- This study was conducted in the Department of Pathology, Mahatma Gandhi Memorial Medical College and M.Y. Hospital, Indore Madhya Pradesh, India. The study was done in 30 biopsy or resected bowel specimens of clinically suspected Hirschsprung's disease

Result- Out of total 30 cases Calretinin expression was seen in 6 cases resulting in diagnosis of NHD. Of 24 cases diagnosed as HD by ganglion cell negativity with IHC, three cases (12.5% cases) showed immunoreactivity in nerve fibers. When nerve fiber immunoreactivity was taken as the criteria for diagnosis, this method had a sensitivity and specificity 87.5% and 100% respectively.

Conclusion- Our present study shows that Calretinin can be a useful adjunct to H&E in the diagnosis of more cases, with accuracy and precision. So Calretinin could prove to be a very valuable adjunct marker in improving diagnostic accuracy in centres with lesser experience.

Keywords- AChE (Acetyl choline esterase), DPX (Dibutylphthalate Polystyrene Xylene), ENS (Enteric Nervous System), ENCC (Enteric neural crest cell), HD (Hirschsprung's disease), H & E (Hematoxylin and Eosin), INF (Intrinsic nerve fibre), IHC (Immunohistochemistry).

INTRODUCTION-

Hirschsprung's disease is a congenital disorder characterised by aganglionosis involving a segment of rectum and proximal bowel. It happens in about 1 in 5000 births, and the inheritance pattern is complicated, frequently non-Mendelian, and exhibits variable penetrance^[1]. The majority of infants present with stomach distension, failure to pass meconium, or constipation in the early stages of infancy^[2,3]. But presentation later in life, with ongoing constipation, is not infrequent.

Hirschsprung's disease is caused by congenital absence of the Meissner's and Auerbach's autonomic plexus (aganglionosis) in the intestinal wall. In 75% of instances, the disease is confined to the distal colon, but it can also affect the entire colon or both the large and small bowels; the denervated area is invariably contiguous. The failure of neuroblasts to migrate from the neural crest is hypothesised to be the cause of aganglionosis. There is a substantial hereditary component to this ailment; Hirschsprung's disease is linked to at least 12 different genetic alterations and other disorders. The enteric

nervous system^[4], the smooth muscle layers, and the interstitial cells of Cajal (ICCs)^[5] regulate the digestive tract's operations. The ganglionated plexuses of the enteric nervous system are composed of neurons that subserve various functions, including motor neurons to the circular and longitudinal muscle layers, sensory neurons, ascending and descending interneurons, and secretomotor neurons^[6]. In the gut wall, ICCs operate as pacemaker cells by generating slow waves that aid in the active propagation of electrical events and neurotransmission^[7]. The myenteric plexus is surrounded by a dense network of ICCs in the normal bowel, but these cells are either absent or sparse in the aganglionic colon of Hirschsprung's disease^[8].

Clinical signs and symptoms, the usual appearance of the intestinal radiography following a contrast enema, and the measurement of recto- anal pressure by rectal manometry can all point to Hirschsprung's disease. The proof of the lack of ganglion cells in the histological sections of the rectal biopsy^[9] is the gold standard, by far. This helps in arriving at the definite diagnosis of Hirschsprung's disease. Hallmark histological finding is aganglionosis and presence of numerous hypertrophic cholinergic nerve fibers^[10,11].

MATERIALS AND METHODS

This study was conducted in the Department of Pathology, Mahatma Gandhi Memorial Medical College and M.Y. Hospital, Indore Madhya Pradesh, India. It is a Prospective study, conducted for a period of Ten months (JUNE 2022 TO MARCH 2023), total 30 Cases are taken. Both biopsy or resected bowel specimens of clinically suspected Hirschsprung's disease, received. Resected bowel specimens were evaluated grossly and representative samples were taken each from distal spastic segment, transitional zone, and proximal ganglionic region. Sampling of proximal margin is most important and we did circumferential sampling of proximal end.

All corresponding tissue sections were stained with Calretinin IHC and evaluated for immunoreactivity. Calretinin immunoreactivity and pattern of staining for ganglion cells and nerve fibers in submucosa and muscularis propria were evaluated for both ganglionic and Aganglionic segments of the IHC stained slides. Results were recorded. If any of the following specific observations were observed, calretinin was regarded as positive:

1. Intense, granular and linear staining of nerve fibres in the lamina propria, muscularis mucosa, submucosa and muscularis propria
2. Diffuse strong cytoplasmic and nuclear staining of ganglion cells in the submucosal and/or in the myenteric plexus, and the supporting Schwann cells and nerve cells (excluding Mast cell immunopositivity).

OBSERVATIONS AND RESULTS

- Most of the patients (43.3%) presented at less than 1 month of age 36.6% patients presented between 1 month to 1 year. And 20.0% patients were more than 1 year of age.
- 73% of the patients presented with clinical diagnosis of HD were male. And 27% of the patients presented with clinical diagnosis of HD were female

- Most common type of specimen (24) received in our lab for diagnosing HD was full thickness rectal/colon biopsy. Six specimen are resected bowel loop.
- Submucosa showed ganglion cells in 9 cases and Muscularis propria showed ganglion cells in 8 cases. The presence of ganglion cases resulted in diagnosis of Non-HD. Hypertrophic nerve fibres were seen in around 66.0% of cases in the study population. In clinical ganglionic segment, ganglion cells could not be in 13.0% cases resulting in sensitivity 87.0%.
- Calretinin expression was seen in 6 cases resulting in diagnosis of NonHD. And 24 cases diagnosed as HD by ganglion cell negativity with IHC, three cases (12.5% cases) showed immunoreactivity in nerve fibres. When nerve fibre immunoreactivity was taken as the criteria for diagnosis, this method had a sensitivity and specificity 87.5% and 100% respectively.

| Number of cases | Initial diagnosis(H&E) | IHC diagnosis(Calretinin) |
|-----------------|------------------------|---------------------------|
| HD | 21 (70%) | 24(80.0%) |
| NHD | 9 (20.0%) | 6 (20.0%) |

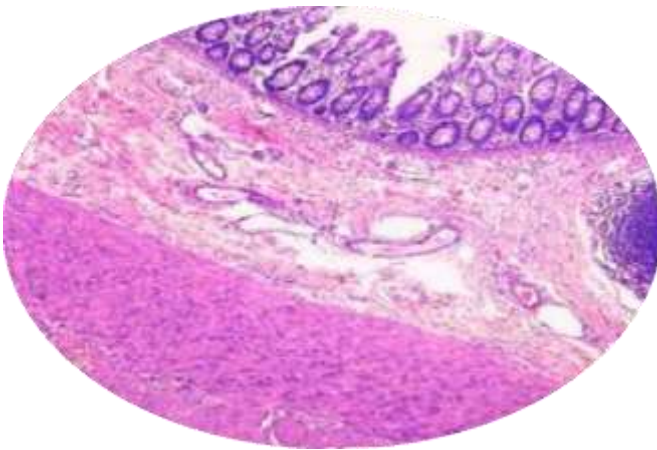


FIG-01 H&E Section Shows Normal Layer Rectal Biopsy

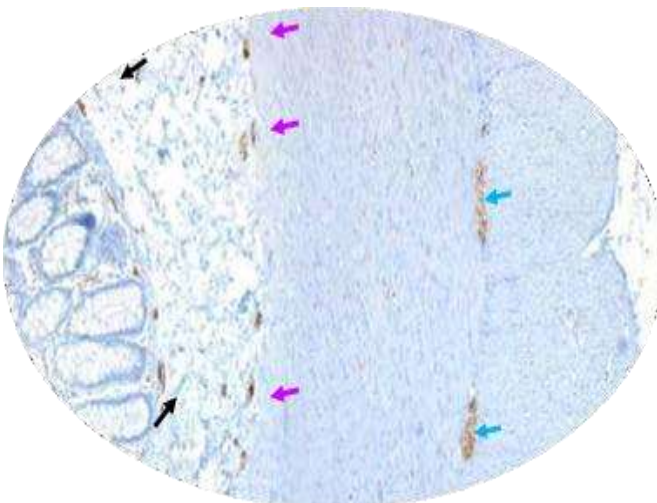


FIG-02 Pattern of staining of CALRETININ in the normal bowel mucosa and to show Meissner's plexus, Henle's plexus and Auerbach's plexus (Black, Pink, Blue arrows respectively).

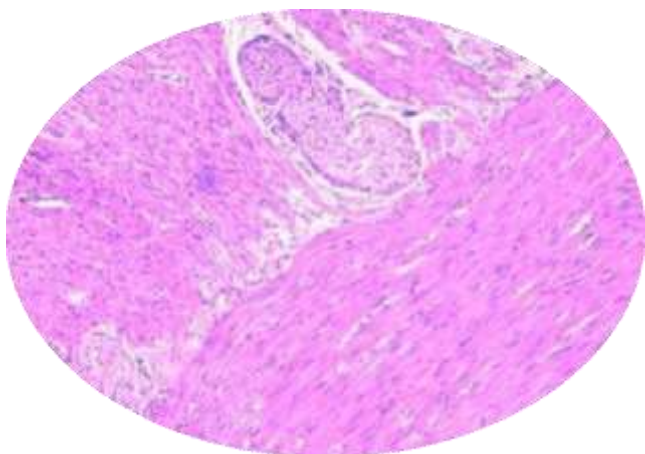


FIG-03 H&E section from a HD case shows Hypertrophic myenteric nerve plexuses (arrows) lacking ganglion cells

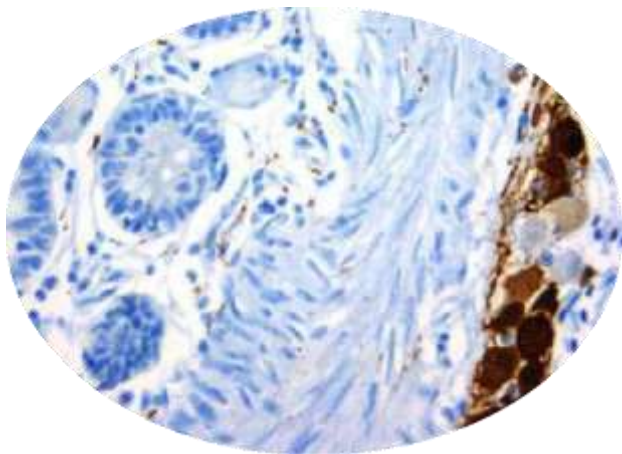


FIG-04 Calretinin IHC section shows diffuse strong (3+) cytoplasmic and nuclear staining in the submucosal ganglion cells

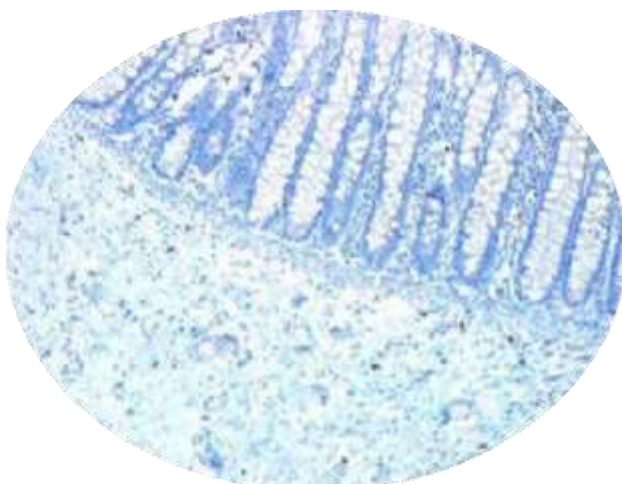


FIG-05 Shows total absent of calretinin staining

DISCUSSION & CONCLUSION-

- Of the 30 specimens, 9 were diagnosed as Non-HD on H&E Calretinin confirmed 6 cases as NHD and 3 cases diagnosed as NHD by H&E confirmed with Calretinin as HD. Of the 21 remaining patients, were diagnosed as HD by both H & E and calretinin. In H&E, nerve fibre

hypertrophy as a predictor of HD showed a sensitivity and specificity of 83.3% and 100% respectively. Nerve immunoreactivity in IHC showed a sensitivity and specificity of 87.5% and 100% respectively.

- Our present study shows that Calretinin can be a useful adjunct to H&E in the diagnosis of more cases, with accuracy and precision. So, Calretinin could prove to be a very valuable adjunct marker in improving diagnostic accuracy in centers with lesser experience.
- Calretinin IHC adds to the cost of diagnosis of HD. This additional cost could be justified by the improvement in accuracy which would save the patient from morbidity and expenses of a second look surgery.
- There was 12.5% false positive immunoreactivity of nerve fibres to Calretinin IHC. Though the pattern of reactivity was different from that of non-HD cases, this warrants further study

REFERENCE-

1. Butler Tjaden NE, Trainor PA. The developmental etiology and pathogenesis of Hirschsprung disease. *Transl Res.* 2013; 162:1–15.
2. Klein MD, Coran AG, Wesley JR, Drongowski RA. Hirschsprung's disease in the newborn. *J Pediatr Surg.* 1984 Aug; 19:370–4.
3. Klein MD, Philippart A. Hirschsprung's disease: three decades' experience at a single institution. *J Pediatr Surg.* 1993 Oct; 28:1291–3.
4. Furness JB, Costa M. The enteric nervous system. Edinburgh: Churchill Livingstone, 1990.
5. U, Piotrowska AP, Nemeth L, et al. Altered distribution of interstitial cells of Cajal in Hirschsprung disease. *Arch Pathol Lab Med* 2002; 126:928–33.
6. Wattoo DA, Porter AJ, Brookes SJ, et al. The polarity of neurochemically defined myenteric neurons in the human colon. *Gastroenterology* 1997; 113:497–506.
7. Thomsen L, Robinson TL, Lee JCF, et al. Interstitial cells of Cajal generate a rhythmic pacemaker current. *Nat Med* 1998; 4: 848–50.
8. Torihashi S, Horisawa M, Tanabe Y. c-kit immunoreactive interstitial cells in the human gastrointestinal tract. *J Auton Nerv Syst* 1999; 75:38–50.
9. Dobbins WO, Bill AH Jr. Diagnosis of Hirschsprung disease excluded by rectal suction biopsy. *N Engl J Med.* 1965; 272: 990– 993.
10. Jay V. Legacy of Harald Hirschsprung. *Pediatr Dev Pathol* 2001; 4:203
11. Swenson O, Rheinlander HF, Diamond I. Hirschsprung's disease; a new concept of the etiology; operative results in 34 patients. *N Engl J Med* 1949; 241: 551–6.