

Diagnostic Utility of Immunohistochemical Expression of Calretinin in Hirschsprung's Disease

Immunohistochemical
Expression of
Calretinin in
Hirschsprung's
Disease

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ABSTRACT

Objective: To identify the ganglionic cells in colonic and rectal biopsies by Calretinin immunostain.

Study Design: Experimental and retrospective study

Place and Duration of Study: This study was conducted at the Life care Molecular & PCR Lab Services, Karachi from January 2019 to December 2021.

Materials and Methods: 100 large intestinal biopsy specimens of 50 patients were collected at Life Care Molecular Lab (between 2019-2021) for confirmation of Hirschsprung's disease and were evaluated with both routine H&E stain and immunohistochemistry using Calretinin.

Results: Children having age from 5 days to 8 years and Male: Female ratio 3:1. 32 cases out of 50 have Hirschsprung disease and 16 cases without Hirschsprung disease. Haematoxylin and Eosin stain showed false positive results in 25 out of 100 biopsies and 15 cases out of 50. Calretinin showed 100% accuracy in all 100 biopsies studied. Both specificity and accuracy of Calretinin was 92% while H&E showed only 83% and 92% respectively.

Conclusion: Diagnosis of Hirschsprung's disease on H&E alone may give false positive result resulting in wrong diagnosis and mismanagement. H&E combined with calretinin within aganglionic segment help to eliminate false positive findings during histological examination of specimens for this disease.

Key Words: Ganglionic Cells colonic, Rectal Biopsies, Calretinin immunostain.

Citation of article: Zafar MS, Rehmatullah A, Qamar N, Ferozuddin N, Nusrat N, Baig AI. Diagnostic Utility of Immunohistochemical Expression of Calretinin in Hirschsprung's Disease. Med Forum 2022;33(5):11-14.

INTRODUCTION

Hirschsprung disease (HD) or congenital aganglionosis involves the hindgut and is caused by failure of migration, imbalanced proliferation and differentiation of tissue ganglionic crest cells due to which meissner's and myenteric plexuses shows absence of parasympathetic ganglion cells. On an average every 1 out of 5000 live birth showed Hirschsprung's disease with male predominance. Infants suffering from this disease are present initially with delay of meconium

passage, abdominal distension and later on vomiting and constipation. Aganglionosis primarily starts from rectum, but later on more proximal areas get involved. On surgically resented specimens three zones are seen in most of the cases like aganglionic zone with constriction, and hypertrophied and dilated zone. A third transition zone, lies at the junction of aganglionic segment and the proximal normal bowel.^{1,2,3}

Although the diagnostic criteria of this disease consists of clinical features and radiological findings but the definitive diagnosis and ultimately surgical resection depends entirely on histopathological examination of large intestinal biopsies for ganglion cells. However, reporting of absence of ganglion on routine hematoxylin-eosin is often misleading and difficult as it requires multiple serial sectioning and there is always a possibility of sampling tissue from the normal intestinal zone resulting in false positive results. Therefore, various Immunohistochemical staining techniques were established to eliminate these false positive results like acetylcholinesterase, PGP 9.5, S-100, synaptophysin and many more. Since these techniques are performed on frozen sections along with special handling which makes them difficult to perform.^{4,5} Recently, calretinin as an immunohistochemical supplement tool with H&E is introduced for the diagnoses of HD. Calretinin is a 29 kD vit-D dependent calcium binding protein and is involved in the development and functioning of the central and peripheral nervous system through calcium

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Received: January, 2022

Accepted: February, 2022

Printed: May, 2022

signaling pathway. Although, the negative expression of calretinin in the aganglionic segment of bowel was initially described by Barshack et al. in 2004, few studies have been done so far investigating the diagnostic utility of calretinin immunohistochemistry. Calretinin not only shows excellent cytoplasmic and nuclear staining of the ganglion or nerve cells but also stains intrinsic nerve fibers of normal bowel in a fibrillary pattern.^{6,7}

MATERIALS AND METHODS

This study was conducted at the Life care Molecular & PCR Lab Services, Karachi from January 2019 to December 2021.

Sample size: An experimental and retrospective study, with 95% confidence level and 5% margin of error. 100 biopsies samples from 50 randomly selected patients with clinical features of Hirschsprung's Disease were collected. H&E and IHC is done on all selected biopsies respectively. 79 out of 100 biopsies showed all layers of intestinal wall i.e., mucosa, submucosa and muscle layer while 21 were partial thickness biopsies comprising of mucosa and submucosa only. (Table 1)

Methodology:

1. Written consent with complete history of all patients was recorded.
2. Radiological examinations and ultrasound reports of abdomen was recorded.
3. Gross as well as histopathological and morphological features of intestinal biopsies was done on slides prepared from paraffin embedded blocks for identification of ganglion cells.
4. At least four to five sections from paraffin block were taken for H&E stain and immunostaining for calretinin.

Inclusion criteria:

Non Hirschsprung's disease;

1. Presence of at least one ganglion cell on routine H&E in biopsy specimen.
2. Immunostaining by calretinin shows positive staining of nucleus and cytoplasm of ganglion cells along with fibrillary expression of nerve bundle in the mucosa and submucosa with upward extensions.

Hirschsprung's disease: Complete absence of ganglions as well as nerve fibers in the biopsy tissue identified by H&E and calretinin immunostain.

RESULTS

Age and sex wise distribution: 19 patients (37.22%) out of 50 were up to 0-1 month age. 16 patients (32.55%) were upto 1 year age and 15 (30.23%) patients were ranges from 1 to 10 years old. M:F ratio is 2.91:1 with 37 (74.42%) out of 50 patients were male and 13 (25.58%) were female.

Interpretation of IHC: Histopathological evaluation was done on all 100 biopsies with H&E and

Immunohistochemical staining by calretinin. Negative discrepancy was showed by positive control. 60 biopsies shows negative expression of calretinin indicating lack of ganglions in intestinal biopsy. (Table 3). H&E stain detected 32 cases while calretinin detects 40 cases as ganglion positive with a difference of 8 biopsies. Likewise H&E stain showed 68 cases while calretinin showed 60 cases as ganglion negative with or without hypertrophied nerve. H&E stain indicate 83% specificity, 88% positive predictive value and 92% accuracy. On the other hand IHC expression of calretinin comparatively give more specific results with 100% accuracy. (Table 4)

Table No.1: Site of Biopsies

| Biopsy site | No of Bx (100) | % |
|------------------|----------------|------|
| Rectum | 55 | 55% |
| Sigmoid colon | 12 | 12% |
| Descending colon | 8 | 8% |
| Transverse colon | 8 | 8% |
| Ascending colon | 5 | 5% |
| Transition zone | 3 | 3% |
| Colostomy | 8 | 8% |
| Ileum | 1 | 1% |
| Total | 100 | 100% |

Table No.2: Classification of Cases (50)

| Stain | HD | Isolated hypoganglionosis | Non HD |
|------------|----|---------------------------|--------|
| Calretinin | 32 | 2 | 16 |
| H&E | 40 | 0 | 10 |

Table No.3: Discrepant result between H&E and calretinin (no of Bx 100)

| | H&E stain | Calretinin |
|---|-----------|------------|
| Ganglion positive | 32 | 40 |
| Ganglion negative with or without hypertrophied nerve | 68 | 60 |

Table No.4: Predictive value & Sensitivity/ Specificity of IHC evaluation

| Indicators | Definition | H&E Stain | Calretinin |
|---------------------------|---------------------|-----------------------|------------------------|
| No of Bx | | 100 | 100 |
| Sensitivity | TP/(TP+FN) | 60/(60+0):100 % | 60/(60+0):100% |
| Specificity | TN/(TN+FP) | 40/(40+8):83 % | 40/(40+0):100% |
| Positive predictive value | TP/(TP+FP) | 60/(60+8):88 % | 60/(60+0):100% |
| Negative predictive value | TN/(TN+FN) | 40/(40+0):100% | 40/(40+0):100% |
| Accuracy | (TP+TN)/TP+TN+FP=FN | (60+40)/60+40+8+0:92% | (60+40)/60+40+0+0:100% |

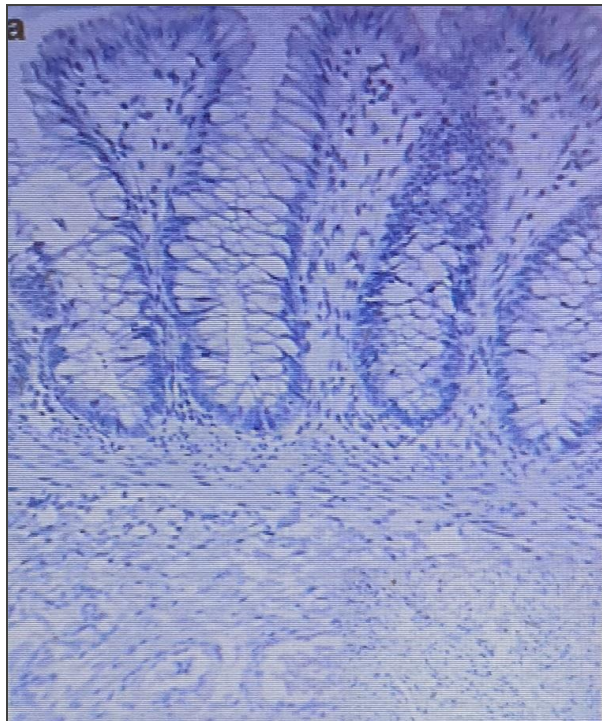


Figure No.1: Calretinin Negative (HD)

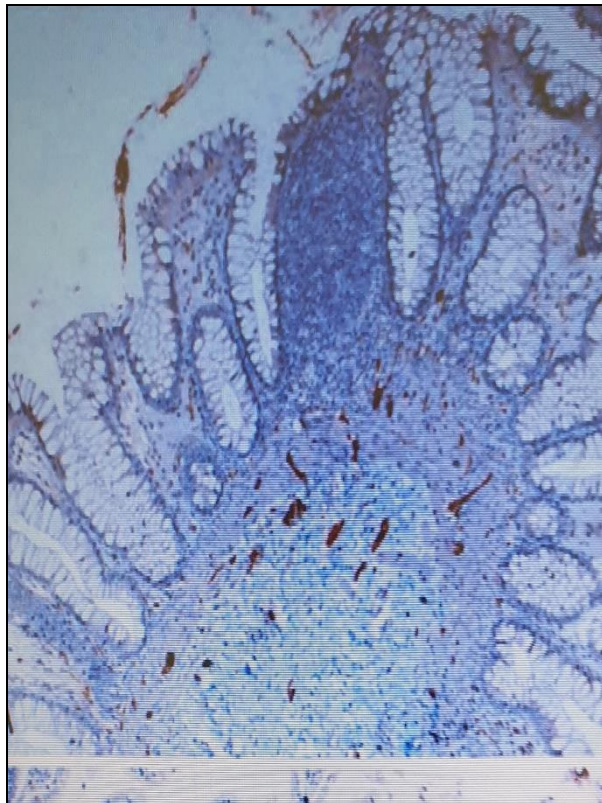


Figure No.2: Calretinin Positive (Non-HD)

DISCUSSION

Hirschprung disease is a birth disorder showing partial or complete absence of nerve cells in large intestine, so

there is lack of peristaltic movements in child's intestine resulting in entrapment of stool in intestine. Studies on molecular levels have identified several genes responsible for agangliogenesis of the enteric nervous system.^{8,9,10}

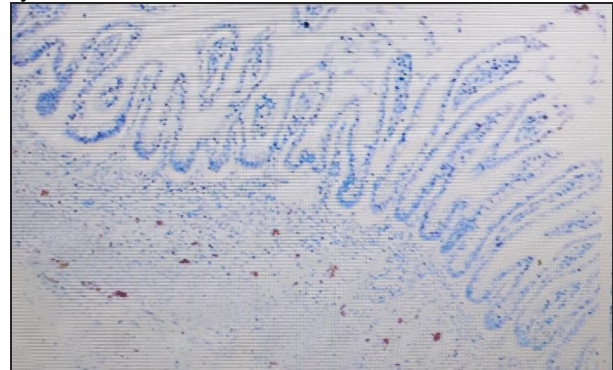


Figure No.3: Calretinin Negative (HD)

Hirschprung disease is a complex multifactorial disorder with no obvious and defined environmental factors. Genetic analysis have shown simultaneous involvement of several genes in concordance with the varying extension of aganglionic segment. Autosomal dominant and autosomal recessive patterns along with incomplete penetrance in HD variants have been found. Familial as well as sporadic cases are caused by those genes which are involved in signaling pathways. Thus, HD is proved to be a complex multigenic disorder and the role of different genes has been explained.^{11,12}

Diagnosis of HD by conventional H&E is still a diagnostic technique for HD in many centers although it has many limitations particularly for those histopathologist which have less interaction with this disease. Several studies have been done so far regarding the combined utility of H&E and IHC for the diagnosis of HD compared with routine H&E. IHC has been proved very beneficial complementary tool to identify nerve and ganglion cells in intestinal tissues. Calretinin, is encoded by the CALB2 gene on human chromosome 8. In humans, calretinin is involved in calcium signaling pathways primarily in whole nervous system, therefore it has very efficient binding ability with ganglions and nerves fibers in enteric nervous system and showed positive fibrillary staining of this nervous fibers as a direct confirmation of presence of ganglionosis.^{13,14} Calretinin immunostain may be used along with H&E for the confirmed diagnosis of Hirschprung disease, since intensity of expression of this immune marker in the ganglions and nerve fibers reflects well with the extent and level of agangliogenesis in intestinal tissue as compared to H&E in terms of false positive and false negative results. Further it has a much better sensitivity and specificity compared with H&E.

Our study showed very comparable results regarding age and sex with other studies like Yadav et al,¹⁵ Anbardar et al,¹⁶

In our study calretinin showed 100% specificity, positive predictive value and accuracy compared with

H&E which showed specificity 83%, positive predictive value 88% and accuracy 92%. This is in concordance with other studies by Guinard et al,¹⁷ Musa et al,¹⁸ Fakhry et al,¹⁹

CONCLUSION

Although histopathological evaluation of Hirschsprung's disease by H&E is in routine practice in most of the centers but it has some limitations. The major drawback of H&E is that it requires multiple serial sectioning and repeated biopsies resulting in false positive and false negative results. By our study it is cleared that calretinin provides in time excellent interpretation and identification of ganglion cell in intestinal tissues compared with H&E without unnecessary serial sectioning. Therefore we recommend use of calretinin IHC along with H&E for diagnosis of HD.

Author's Contribution:

Concept & Design of Study: Mohammad Salman Zafar
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 Revisiting Critically: Mohammad Salman Zafar, Nadeem Nusrat
 Final Approval of version: Mohammad Salman Zafar

Conflict of Interest: The study has no conflict of interest to declare by any author.

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