



ROLE OF IMMUNOHISTOCHEMISTRY IN THE DIAGNOSIS OF HIRSCHSPRUNG DISEASE.

Medical Science

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ABSTRACT

Hirschsprung disease constitutes one of the important causes of intestinal obstruction in the pediatric age group. Males are affected more than females. The classic and short segment Hirschsprung disease is the most common type of Hirschsprung disease and the rectosigmoid colon is the most common site for this disease, the patients most commonly present with clinical manifestation of intestinal obstruction such as delayed passage of meconium, constipation etc. Though histopathological examination is the gold standard, Calretinin is reliable in detecting the presence or absence of ganglion cells. It has many advantages that it can be carried out in paraffin embedded tissue sections. Also binary pattern of interpretation; negative indicates a diagnosis of Hirschsprung disease and positive rules out the diagnosis of Hirschsprung disease. Thus calretinin can be a valuable cost effective diagnostic aid for diagnosis of Hirschsprung disease.

KEYWORDS

Hirschsprung Disease, Ganglion Cells, Immunohistochemistry, Calretinin.

INTRODUCTION:

Hirschsprung disease is a congenital disorder that results from lack of coordinated propulsive movement of the distal portion of the large bowel due to the loss of intrinsic inhibitory innervation.¹ It occurs in approximately 1 of 5000 live births. Hirschsprung disease also known as congenital aganglionic megacolon, results when the normal migration of neural crest cells from caecum to rectum is arrested prematurely or when the ganglion cells undergo premature death. This produces a distal intestinal segment that lacks both the Meissner submucosal and the Auerbach myenteric plexus. The mechanism underlying defective neural crest migration are unknown, but a genetic component is present in nearly all cases and 4% of patients siblings are affected². It is diagnosed during the first year of life in most patients. Approximately 80% of the patients are male; 10% have Down syndrome and another 5% have other serious neurologic abnormalities¹. The earliest and most common presentation is delayed (>48 hours) passage of meconium in the newborn. Infants and older children tend to present with chronic constipation, frequently accompanied by abdominal by abdominal distension and vomiting³. The most important complications are acute intestinal obstruction and enterocolitis¹. Contrast enema and anorectal manometry have been used to attempt diagnosis of Hirschsprung disease. However, the histological study remains the gold standard using paraffin embedded biopsies⁴. Although hypertrophy of nerves, by itself should not be considered sufficient evidence to establish the diagnosis, it may represent a useful clue to support this possibility³.

Immunohistochemical stains have been used as a supplementary method to identify ganglion cells and nerve hypertrophy. An advantage of IHC is that it uses paraffin embedded biopsies⁴. Calretinin is a calcium-binding protein which plays an important role in the organization and functioning of the ENS. Calcium binding proteins (calretinin, calbindin) are involved in physiological calcium hemostasis. Ganglion cells and their projections express calretinin within the submucosal and myenteric plexus of normal bowel and ganglionic bowel of Hirschsprung disease whereas in aganglionic segments of Hirschsprung disease a lack of calretinin expression has been shown. The absence of calretinin immunostaining in the nerve fibres also represents a lack of calretinin in related nerve cells, which may serve as a diagnostic tool in the diagnosis of aganglionic segments⁵.

MATERIALS AND METHODS:

This is a cross sectional study. The duration of study is 1 year from December 2014 to November 2015. It was carried out in the Department of Pathology, Gauhati Medical College. The materials for the study consists of resected specimens and biopsies of intestine

received in the Department of Pathology, Gauhati Medical College during the period of one year from the Department of Paediatric Surgery, Gauhati Medical College. Inclusion criteria: Patients of paediatric age group and both sexes who are clinically suspected of Hirschsprung disease are included in this study. 1) Specimens which are adequate. 2) Properly resected surgical specimens are included. Exclusion criteria: 1) inadequately preserved specimens. 2) improper clinical record.

METHODOLOGY:

- 1) Formalin fixed specimens were received in the Department of Pathology, GMCH.
- 2) The specimens were subjected to detailed gross examination and subjected for histopathological processing and paraffin blocks prepared. Sections were cut at 3-5 micron thickness and stained by H&E and mounted in DPX.
- 3) Immunohistochemistry was done in each case using Calretinin of Biogenin make and was examined.

RESULTS AND OBSERVATIONS:

A total of 54 cases of suspected Hirschsprung disease were received in the department of pathology, GMCH. Histopathological examination and Immunohistochemistry was carried out in the department of pathology of GMCH, Guwahati. Out of the 54 cases, 33 cases [61.1] were diagnosed as Hirschsprung disease and 21 cases [38.9] were diagnosed as non Hirschsprung disease. The results and observations of the data were recorded in tables, bar and pie diagrams, statistically calculated and presented. Total number of resected specimens were 42 [77.8%], biopsy specimens were 12 [22.2%]. Out of the resected specimens, 30 were Hirschsprung disease and 12 were non Hirschsprung disease. Of the 12 biopsied specimen, 3 cases were diagnosed as Hirschsprung disease and in rest ganglion cells were noted. The age ranged from 3 days to 12 years. Out of these, 26 [78.8%] were males and 7 [21.2%] were females.

Observations : Maximum cases were seen in 1 month to 1 year age group (42.4%) followed by >1 year (39.4%) in this present study. The patients ranged from 3 days -12 years with mean age at presentations at 22.54 months. Male preponderance was seen with male:female ratio of 3.7:1. Classic and short segment Hirschsprung disease is the most frequently occurring type in this study. Rectosigmoid is the most common site involved in Hirschsprung disease. Associated conditions are observed in only 6.1% cases of Hirschsprung disease. Resected specimens comprises of majority of the cases (77.8%) in the present study.

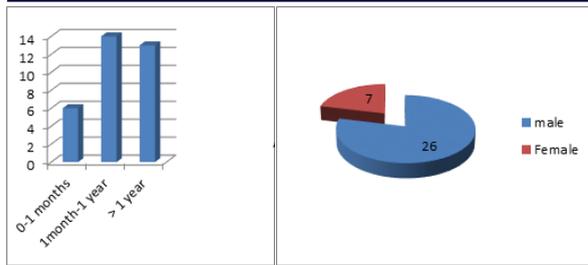


Figure 1: Age distribution in Hirschsprung disease

Figure 2: Gender distribution of Hirschsprung disease

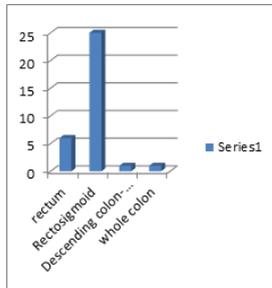


Figure 3: Frequency of different sites of affected colon



Figure 4: Constricted part of colon (arrow)

DISCUSSION: The present study entitled “Role of Immunohistochemistry in the diagnosis of Hirschsprung Disease” was carried out in the Department of Pathology of GMCH, Guwahati in association with the Department of Pediatric Surgery, GMCH for a period of one year from December 2014 to November 2015. A total of 54 cases of suspected Hirschsprung disease were received in the department of pathology, GMCH. Histopathological examination and Immunohistochemistry was carried out in the department of pathology of GMCH, Guwahati. Out of the 54 cases, 33cases[61.1] were diagnosed as Hirschsprung disease and 21 cases[38.9] were diagnosed as non Hirschsprung disease. Our study shows that Hirschsprung disease was found to be in the pediatric age group ranging from 3 days to 12 years which was similar to some other studies like Rahman Z et al., Hiradfar M et al., and Yadav L et al. In our study the mean age of presentation was 22.54 months which is similar to studies by Hiradfar M et al., and Yadav L et al. In our study we found that 78.8 % cases were males and 21.25 were females which is similar to studies by Rahman Z et al., Kacar A et al and Yadav L et al. In our study classic and short segment Hirschsprung disease is the most common, it constitutes 94% cases of Hirschsprung disease similar to studies by Ryan ET et al., Russell MB et al. In our study 75.7% cases were present in the rectosigmoid colon which is the most common site similar to studies by Hiradfar M et al., and Yadav L et al and Suita S et al. In our study we found that sensitivity, specificity, positive predictive value, negative predictive value and accuracy were 93.9%, 100%, 100%, 91.3% and 94.4% respectively for calretinin immunostaining for Hirschsprung disease which is similar to studies by Hiradfar M et al. Kacar A et al, and Yadav L et al .

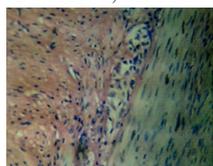


Figure 5: Presence of ganglion cells excludes Hirschsprung disease

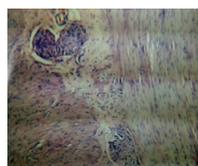


Figure 6: Absence of ganglion cells represents a case of Hirschsprung disease



Figure 7: Calretinin positivity in ganglion cells excludes Hirschsprung disease.

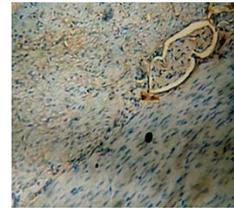


Figure 8: Total absence of calretinin staining and unstained hypertrophied nerve fibres represents a case of Hirschsprung disease

CONCLUSION:

Hirschsprung disease constitutes one of the important causes of intestinal obstruction in the pediatric age group. From the present study we can conclude that Hirschsprung disease is diagnosed in the first year of life. Males are affected more than females. The classic and short segment Hirschsprung disease is the most common type of Hirschsprung disease and the rectosigmoid colon is the most common site for this disease, the patients most commonly present with clinical manifestation of intestinal obstruction such as delayed passage of meconium, constipation etc. congenital anomalies and positive family history can be seen in a small percentage of patients. Calretinin is reliable in detecting the presence or absence of ganglion cells. It has many advantages that it can be carried out in paraffin embedded tissue sections. Also binary pattern of interpretation; negative indicates a diagnosis of Hirschsprung disease and positive rules out the diagnosis of Hirschsprung disease. Thus calretinin can be a valuable cost effective diagnostic aid for diagnosis of Hirschsprung disease.

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