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Comparative Study of Immunohistochemical, Hematoxylin & Eosin Staining and its Diagnostic Importance in Hirschsprung's Disease

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Abstract

Material and Methods: The study of 510 patients comprised colorectal, appendicectomy biopsies and myectomy specimens at various levels. The study included both ganglionic and aganglionic segments of intestine. The specimens were fixed in 10

Index terms— hirschsprung?s disease, immunehistochemi-cal stain, H and E stain.

Introduction arald H irschsprung first described in 1888 two unrelated boys who died from chronic severe constipation with abdominal distension resulting in congenital megacolon. **??** Hirschsprung's disease (HD) is defined as the absence of ganglion cells in submucosal (Meissner's) and myenteric (Aurbach's) plexuses in distal bowel extending proximally from internal anal sphincter for variable distances that result in functional obstruction caused by dysmotility of the diseased segment. **??** It is one of the most common diseases in the field of pediatric surgery. Occurrence of the disease is 1 in 5000 live births. 70-80 percent of them are boys. Based on the age of diagnosis, the most cases of Hirschsprung's disease are diagnosed in neonatal period and the rest are discovered uptil 2 years of age. It is believed to result from the failure of ganglion cells to migrate caudally during the embryonic life. The loss of ganglion cells extends for a variable distance above the anorectal junction. The classical Hirschsprung's disease was found restricted to rectosigmoid junction in 75% of cases; long segment disease in 15% of cases, ultra short segment disease in 5% of cases and variable length was found in 5% of cases. **??** The aganglionic bowel in Hirschsprung's disease was diagnosed using HSCR in most of the newborn cases owing to intestinal obstruction with the following features are failure to pass meconium within the first 48 hours of life, vomiting, abdominal distension lacks the normal motility, functional obstruction that leads to neonatal enterocolitis. **??** The diagnostic accuracy of various modalities for Hirschsprung's disease are radiology 60% (Barium enema) manometry 90%, biopsy 95% and immunohistochemistry has 99% accuracy. **??** Present our study is to evaluate the diagnostic difficulties in identifying ganglion cells and to compare the utility of seromuscular biopsy over sub mucosal biopsy.

1 II.

2 Material and Methods

This prospective study was carried out at Niloufer hospital, Hyderabad for a period of 6 years (from January 2000 to December 2005). The total number of surgical specimens and biopsies received at pathology Department of niloufer hospital, Hyderabad for 6 year period were 3844 out of which 357 cases were Hirschsprung's disease and rest 153 cases are other causes of constipation in pediatric age group [Table 1]. The surgical specimen's, colorectal specimens, appendicectomy, myectomy, biopsies at various levels of intestine were taken. The cases that presented with various causes of chronic constipation and intestinal obstruction such as Hirschsprung's disease, meconium ileus, ileal atresia, intestinal neuronal dysplasia and hypoganglionosis were examined by surgical biopsies and specimens [Table 2]. The study of 510 patients comprised colorectal, appendicectomy biopsies and myectomy specimens at various levels. The study included both ganglionic and aganglionic segments of intestine. The specimens were fixed in 10% formalin solution. In the laboratory, after preparing sections of paraffin embedded

tissues, H and E staining slides were compared with Cathepsin D. Cathepsin D is a specific, sensitive marker that detects immature ganglion cells. Acetylcholine esterase is equally specific and sensitive, but neuron specific enolase (NSE) is a histochemical and IHC method, it will not help the detection of immature ganglion cells.

3 III.

4 Results

Based on the age of diagnosis, most cases of Hirschsprung's disease are diagnosed in neonatal period and the rest are diagnosed until 2 years of age [Graph 1]. In our study of 357 cases, 223 are male children and 74 are female children (Male: Female ratio-3:1). Short segment was the most commonly occurring type constituting 229 cases (64%). The less common is the total colonic aganglionosis constituting 21 cases (5.8%); while long segment was 77 cases (21.5%)[Graph 2].

There were 20 cases of Hirschsprung's disease among the 96 subjects, 15 cases showed a positive pattern -A. In 13 of these patients, the fresh frozen, cryostat cut, and H & E stained sections showed the absence of neurons and the presence of hypertrophic nerve bundles in the submucosa [Table 3]. The H & E stain pointed to the diagnosis of Hirschsprung's disease in five other cases when the AChE pattern was other than pattern-A. The full thickened biopsies from the aganglion areas at the time of colostomy confirmed the diagnosis in all the 20 cases.

In Immuno-histochemistry (Cathepsin D) stains both immature and mature ganglion cells. Nerve fibers are not stained. Intense granular cytoplasmic staining is produced. This forms a collarets around the nucleus [Figure **??**]

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6 Discussion

Study of Hirschsprung's disease in pediatric age group was undertaken to observe the age and sex incidence, to study the various types of Hirschsprung's disease, the utility of seromuscular biopsy over sub mucosal biopsy and identify the diagnostic difficulties in detecting immature ganglion cells especially in total colonic aganglionosis. Detection of ganglion cells in H and E sections can be a difficult process for the pathologist. **??** The maturation of ganglion cells is incomplete at the time of birth, especially in the sub mucosal area. 7 Immature ganglion cells may be unipolar or bipolar and can be mistaken for stromal cells. 7 Sub mucosal ganglion cells are smaller than myenteric plexus ganglion cells, 8 and pathologists have to prepare between 50 to 400 sections of H and E stained slides to find ganglion cells. 9 On the other hand, although AChE staining is the chosen technique for some pathologists 10 its diagnosis needs experience and its interpretation is difficult in some instances. 11 One of the problems is the interference of red blood cell (RBC) is acetyl cholinesterase due to hemorrhage in lamina propria. **??** Also, false positive 9 and false negative 6 reactions were reported using this staining technique. Technical difficulties and storage problem of reagents is also reported. 10,12,13,14 In our study, short segment Hirschsprung's disease is the most common type involving 64.5% cases; lowest incidence is occupied by total colonic aganglionosis i.e., 6% [Graph 2]. In our study, almost 1/3 rd (33%) of cases were established by the first 3 months of life, only 17% by the first year, from 1-6yrs, they are almost 40%. Beyond 6yrs i.e., 6-14 yrs is only 8% are reported [Graph 1]. The histochemical technique must be affordable with specificity and sensitivity for the detection of ganglion cells. In our study, cathepsin D was performed on several formalin fixed paraffin embedded blocks. It involved both aganglionic [Figure **??**] and ganglionic segments of intestine.

Cathepsin D and AChE are the only stains to detect immature ganglion cells [Figure **??**]. In total colonic aganglionosis this is the only stain helps for a definite diagnosis. Cathepsin D is the only stain which stains immature and mature ganglion cells along with AChE but in cases of total colonic aganglionosis [Figure **??**], this panel can detect smaller or immature ganglion cells and also small cytoplasmic portions of those cells [Figure **??**]. Hence, the sensitivity and specificity is increased with false negative and decreased with false positive results.

V.

7 Conclusion

Comparing the results of Cathepsin D with Acetyl cholinesterase, Cathepsin D was found to be equally good like acetyl cholinesterase and useful as a reliable immune-histochemical stain in detecting immature and mature ganglion cells. Following colostomy in patients with Hirschsprung's disease, few of them are prone to develop neonatal enterocolitis and perforation. This enterocolitis may be due to improper level colostomy. So to detect this it is essential that the presence of ganglion cells should be looked for in the colostomy site biopsy which helps in differentiating neonatal enterocolitis due to improper colostomy from other etiologies.

Figure 1:

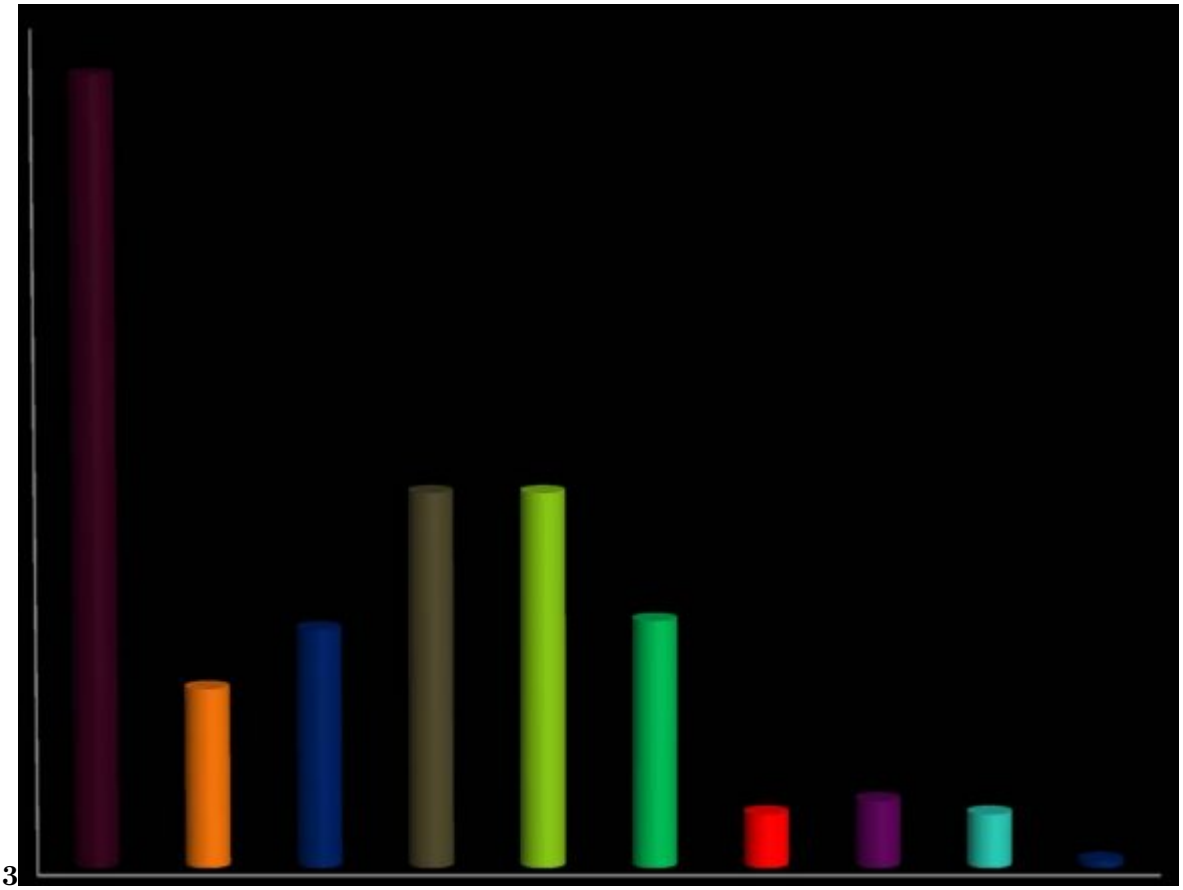

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Figure 2: Figure 1 :Figure 3 :

1

Signs, Symptoms and Diagnostic Studies	Idiopathic Constipation	Hirschsprung's disease
1. Soiling	Common	Unusual
2. Still in ampulla	Common	Unusual
3. Obstructive symptoms	Rare	Common
4. Stool retentive behavior	Common	Rare
5. Enterocolitis	Never	Possible
6. Anorectal examination findings	Dilated ampulla	Narrow
7. Contrast enema findings	Dilated ampulla	Narrowed distal segment

Figure 3: Table 1 :

2

Disease	(2000-2005) Total No. of Cases	% of Cases
Hirschsprung's disease	357	70%
Meconium Ileus	11	2.3%
Intestinal Atresia	21	4.1%
Intestinal neuronal Dysplasia	02	0.4%
Hypoganglionosis	10	2%
Normal	93	18.2%
Inadequate Biopsies	16	3.4%
Total	510	100%

Figure 4: Table 2 :

3

	Fresh frozen, cryostat cut, H & E stained section Results			
AChE pattern	With Hirschsprung's disease Neurons Absent	Hypertrophic Nerves present	Without Hirschsprung's	Nerv pres
Pattern A (n=15)	13	13	0	0
Pattern B (n=3)	3	3	7	0
Equivocal (n=2)	2	2	5	0
Negative (n=0)	0	0	50	0

Figure 5: Table 3 :

Figure 6: AGE INCIDENCE OF HIRSCHSPRUNG'S DISEASE 0-3months 3-6months 6-12months 1-2yrs 2-4yrs 4-6yrs 6-8yrs 8-10yrs 10-12yrs 12- Ultrashort segment 8% Short segment 64% Long segment 22% Total Colonic aganglionosis 6%

Therefore it is emphasized that correct level for colostomy surgery is to be checked with biopsy of the colostomy site and this biopsy must also be subjected to immuno-histochemistry. [1] [2]

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