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HIRSCHSPRUNG DISEASE AND HEMATOXYLIN-AND-EOSIN-STAINED SECTION-BASED DIAGNOSIS

Histopathology

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ABSTRACT

The Hirschsprung disease is defined as a rare congenital intestinal disorder which is characterized by the bowel obstruction due to the presence of aganglionic cell. Sometimes this disease is termed as Neurocristopathy, implying the disorder as a consequence of abnormality in cells and tissues arising from the neural crest. A vast variety of diagnostic test including contrast enema used as screening test; however, the finalopinion rely upon rectal biopsy interpretation. Moreover, the conventional hematoxylin-and-eosin-stained sections is widely used by the experienced pathologist to establish the diagnosis of this disease. In view of this, the given article reviews the clinical and pathological features of Hirschsprung disease, highlighting the prominent features found in patients. Further, it presents a discussion on addressing the challenging aspects of its diagnosis.

KEYWORDS

Epidemiology and Clinical presentation

Hirschsprung disease is the congenital absence of the enteric ganglion neurons at the end of the intestinal tract. The enteric nervous system (ENS) is responsible for most of the bowel function in the human body and its absence led to no neural mediated propulsive motility patterns, causing intestinal spasticity and chronic bowel obstruction (Heuckeroth, 2018). The presence of aganglionic cell can be of variable extent in the distal intestinal part. In 80% of affected patients, the aganglionic segment is short-spreaded (limits to rectosigmoid), in 3 to 10% patients, entire colon is a ganglionic whereas for a very tiny fraction of patients, the aganglionic extend upto small intestine (Amabartsumyan*et al*., 2019).The estimated prevalence of disease is 1-5/10000 with a male dominance of 4:1, worldwide (*Orphanet*, 2023). The disease has rare prevalence in older children whereas the onset age was reported as infancy or neonatal. For the 65% of the cases, the diagnosis is made in children aged less than a month, whereas for 95% of cases, the diagnosis is made in children with age before one year.

Clinical Presentation

The clinical symptoms of the Hirschsprung disease may vary by age in terms of its presentation and extent of the aganglionic segment. In neonates, the classical symptoms of disease observed are the failure to pass meconium within 48 hour of life, abdominal pain, feeding intolerance, constipation and bilious emesis. The complicated symptoms may include the massively distended proximal colon with muscular wall thinning followed by perforation. Additionally, it may present with the acute intestinal obstruction, enterocolitis with fluid and electrolysis imbalance.

Beyond neonatal periods, the symptoms in infants and children may present with mild to severe constipation unmanageable with oral laxatives, rectal therapies, vomiting, abdominal distention and often bloody diarrhea (Amabartsumyan *et al*., 2019). The presentation of disease symptoms beyond childhood and adulthood is considered rare.

Diagnostic procedure

The two screening tests, Contrast enema (CE) and Anorectal manometry (ARM) are available for diagnostic workup of Hirschsprung disease. The screening using CE requires exposure to radiation and thus, need a radiologist to perform accurately. The procedure of CE involves injection of water-soluble contrast into colon using catheter placed inside anus following live fluoroscopic imaging. In contrast, the ARM is less invasive and do not involve radiation exposure. The ARM aims to evaluate the voluntary and involuntary properties of anorectum by measuring intra-anal pressure measurement.

Patients with high suspicion of Hirschsprung disease are suggested to undergo either Rectal suction biopsy (RSB) or Incisional biopsy. If the results from RSB are unclear, then a full thickness biopsy (FTB) is recommended. In some cases, ancillary diagnostic tests such as acetylcholinesterase histochemistry or calretinin immuno histochemistry are extremely helpful.

Diagnostic Pathology

The most critical variable is the pathologists experience and familiarity with strengths and limitations of the approach used in their clinical and laboratory settings. This article describes the most commonly applied practices based on formalin-fixed paraffin-embedded (FFPE) tissue samples followed by histopathological examination for the diagnosis of Hirschsprung disease.

The examination of the anorectal specimen from 25 patients ranged between premature infants to 15years of age is performed. For each sections interpretation from distal, transitional and proximal rectum with hematoxylin-and eosin (H&E) followed by microscopic examination (refer Fig. 1,2, and 3). Further, the confident diagnosis of suction rectal biopsy depends on following variables:

Identification of ganglion cell in both plexus. The recognition of ganglion cell in an H&E-stained section is not difficult for experience pathologist, if the staining quality of tissue is good. The classic cytological features include abundant cytoplasm, an eccentric round nucleus, conspicuous nucleolus with peripheral clump chromatin and perinuclear pallor. Premature infant have immature ganglion cells with less cytoplasm, round nucleus with stippled nuclear chromatin can misinterpret with absence of ganglion cell. H&E-based diagnosis by an experienced pathologist is more reliable and justified, if adequate submucosa biopsy taken. Diagnostic findings are present in sections from desirable site of suction biopsies. However, when sections from biopsy is very tiny, it is difficult to interpret nerve bundle in both plexus, it is often advise and necessary to apply immuno histochemistry (IHC) to facilitate diagnosis.

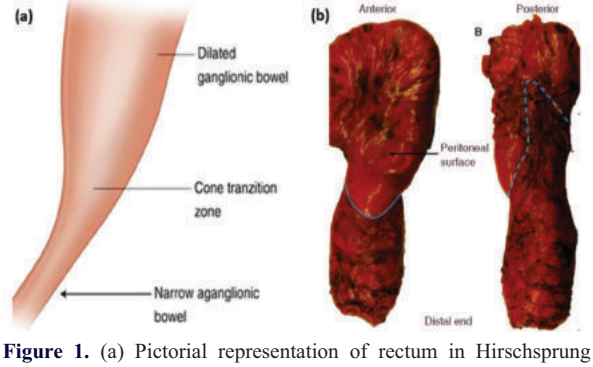

Figure 1. (a) Pictorial representation of rectum in Hirschsprung (*Source*: Choudhury, 2018), (b) Pictorial representation of rectum. The anterior peritoneal reflection separates intraperitoneal part of rectum, considered important landmark during surgery (*Source*: Westwood and West, 2018)

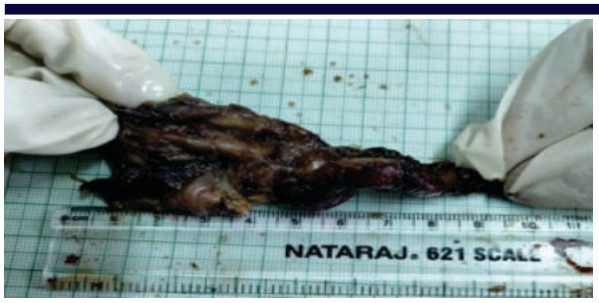

Figure 2. Anorectal specimen of dilated proximal ganglionic bowel with funnel shaped transition interface and constricted distal end (Pathology Dept., MGMMC Indore, 2023)

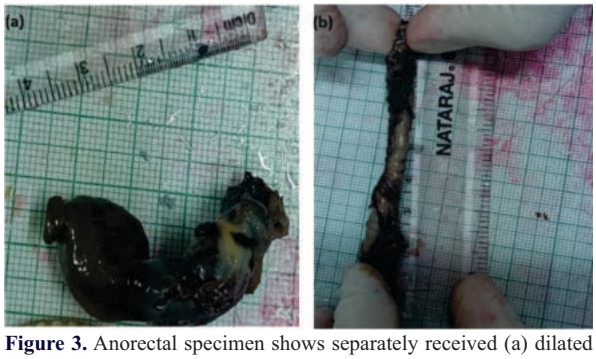

Figure 3. Anorectal specimen shows separately received (a) dilated bowel segment, and (b) atretic bowel segment (Pathology Dept., MGMMC Indore, 2023)

The microscopic examination images of the rectal tissue obtained from biopsy for different cases is shown in Fig. 4-9 to facilitate the diagnosis using H&E based samples. Figure 4 shows the presence of the ganglion cells and the submucosal cells nerves in a neonate, which do not have Hirschsprung disease. The microscopic image of stoma site, dilated segment, transition segment from confirmed Hirschsprung case (IHC confirmed) is shown in Fig 5, 6 and 7, respectively. The microscopic imaging of rectal biopsy sample (Fig. 9) obtained from neonate having Hirschsprung disease indicates the absence of ganglion cells and the presence of hypertrophic submucosal nerves. IHC examination-such positivity will be absent in confirmatory evaluation of Hirschsprung disease (by absence of calretinin in submucosal nerve).

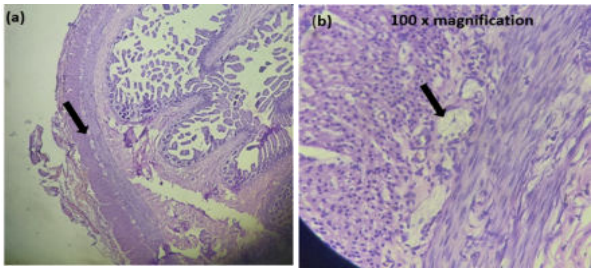

Figure 4. Typical microscopic image of the ganglionic rectal biopsy from a neonate in different resolution (a) 10 x magnification, and (b) 100 x magnification showing presence of ganglion cell between muscle fiber.

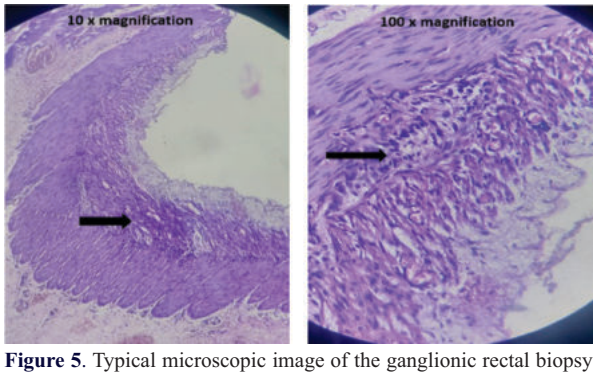

Figure 5. Typical microscopic image of the ganglionic rectal biopsy from **stoma** site of Hirschsprung patient, show the presence of ganglion cell between muscle fiber.

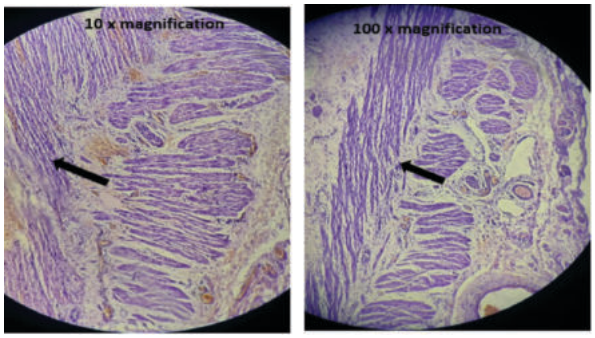

Figure 6. Typical microscopic image of the aganglionic rectal biopsy from **dilated** segment of bowel from Hirschsprung patient, show the presence of thinning of muscle fiber in different resolution

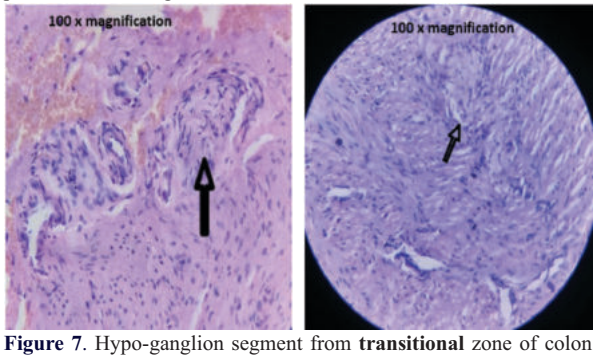

Figure 7. Hypo-ganglion segment from **transitional** zone of colon sometime misinterpret as aganglionosis (absent ganglion cell)

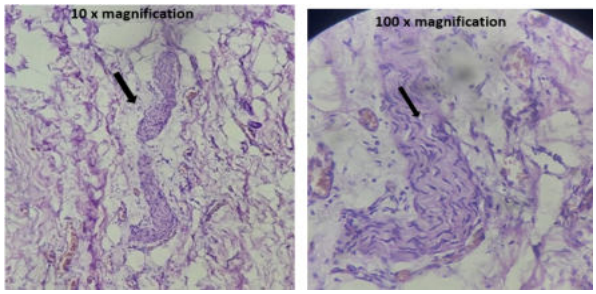

Figure 8. Microscopic image of the rectal biopsy from a neonate having Hirschsprung disease show **hypertrophied nerve bundle.**

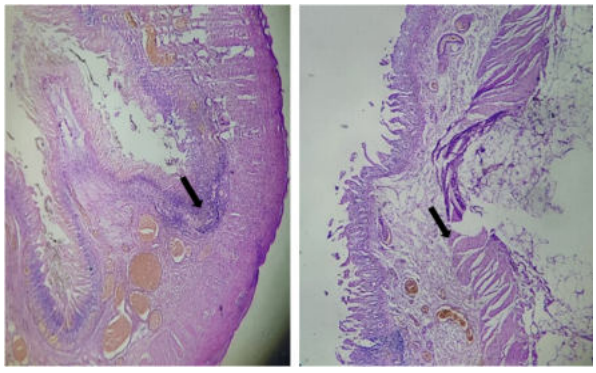

Figure 9. H & E section study show dense chronic inflammatory cell infiltrate in mucosa, submucosa, muscularis propria along with thinning of muscularis with presence of ganglion cells, suggestive of enterocolitis is an important **differential** while reporting Hirschsprung with similar clinical presentation. (10x,40x magnification)

Diagnostic findings

The confirm diagnosis of Hirschsprung disease shows following pathological findings – absence of ganglion cells, presence of submucosal nerve hypertrophy, intramural nerve hypertrophy and absent of calretinin in IHC examination (for confirmatory evaluation).

Differential diagnosis

Enterocolitis with gangrenous bowel (Fig. 9)
Meconium ileus

Pseudoobstruction syndrome characterized by congenital thinning of muscle

Reporting of Rectal biopsy

The surgical pathology report of rectal biopsies should incorporate the site from which biopsy has been taken, if ganglion or hypertrophied nerve identified or not along with any ancillary staining results (if conducted). An exemplary format of the report according to standard protocol given below:

Rectum suction biopsy from dilated end: findings consistent with Hirschsprung disease

- Intramural plexus -Ganglion cells present
- Submucosal plexus-Ganglion cells present
- Hypertrophied nerve fiber-absent
- Normal pattern of calretinin-immunoreactive mucosal innervation

Rectum suction biopsy from transition zone: findings consistent with Hirschsprung disease

- Intramural plexus -Few ganglion cells present (hypoganglionic)
- Submucosal plexus-Few ganglion cells present (hypoganglionic)

Hypertrophied nerve fiber-absent

- Normal pattern of calretinin-immunoreactive mucosal innervation

Rectum suction biopsy from constricted end: findings consistent with Hirschsprung disease

- Intramural plexus -Ganglion cells absent
- Submucosal plexus-Ganglion cells absent
- Hypertrophied nerve fiber-Present
- Normal pattern of calretinin-immunoreactive mucosal innervation – Absent

Resection report can be used for diagnosis or as **comment** after diagnosis

Note – Choline transporter immunohistochemistry: formalin fixation and paraffin embedding are relatively new and has not been validated in laboratory (Department of Pathology, MGMMC Indore).

Management

In most clinical cases, aganglionic part resected followed by end-to-end anastomosis of proximal and distal ganglionic physiological normal segment of bowel (Pull-through technique). Also, some surgeon prefers to perform ostomy surgery where temporary stoma opening formed followed by closure after fixed duration (Fig. 10 and 11).

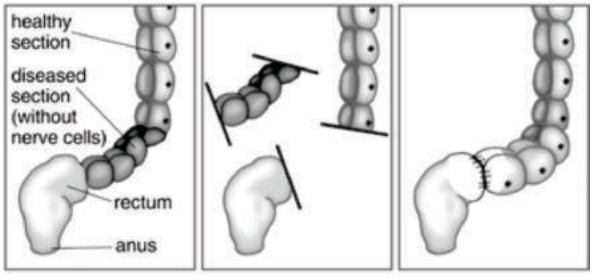

Figure 10. Schematic of resection of aganglionic bowel followed by end-to-end anastomoses of ganglionic segment (*source*: National institute of health, NIH, 2023)

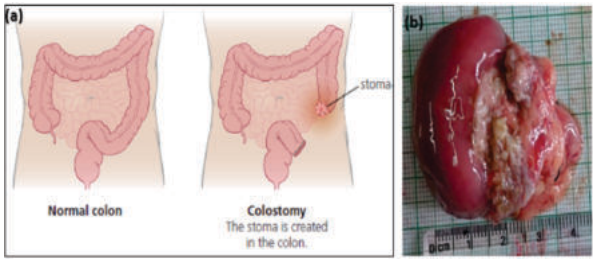

Figure 11. (a) Schematic diagram of ostomy procedure followed by stoma formation (*Source*: IH, 2023), (b) Sample received in our department after successful closure of stoma from Hirschsprung patient managed through ostomy procedure (*MGMMC, Indore)*.

RESULTS AND DISCUSSION

The examination of anorectal specimen of 25 patients is done with age ranging between premature infants to 15 years. It is observed that 15 patients were below age of one year with ganglion cells absent and hypertrophied nerve present in aganglionic dilated segment in both plexus, 5 patients below one-year age showed feature similar to Hirschsprung with contrasting presence of ganglion cells (later Hirschsprung is confirmed based on IHC). Thus, the H&E-based diagnosis of Hirschsprung shows the more than 70% accuracy for patients age below one year.

For the patients age above one year, four showed absence of ganglion cells and presence of hypertrophied nerve in aganglionic dilated segment in both plexus, whereas one patient showed presence of ganglion and absence of hypertrophied nerve (later confirmed Hirschsprung based on IHC). Thus, for patients age above one year, H&E-based diagnosis showed 80% sensitivity. Based on above, it can be said that the H&E-based examination stimulate diagnosis whereas the conflicting cases can be confirmed using IHC study.

Many studies regarding pathophysiology and diagnosis of Hirschsprung has been done, ultimately put emphasis on H&E based examination, followed by confirmation on IHC. This study give strength to H&E based examination exclusively, making it more diagnostic, reliable, cost effective and reachable to patient. Moreover, it rules out all possible differential with adequate biopsy sample, staining and experienced pathologist.

Challenges in Diagnosis

The exclusion of Hirschsprung based on rectal biopsy impart simple way to rule out all differentials with similar clinical presentation. As per IHC, the classic findings of rectal biopsy include absent ganglion cell, presence of large submucosal nerves, absence of calretinin-immunoreactive mucosal innervation. However, variation of diverging findings may indicate the unusual biopsy interpretation in Hirschsprung or related conditions which occur in significantly lesser percent of cases and additional ancillary studies will be helpful for such cases.

Acknowledgement

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