

Diagnosis of Hirschsprung Disease by Frozen Section Biopsy Using Routine Hematoxylin-Eosin (HE) Stain: A Year's Study

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Abstract

Background: Frozen section biopsy is an essential intraoperative histopathology procedure for diagnosis and management of Hirschsprung disease (HD). Interpretation of this procedure depends on identification of ganglion cells or hypertrophied nerve bundles in the colorectal wall tissue. A number of rapid stains are available to identify the ganglion cells and the nerve fibres in frozen sections; however, in many countries including Bangladesh, hematoxylin and eosin (HE) is the only or predominant stain being used for long.

Method: This study has been done retrospectively to see the effectiveness of HE stain used in frozen sections for diagnosis of Hirschsprung disease. 34 frozen sections were done for Hirschsprung disease during January through December, 2019. Remaining tissue in 31 cases were routinely processed afterwards. All the frozen and routine sections were stained with HE stain.

Result: In 29 (93.55%) cases, ganglion cells were identified both in frozen and routine sections. In 2 (6.45%) cases, ganglion cells could not be detected in frozen sections; however, they were detected in routine sections with HE stain.

Conclusion: Addition of histochemical and/or immunohistochemical stain to hematoxylin and eosin method is recommended to increase the efficiency of diagnosis of Hirschsprung disease.

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Keywords: Hirschsprung disease, ganglion cells, hematoxylin and eosin, frozen section

Introduction

Frozen section is a multistep technique involving surgical and histopathology laboratory procedures. It involves intraoperative preparation of slides, microscopic examination of the sections and their interpretation, and finally completion of the ongoing surgery on the basis of the pathological interpretation.¹ The technique was first used by William H Welch of John Hopkins Hospital in 1891. The aim of this technique was to guide immediate surgical management.² Though primarily developed for intraoperative diagnosis of malignancy status of tumours, frozen sections also

effectively aid in diagnosis and management of Hirschsprung disease cases. Hirschsprung disease is a developmental disorder of distal colon manifested in early childhood with delayed passage of meconium and constipation. It is characterized by absence of ganglion cells in the submucosal and myenteric nerve plexuses of colorectal wall. The management includes resection of the aganglionic segment with pull-through operation or temporary colostomy which largely depends on intraoperative assessment of colorectal tissue for presence or absence of ganglion cells.^{3,4}

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Now-a-days, several rapid stains are available to identify ganglion cells and hypertrophied nerve bundles in the colorectal biopsy specimens of suspected Hirschsprung disease patients on frozen section. In addition to the universally used routine stain hematoxylin and eosin (HE), other less commonly used stains are Diff-Quik, toluidine blue, Giemsa and cresyl violet. Though one or the other of these stains sometimes proves better, in many countries of the world, the HE stain is still the only or predominant stain used because of its availability, simplicity of use, low price, wide range of tissue structures it stains and its diagnostic capability.^{5,6}

This study was done to see the effectiveness of HE staining used in frozen section procedure for intraoperative diagnosis or exclusion of Hirschsprung disease.

Method

This is a retrospective observational study carried out in Pathology department, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. The colorectal biopsies (taken from suspected Hirschsprung disease patients) received for frozen section and reported in the department of Pathology, BSMMU from January to December, 2019 were the study cases. A total of thirty four frozen sections for Hirschsprung disease diagnosis had been performed throughout the year. The slides were collected from archive and reviewed. HE-stained sections of both frozen section procedure and subsequent routine histopathology procedure were examined under light microscope. Presence or absence of ganglion cells and hypertrophied nerve bundles in the submucosa and muscle coat of the colorectal wall were looked for and evaluated. Tissue inadequacy, procedural artifacts and diagnostic discrepancies between frozen and routine sections were assessed.

Results

Among the 34 cases of frozen section, 10 cases were diagnosed as Hirschsprung disease (HD) on the basis of absence of ganglion cells and presence of hypertrophied nerve bundles in the submitted colorectal tissue. Each of these cases was reported after processing two or more consecutive samples, latter ones from more proximal sites, being failed to identify ganglion cells in the earlier samples. The final samples of these cases were from more proximal ganglionic segments where ganglion cells were found and hypertrophied nerve bundles were absent. Twenty three of remaining 24 cases had been previously diagnosed as Hirschsprung disease followed by pull-through operation or colostomy. These cases showed presence of ganglion cells and absence of hypertrophied nerve fibres in the submitted tissue. One case was diagnosed as non-HD as it showed presence of ganglion cells and absence of hypertrophied nerve fibres and had no previous history of biopsy or surgery. No inadequate tissue was found in any case.

In all cases except 3 of 23 postsurgery cases, subsequent routine histopathology was done with HE staining. In those 3 cases, routine histopathology procedure could not be done because no tissue was left after frozen section. Remaining 20 postsurgery cases as well as the only non-HD case showed well recognized ganglion cells in the colorectal wall on routine procedure. Among the 10 cases diagnosed as HD on frozen section, 8 cases were confirmed as HD on routine HE stain, whereas 2 cases showed presence of ganglion cells. Hypertrophied nerve bundles, however, were found in routine stain also. The failure rate in detecting ganglion cells on frozen section was 6.45%. On review, one of these two cases had

marked freezing artifact and the other showed repeated oblique cuts on frozen section. On routine histopathology these cases showed recognizable mature ganglion cells. The patients in these two cases were 2 years and 22 months by age.

The age range of the patients having their biopsies sent for frozen section was 3 months to 12 years. There was no neonate patient but 6 patients were infants. Among them the youngest one, diagnosed HD on frozen section, was 3 months old. Immature ganglion cells could be identified in HE-stained frozen and routine sections in more proximal biopsy (ganglionic segment) of this patient.

Regarding gender of the patients, 23 (67.6%) cases were male and 11 (32.4%) cases were female. Male : female ratio was 2.1:1.

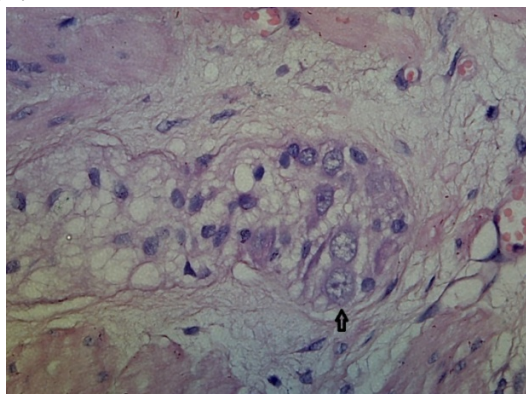


Figure 1. Mature ganglion cells in HE-stained frozen section (x40 objective)

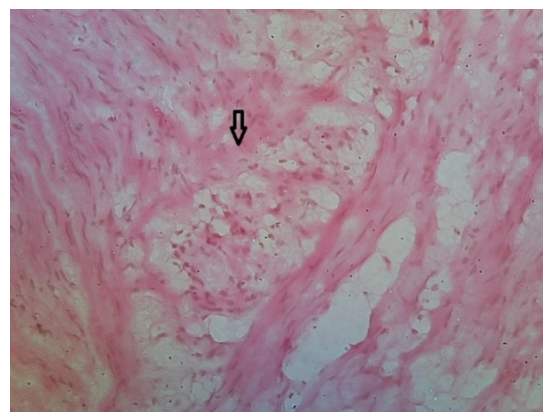


Figure 2. Immature ganglion cells in HE-stained frozen section (x20 objective).

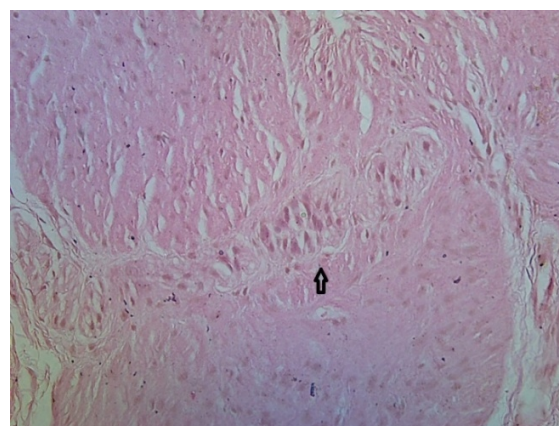


Figure 3. Immature ganglion cells in HE-stained routine section (x20 objective).

Table I: Findings in frozen and routine sections of the study cases.

Frozen section findings				Routine procedure findings			
Number of cases (n=34)	Ganglion cell	Hypertrophied nerve bundle		Number of cases (n=34)	Ganglion cell	Hypertrophied nerve bundle	
New HD case, 10	Absent = 10	Present = 10		New HD case, 08	Absent = 08	Present = 10	
Postsurgery case, 23	Present = 23	Absent = 23		Postsurgery case, 20	Present = 20	Absent = 20	
Non-HD, 01	Present = 01	Absent = 01		Non-HD, 01	Present = 01	Absent = 01	

Correct diagnosis = 93.55%; Correct diagnosis = 93.55%; Incorrect diagnosis = 6.45%; Correct diagnosis = 100%

Discussion

Hematoxylin-Eosin (HE) is the most commonly used stain in frozen section procedure. This stain contains two main dye components - hematoxylin and eosin. Hematoxylin is a basic dye that stains acidic components of a cell e.g. nucleic acids, into blue-purple.⁷ Eosin is an acidic dye that stains the cell cytoplasm and its contents like mitochondria and secretory granules into pink. HE yields excellent cellular morphology and contrast between different cellular components.⁸ However, it also has some drawbacks like under- or overstaining, especially in procedures like frozen section where tissue fixation is lacking. Regarding Hirschsprung disease diagnosis, HE stain often fails to identify immature ganglion cells present in neonates and renders a false diagnosis.^{9,10}

In the present study, 34 cases of HE-stained frozen sections and 31 routine sections are reviewed. Among these, 10 cases were diagnosed as Hirschsprung disease (HD) intraoperatively on the findings of absence of ganglion cells and presence of hypertrophied nerve bundles. On subsequent routine histopathology procedure, 8 cases were confirmed as HD but 2 showed presence of ganglion cells. On review, one of these latter two cases showed marked freezing artifact and the other showed oblique cuts in frozen section slides. As the biopsies also showed hypertrophied nerve bundles, they might have been taken from the transition zone or distal rectum close to the pectinate line. It was long established that the transition zone and rectal wall within 1-2 cm of the pectinate line contain reduced number or even no ganglion cells and significant hypertrophy of the nerve fibres.^{11,12}

In the present study, ganglion cells were detected in 20 cases of previously diagnosed Hirschsprung disease with history of surgery

in both frozen and routine HE-stained sections. Samples from these cases were sent for frozen section to re-check the presence of ganglion cells either at the time of colostomy closure or for reappearance of some symptoms following pull-through operation. The only non-HD case also showed well recognized ganglion cells in both frozen and routine sections.

In young children especially the neonates, immature ganglion cells are a common finding. Enteric nerve cells begin to mature during the last trimester of pregnancy and complete their maturation after birth.¹³ At birth there are both mature and immature cells. The immature ganglion cells mature over time, usually by 2 to 5 years of age.^{4,15} These cells are small, have little cytoplasm and dark crowded nuclei, and are often irregularly distributed in the neonatal gut wall.¹⁶ It is a challenge to detect these cells with HE stain on frozen section. Immunohistochemical staining, especially using neurone specific enolase (NSE) and ret oncoprotein can effectively diagnose these cells.^{17,18} In this study, however, immature ganglion cells were detected with HE stain in a 3-month old infant.

A study by Mala DM¹⁹ performing 93 frozen sections for 80 suspected HD cases showed 89% concordance rate between frozen and routine sections. HE stain and anti-neurofilament monoclonal antibody stain were proved equally effective for identification of myenteric ganglion cells in another study done by Yu CS et al.²⁰

RakhshaniN et al.²¹ termed hematoxylin and eosin (HE) the most relied stain so far for histopathology. They carried out a study where they compared HE stain with calretinin immunostain in diagnosis of Hirschsprung disease. They found the two stains comparable by Kappa test. According to study

by Harjai MM, biopsy with routine stain for Hirschsprung disease diagnosis confers 95% accuracy. When supported by immunohistochemistry, it has a high sensitivity (99.7%). HE along with acetylcholinesterase (AChE) histochemical stain is another method of choice to diagnose HD.^{5,9,22} While AChE passively supports HD diagnosis by staining the increased cholinergic nerve fibres, it can be done as a rapid stain in the intraoperative diagnostic procedure.^{5,23}

Conclusion

In many centers with limited facility, hematoxylin and eosin stain has been being used as the lone stain in diagnostic frozen section method for Hirschsprung disease. It is found quite effective in detecting presence or absence of ganglion cells with low failure rate. However, addition of histochemical and/or immunohistochemical techniques are recommended whenever possible to avoid every single false-positive or false-negative diagnosis. Representative biopsy, technical skill, and experience of the pathologist are other important factors in correct diagnosis of Hirschsprung disease.

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