

## ORIGINAL ARTICLE

# Punch and Full-thickness Rectal Biopsy in Diagnosis of Hirschsprung's Disease: A Comparative Study

Md. Abdus Sattar<sup>1</sup>, Muhammad Rashedul Alam<sup>2</sup>, Md. Rayhanur Rahman<sup>3</sup>, Poritosh Kumar Palit<sup>4</sup>, Md. Mahfuzul Haque<sup>5</sup>, Md. Jainul Abedin<sup>6</sup>, M. Kabirul Islam<sup>7</sup>, Md. Abdul Aziz<sup>8</sup>

### Abstract

**Background:** Hirschsprung's disease is a common cause of neonatal & infantile intestinal obstruction. The confirmed diagnosis of HD depends on histopathological demonstration of the complete absence of ganglion cells with the presence of hypertrophied nerve fibers in the distal bowel. So, rectal biopsy is the gold standard for diagnosis of HD no doubt.

**Objectives:** This study is an attempt to see the comparison between punch and full-thickness rectal biopsy to find out a low cost, easily performed technique with accurate histopathological results for diagnosis of HD, specially for poor and developing countries of the world.

**Methods:** This was a cross sectional comparative study. Total 50 patients of suspected Hirschsprung's disease were included in this study after fulfillment of inclusion criteria from November 2010 to March 2012 at Dhaka Shishu (Children) Hospital, Dhaka, Bangladesh.

**Results:** Out of 25 cases, 17 were diagnosed as having HD in punch biopsy but in case of full-thickness rectal biopsy out of 25 cases 20 were diagnosed as having HD. The sensitivity was 80% and positive predictive value was 94.11% of punch rectal biopsy in relation to full-thickness rectal biopsy. Operation time was less in punch rectal biopsy (14 minutes) than full-thickness rectal biopsy (20.5 minutes). Cost was less in punch rectal biopsy (1850 taka) than full-thickness rectal biopsy (3500 taka). Post procedure hemorrhage occurred 1 case in punch rectal biopsy, whereas 2 cases occurred in full-thickness rectal biopsy. No perforation occurred in case of punch biopsy but 1 case developed perforation in full-thickness rectal biopsy.

**Conclusion:** Punch rectal biopsy for diagnosis of HD is advantageous over full-thickness rectal biopsy in the form of less time, less cost, less complication but diagnostic efficacy is almost similar.

**Keywords:** Hirschsprung's disease, rectal biopsy, ganglion cell.

1. Assistant Professor, Department of Pediatric Surgery, Pabna Medical College, Pabna.
2. Assistant Professor, Department of Pediatric Surgery, Bangladesh Institute of Child Health (BICH), Dhaka Shishu (Children) Hospital, Dhaka.
3. Associate Professor, Department of Surgery, Pabna Medical College, Pabna.
4. Assistant Professor, Department of Pediatric Surgery, Shaheed Suhrawardy Medical College, Dhaka.
5. Assistant Professor, Department of Pediatric Surgery, Rangpur Medical College, Rangpur.
6. Assistant Professor, Department of Urology, Pabna Medical College, Pabna.
7. Professor, Department of Pediatric Surgery, Bangladesh Institute of Child Health (BICH), Dhaka Shishu (Children) Hospital, Dhaka.
8. Professor, Department of Pediatric Surgery, Bangladesh Institute of Child Health (BICH), Dhaka Shishu (Children) Hospital, Dhaka.

**Correspondence to:** Dr. Md. Abdus Sattar, Assistant Professor, Department of Pediatric Surgery, Pabna Medical College, Pabna. Cell: 01712-039265, E-mail: dr.mdabdussattarpaed@gmail.com.

**Received:** 18 December 2018; **Accepted:** 20 January 2019

## Introduction

Hirschsprung's disease is a common cause of neonatal and infantile intestinal obstruction which is due to absence of ganglion cells with presence of hypertrophied nerve bundles along a variable portion of distal intestine. The incidence of HD is 1 in 5000 children with sex ratio in classic pattern male female is 4:1. From the beginning period of its description the diagnosis of Hirschsprung's disease is a challenging matter to pediatrician and pediatric surgeons. Complete history taking, careful clinical examination, radiological investigation and anorectal manometry may be sufficient in many cases to suggest Hirschsprung's disease but the unique criteria for diagnosis of Hirschsprung's disease is the histopathological demonstration of the complete absence of ganglion cells with presence of hypertrophied nerve fibers in the distal bowel. Historically, rectal biopsy is the gold standard for diagnosis of Hirschsprung's disease. Upto this, there are 3 ways of rectal biopsy 1. Full-thickness rectal biopsy, 2. Rectal suction biopsy and 3. Punch rectal biopsy.

Swenson et al<sup>1</sup> first described the method of full-thickness rectal biopsy that requires admission into hospital, general anesthesia and suturing of biopsy site. It may result in bleeding, sepsis, inflammation and scarring which may interrupt subsequent pull through operation.

Dobbin et al<sup>2</sup> and Noblett et al<sup>3</sup> introduced rectal suction biopsy for obtaining rectal mucosa and submucosa for histopathological diagnosis. It is easy to perform without anesthesia but it needs a special instrument (Great Ormond street flexible rectal suction biopsy instrument) and there may be some notifiable complication's like bleeding, perforation etc.<sup>4</sup>

Punch rectal biopsy, this is another method, first described in 1972 by Shanding et al<sup>5</sup>. It is also simple, safe, no need of anesthesia, done on bedside as a day case procedure with a simple biopsy forcep, or nasal cutting forcep or laryngeal biopsy forcep Hirose et al<sup>6</sup> with the help of proctoscope, nasal speculum Schmitt et al<sup>7</sup> or hand made biopsy tube (plastic centrifuge tube).

Both (suction and punch biopsy) are easy and safe but advantages of punch rectal biopsy over full-thickness rectal biopsy is noted. To the best of our knowledge primary work on full-thickness, suction and punch rectal biopsy have been performed in

different institutions and hospitals of the world but there is few comparative study have been performed. So, we are interested to perform a comparative study between punch and full-thickness rectal biopsy to see further and find out a low cost, less time consuming, easily performed method of rectal biopsy with accurate histopathology results and less complications.

## Materials and Methods

This was a cross-sectional, prospective, comparative study. Total 50 patients of suspected Hirschsprung's disease, age ranging from 1 day to 12 months were included in this stud, after fulfillment of inclusion criteria from November 2010 to March 2012 at Bangladesh institute of child health (BICH) and Dhaka shishu (children) hospital, Dhaka. Patients with neonatal intestinal obstruction that needed immediate surgical exploration and patient's parents who were unwilling to subject their child to undergo the biopsy procedure were excluded from the study.

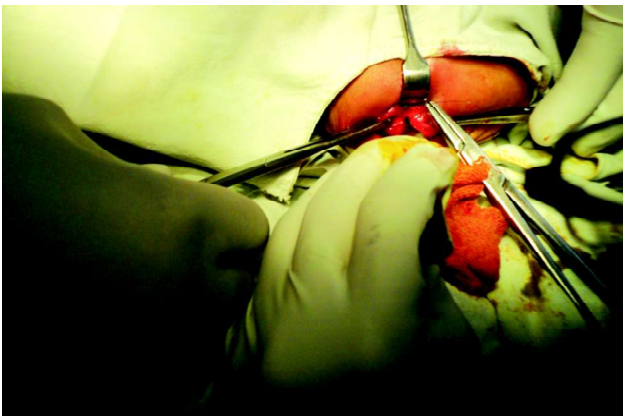
Group A: Punch rectal biopsy was done n=25 and Group B: Full-thickness rectal biopsy was done n=25. After biopsy, all the specimens of 50 patients were evaluated by histopathological examination at pathology department of DSH. Specimen was collected from one site of rectum from each patient and serial sections (50-60) were done from all the samples and were stained with H & E. Two patients who underwent punch rectal biopsy but tissue was inadequate, again full-thickness rectal biopsy was performed before definitive procedure. Remaining 23 patients of punch rectal biopsy, 17 patients were diagnosed as having HD and 6 patients were diagnosed as having no HD, again full-thickness rectal biopsy were done of this 6 patients prior to definitive procedure.

Punch rectal biopsy: We used Takahashi biopsy forcep whose tip was cup likes with sharp cutting edge in both lips measuring 3mm x 3mm x 5mm and a hand made biopsy tube made from plastic centrifuge tube whose length was 6-8 cm, diameter 8 mm, side hole diameter was 8 mm to perform it. The rectum was cleaned with povidone iodine soaked gauze piece before biopsy & the biopsy was performed in the lithotomy position without anesthesia.



**Fig 1** *Hand made biopsy tube*

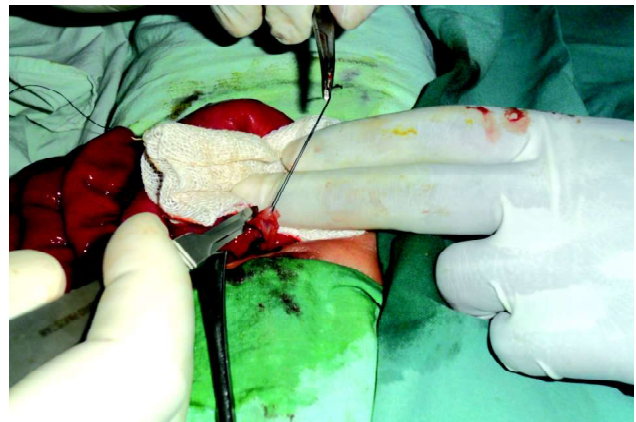
The hand made biopsy tube was inserted into the rectum with the side hole facing the posterior wall of the rectum. Slight lateral pressure was applied so that the side aperture rests firmly against the mucosal surface. The rectal mucosa protruding from the hole can be seen in the biopsy tube using a spot light as a source of light. The protruded mucosa was grasped by the biopsy forcep and pulled off (Fig-1). The edge of the side hole acts as the traction fulcrum, therefore making a small specimen of the mucosa with submucosa easily obtainable. Thereafter, the biopsy tube was turned so that pressure was applied to the biopsy site by the wall of the tube for purpose of the hemostasis. Then we kept a normal saline soaked gauze piece over the biopsy site for 3 minutes. After 3 minutes we removed the gauze piece and observed for bleeding per anus. Subsequently, we also observed features of perforation like abdominal distension, vomiting, temperature & plain x-ray abdomen in erect posture, if needed.



**Fig 2** *Collection of rectal mucosa during biopsy*

Full-thickness rectal biopsy: No formal intestinal preparation was given & done under general anesthesia in lithotomy position. The patients rectum was cleaned with normal saline then povidone iodine soaked gauze piece at the time of procedure. A povidone iodine soaked gauze piece was placed into the proximal rectal vault to prevent stool from entering the surgical field. The buttocks were positioned at the end of the table and supported with a folded towel. The feet were placed together (planter surfaces adjoined) with a cotton roll and both legs were suspended on a metallic screen stand and flexed at the hips. Digital dilatation followed by the placement of two narrow anal retractors, the posterior aspect of the dentate line was identified and a vicryl suture (4/0) round body was placed 1 cm above this and used for traction.

Two additional sutures were placed on the posterior wall of rectum, at 2.5 cm & 3.5 cm proximal to the dentate line. The surgeon's non dominant hand holds the middle suture, using sharp curved scissor, a full-thickness incision was made along the lower



**Fig 3** *Application of normal saline soaked gauze piece for hemostasis*

half of the rectal wall between the dentate line and the midline suture. Once this was done, the scissor can be placed in the presacral space and gently spread. Bleeding may obstruct the view at this point, however, by maintaining traction on the middle suture, the upper half of the rectum can be incised with two smooth cuts of the scissors. Each cut should sweep, approximately half the tissue suspended by the middle suture. The specimen was inspected and if satisfactory, submitted for histologic study.

The rectal defect was closed with interrupted layer with 4/0 vicryl. After suturing, a normal saline soaked gauze piece was kept over biopsy site for 3 minutes, after 3 minutes it was removed and observed for bleeding per anus.

For the validity of the study outcome sensitivity and positive predictive value of punch rectal biopsy was done. For significance of differences Pearson's  $\chi^2$  test, Fisher's exact test and unpaired 't' test were applied, p value <.05 was taken as statistically significant.

Prior to the commencement of the study, the study protocol was approved by the ethical review committee of BICH. The objectives of the study, the procedure, its assistance in making rational approach of the case management and potential complications were explained to the parents/legal guardians in easily understandable local language and then informed written consent was taken.

## Results

Out of 25 cases, 17 were diagnosed as having HD in punch rectal biopsy, but in case of full-thickness rectal biopsy out of 25 cases 20 were diagnosed as having HD.

Sensitivity and positive predictive value of punch rectal biopsy was 80% and 94.11% respectively in relation to full-thickness rectal biopsy. Time was less in punch rectal biopsy (14 minutes) than full-thickness rectal biopsy (20.5 minutes). Cost was less in punch rectal biopsy (1850 taka) than full-thickness rectal biopsy (3500 taka). Post procedure hemorrhage occurred 1 case in punch rectal biopsy whereas 2 cases occurred in full-thickness rectal biopsy. No perforation occurred in punch biopsy but 1 patient developed perforation in full-thickness rectal biopsy.

**Table I**  
*Efficacy of punch biopsy in relation to full-thickness biopsy*

		(Gold standard)		
		Disease +ve	Full-thickness Disease -ve	
(Test outcome) Punch	+ve 17	True +ve 16	False +ve 1	17
	-ve 8	False -ve 4	True -ve 4	8
Total	25	20	5	25

\*Sensitivity 80%, Positive predictive value 94.11%

**Table II**  
*Time, cost & complications of biopsy procedures*

Variables	Group-A	Group-B	p value
Procedure time (min)			
Range	11-17	15-26	0.0001
Mean±SD	13.04±15.32	24±2.88	
Cost (BDT)			
Mean±SD	1850.00±0.00	3500.00±0.00	
Complications			
Hemorrhage	1(4%)	2(8%)	1.000
Perforation	0(0%)	1(4%)	

## Discussion

Diagnosis of HD was definitively based on the histopathological confirmed absence of ganglion cells and the presence of hypertrophied nerve bundles. Thus, rectal biopsy which requires an appropriate and representative tissue sample is mandatory for definitive diagnosis of HD. Full-thickness biopsy, suction biopsy and punch biopsy are currently available as rectal biopsy methods. Swenson et al<sup>1</sup> in 1959 first described the method of full-thickness rectal biopsy, it is the oldest method of biopsy and represents the tissue adequately no doubt but it requires hospital admission, general anesthesia, suturing of biopsy site that may lead to bleeding, sepsis, inflammation, scarring of biopsy site and interruption of subsequent operative procedure. In common sense, it is time consuming and costly one. So, several attempts and works are going through the world to find out a easily performed, most accurate, less time consuming and cheap procedure of biopsy. On this regard, Dobbin et al<sup>2</sup> and Noblett et al<sup>3</sup> introduced rectal suction biopsy, Shandling et al<sup>5</sup> also introduced rectal punch biopsy. Suction and punch biopsy are popular and acceptable due to their low complication rates as reported by Hirose et al<sup>6</sup> in 1993. At least 25 punch biopsy was performed during this study period and the low complication rates have thus far been maintained. Our study population was 50 and among those 32 (64%) were neonates and 18 (36%) were infants. Another study showed 34% neonates conducted by Islam et al<sup>8</sup> at Dhaka Shishu (Children) Hospital, Dhaka. More recent series have noted that HD is diagnosed in the newborn period in more than 90% of patients.<sup>4</sup> Russel et al<sup>9</sup> found one out of 5,000 children (varying 4,400 to 7,000) is born with HD with sex ratio in classic pattern male female 4:1. In our study series, male predominates over female at a ratio of 5:2 but previous study in Dhaka shishu (Children) hospital having the ratio of 3.8:1.<sup>10</sup> Other previous demographic study showed around the same type of results, where male female ratio was found 3:1 by Ikeda et al<sup>11</sup> and 4.32:1 by Goldberg et al.<sup>12</sup> So, the sex ratio of HD patient in our study was well within the expected range of different published series.

In case of punch rectal biopsy out of 25 patients, post biopsy hemorrhage occurred in 1 case (4%) which was insignificant and managed by wet gauze pack pressure and Vit-K intravenous injection, but

have no need of stitch or fresh blood transfusion. This baby was preterm and low-birth weight (1.5 kg). Coagulation profile of this baby was within normal limit. We have not encountered any case of perforation. There was no other complication such as infection or abscess at biopsy site which are shown by Hirose et al<sup>6</sup>. Yet, we feel that this type of complications can readily be avoided by proper evaluation of patients including coagulation profile before punch biopsy and more gentle approach during the procedure. In case of full-thickness rectal biopsy out of 25 cases, 2 (8%) had post biopsy hemorrhage that was also insignificant and managed with wet gauze pack pressure without stitch or fresh blood transfusion. We have encountered 1 case (4%) of perforation that was managed subsequently with colostomy. There was no other complications such as biopsy site infection or abscess. Mean time was less in punch rectal biopsy (14 minutes) than full-thickness rectal biopsy (20.5 minutes). Cost was less in punch rectal biopsy (Tk. 1850) compared to full-thickness rectal biopsy (Tk. 3500). Out of 25 cases of punch rectal biopsy 17 (68%) were proved to be HD positive and 8 (32%) to be HD negative. Two patients had inadequate sample, these 2 cases again underwent full thickness rectal biopsy and found 1 was HD positive and 1 was HD negative. The remaining 23 cases also underwent full-thickness rectal biopsy before definitive procedure to see the effectiveness of punch rectal biopsy in relation to full-thickness rectal biopsy. Hirose et al<sup>6</sup> showed inadequate specimen was 11.2%. This inadequate specimen may be due to inexperience in obtaining tissue in early period of study. Out of 25 cases of full-thickness rectal biopsy 20 (80%) were proved to be HD positive and 5 (20%) were to be HD negative.

## Conclusion

Punch rectal biopsy is a simple, safe, cost effective, less time consuming and effective procedure to diagnose the HD easily and accurately. It does not need hospitalization, pre-operative preparation, operation theater setup or general anesthesia. So, it can be taken as alternative to full-thickness rectal biopsy.

## References

1. Swenson O, Fisher JH, Gherardi GJ. Rectal biopsy in the diagnosis of Hirschsprung's disease. *Surgery* 1959;45:690-95.

2. Dobbins WO, Bill AH. Diagnosis of Hirschsprung's Disease excluded by rectal suction biopsy. *The New England Journal of Medicine* 1965;**272**:990-93.
3. Noblett H. A Rectal suction biopsy tube for use in the diagnosis of Hirschsprung's Disease. *Journal of Pediatric Surgery* 1969;**4**:406-09.
4. Grosfeld JL. HD & related neuromuscular disorder of intestine. Pediatric surgery. 6<sup>th</sup> ed. St Lous: Mosby, Philadelphia, USA: ELSEVIER. p.14-14.
5. Shandling B, Auldish W. Punch biopsy of the rectum for diagnosis of Hirschsprung's Disease. *Journal of Paediatric Surgery* 1972;**7**:546-52.
6. Hirose R, Hirata Y, Yamada, Kawana T, Taguchi T, Suita S. The simple technique of rectal mucosal biopsy for the diagnosis of Hirschsprung's disease. *Journal of Pediatric Surgery* 1993;**28**:342-44.
7. Schmitt. Biopsy of the rectal mucosa in paediatric surgery, technic- diagnostic value. A propos of 193 biopsies. *Chir-pediatric* 1994;**25**:98-101.
8. Islam T. Rectal punch biopsy in Hirschsprung's Disease, MS Thesis, BICH, University of Dhaka, Dhaka. 2004.
9. Russel MB, Russell CA, Niebuhr E. An epidemiological study of Hirschsprung's disease and additional anomalies. *Acta Pediatrics* 1994;**83**: 68-71.
11. Ikeda K, Goto S. Diagnosis and treatment of Hirsch's disease in Japan: An analysis of 1628 patients. *Annals of Surgery* 1984;**199**:400-05.
12. Goldberg EL. An epidemiological study of Hirschsprung's disease. *International Journal of Epidemiology* 1984;**13**:479-85.