Original Article

Histopathological Comparison between Punch and Full Thickness Rectal Biopsy in Diagnosis of Hirschsprung's Disease

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Abstract

Background: *The diagnosis of Hirschsprung's disease (HD) is dependent on the histological study of rectal ganglion cells. Open rectal biopsy is the mainstay that requires general anesthesia (GA) and carries risk of many complications. Rectal punch biopsy has gained wide acceptance and has become the choice as it is simple, safe, having no need of anesthesia, done bedside with a simple biopsy forcep with high degree of accuracy and virtual absence of any complications.* ***Objective:*** *To find out the efficacy of rectal punch biopsy in the diagnosis of Hirschsprung's disease.* ***Materials and Methods:*** *A cross sectional descriptive observational study was carried out with the histological findings of 60 rectal punch biopsies with corresponding 60 full thickness rectal biopsies from 60 suspected HD patients from March 2018 to February 2020. Sections made from the paraffin blocks were stained with H&E and were examined for the presence or absence of ganglion cells and hypertrophic nerve fibers in the submucosa. Then the relations were studied. These findings were compared with full thickness rectal biopsy.* ***Result:*** *There were 40(66.7%) cases of male and 20(33.3%) cases of female including 26 (43.3%) cases of infants. Out of 60 cases, 10 cases (seven inadequate and three suspicious) of punch biopsy were not compared with full thickness rectal biopsy. Out of 50 cases, 38(63.3%) cases were HD in punch biopsy; among these 36(92.3%) cases were HD and 2(18.2%) cases were Non-HD in full thickness rectal tissue biopsy. The accuracy of punch biopsy was measured by sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and efficiency of the test which were 92.3%, 81.8%, 94.7%, 75.0% and 90.0% respectively.* ***Conclusion:*** *The rectal punch biopsy is simple, safe, no need of anesthesia and can be done with a simple biopsy forcep with high degree of accuracy.*

Introduction

Hirschsprung disease (HD) is a developmental disorder of the enteric nervous system characterized by absence of ganglion cells in the myenteric and submucosal plexuses and presence of hypertrophied nerve terminal along variable portion of the distal intestine.[1] The most accepted theory of the cause of Hirschsprung's disease is that there is a defect in the craniocaudal migration of neuroblasts originating from the neural crest that occurs during the first 12 weeks of fetal development. Defects in the differentiation of neuroblasts into ganglion cells and accelerated ganglion cell destruction within the intestine may also contribute to the disorder.[2] Hirschsprung disease occurs in about one in 5000 births.[3] Full thickness rectal biopsy is the gold standard for the diagnosis of Hirschsprung's disease.

Swenson, Fisher and Gherardi (1959) first described the method of full thickness rectal biopsy that includes the superficial submucosal plexus (Meissner plexus), the deep submucosal plexus (Henle plexus) and the myenteric plexus (Auerbach plexus). The advantage of this procedure is that adequate tissue is available for histopathology and other neurocristopathies (i.e Intestinal neuronal dysplasia and hypoganglionosis) can be diagnosed but the disadvantage of this method is that need for general anesthesia and carry risk of postoperative many complication.[4]

Ideally biopsy should be performed at a facility that can provide complete patient care with maximum diagnostic accuracy. The

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type of biopsy performed must be tailored to the lesion so that adequate tissue is obtained without prematurely removing important structures or deforming the patient. In an attempt to provide an alternative of this, rectal punch biopsy is an effective technique.[5]

Rectal punch biopsy first described in 1961 by Shandling and Auldist.[6] It is simple, safe, no need of anesthesia or suturing, done on bedside with a simple biopsy forceps with avoiding many complications.[7] In punch biopsy only rectal mucosa and submucosa is taken 2-3 cm above the dentate line on the posterior wall of the rectum. Diagnostic accuracy of the biopsy specimens depends on the adequacy of biopsy, number of the sections studied, levels at which they are obtained, a skilled pathological team including the technician. When all these criteria are met, diagnostic accuracy as high as 99.78%.[8] Hence, rectal punch biopsy becomes the initial and definitive modality used to evaluate the patient with constipation. This study was carried out to find out the efficacy of rectal punch biopsy in the diagnosis of Hirschsprung's disease.

Materials and Methods

This descriptive cross-sectional study was carried out from March 2018 to February 2020, in Mymensingh Medical College, Mymensingh, Bangladesh. All specimens of rectal punch biopsy from children suspected to be having HD were studied. The specimens were received in 10% formalin solution and processed for paraffin embedding. Multiple serial sections were cut at 3–5 µm thickness. About 4–6 sections per slide were mounted and slide was made and stained with H&E then examined under light microscope.

On microscopic examination, biopsy specimens were classi-fied as:

(A) Ganglionic (non-HD): clearly definable characteristic clusters of ganglion cells could easily be discerned in the sub-mucosa just beneath the mucosa.
(B) Aganglionic (HD): when ganglion cells were absent. On an average 10 sections were examined before a comment being made as absence of ganglion cells.
(C) Inadequate: biopsies were reported inadequate when:
(i) No or very minimal sub-mucosa is present;
(ii) Sub-mucosa is occupied by a lymphoid follicle;
(iii) Specimen is decomposed;
(iv) Specimen reveals stratified squamous epithelium (biopsy from too low position).
(D) Suspicious: ganglion cell like structure seen but report could not be conclusive.

H&E -stained sections were examined for the presence or absence of ganglion cells and hypertrophic nerve fibers in the submucosa then the relations were studied and compared with full thickness rectal tissue biopsy.

Statistical analyses of the result were obtained by using window based computer software devised with Statistical packages for Social Science (SPSS-23). The association between the punch and full thickness biopsy procedure was evaluated with Chi-Square (χ2) test. P value <0.05 was considered as statisti-cally significant.

Results

A total 60 rectal punch biopsy specimens were studied and compared with corresponding 60 full thickness rectal biopsy, specifically for the evaluation of HD. Analysis of 60 rectal punch biopsies revealed 38 (63.3%) were aganglionic (HD), 12 (20.0%) were ganglionic (Non-HD) and 7 (11.7%) specimens were inadequate and 3(5.0%) specimens were suspicious for proper histopathological diagnosis. Age at the diagnosis ranged from 1 day to 10 years. Among them 26 (43.3%) were infant most common age group. Among 60 patients, 40 were males and 20 were females [Table I].

Table I: Distribution of the patients according to age group and sex (n=60)

Age (Months)	Frequency	Sex	
		Male	Female
≤1	14 (23.3)	7 (17.5)	7(35.0)
1-12	26 (43.3)	18 (45.0)	8 (40.0)
>12	20 (33.3)	15 (37.5)	5(25.0)
Total	**60 (100.0)**	**40 (100.0)**	**20(100.0)**

Figure within parenthesis indicates percentage.

Out of 60 cases, 10 cases (seven inadequate and three suspicious) of punch biopsy were not compared with full thickness rectal biopsy. Out of 50 cases, 38 cases were HD in punch biopsy among which 36 cases were HD and 2 cases were Non-HD in full thickness rectal tissue biopsy. 12 cases were Non-HD in punch biopsy among which 9 cases were Non-HD and 3 cases were HD in full thickness rectal tissue [Table II].

Table II: Distribution of the patients according to punch biopsy by full thickness biopsy (n=50)

Punch biopsy (n=50)	Full thickness biopsy		p value*
	HD	Non-HD	
HD (n=38)	36 (92.3)	2 (18.2)	<0.001
Non-HD (n=12)	3 (7.7)	9 (81.8)	
Total (n=50)	**39 (100.0)**	**11 (100.0)**	

*Chi-square test was done to measure the level of significance. Figure within parenthesis indicates percentage.

Table III: Validity test

Variables	Value	95% CI	
Sensitivity	92.3%	84.3	- 96.4
Specificity	81.8%	53.4	- 96.2
PPV	94.7%	86.5	- 98.9
NPV	75.0%	49.0	- 88.2
Accuracy	90.0%	77.5	- 96.3

CI = Confidence Interval, PPV = Positive Predictive Value, NPV = Negative Predictive Value

In H&E stain, out of 50 punch biopsy cases 39(78.0%) cases show absence of ganglion cells (GC) and 11(22.0%) cases show presence of ganglion cell (GC). And 33(66.0%) cases show presence of hypertrophic nerves (HN) and 17(34.0%) cases show absence of hypertrophic nerves (HN) [Table IV].

Table IV: Distribution of the patients according to identification of ganglion cells (GC) and hypertrophic nerves (HN) with H&E stain in punch biopsy (n=50)

Punch biopsy	H&E stain	
(n=50)	Frequency	Percent
GC		
• No ganglion cells seen	39	78.0
• Ganglion cell seen	11	22.0
HN		
• Present	33	66.0
• Absent	17	34.0

Discussion

Swenson established rectal biopsy for the histological diagnosis of HD, which was supported in the follow up studies with a 98% diagnostic accuracy.[9] Since then, rectal biopsy was done frequently to diagnose HD definitively as well as to differentiate HD from other causes of constipation. However, a full thickness rectal biopsy was needed to be done after a pre-operative preparation, GA and occasional hospitalization for rectal bleeding.

Gherhardi showed identical aganglionosis in the submucosal and myenteric plexuses.[10] Smith demonstrated sequential maturation of myenteric plexus in a cranial caudal direction and pointed the importance of degree of maturation as well as presence or absence of ganglionic cells in the diagnosis of HD.[11] Aldridge and Campbell confirmed the presence of hypoganglionic zone within 1–2 cm of anal verge and demonstrated that the density of ganglion cells in the submucosal plexus was sufficient above this level.[12] All these works contributed to develop an ideal rectal biopsy procedure for more easier and accurate diagnosis of HD.

Bodian showed that full thickness biopsy was not necessary for the diagnosis of HD and histological examination of submucosal plexus only sufficient by using punch biopsy forceps.[13]

Hirose et al., in their study explained the rectal punch biopsy procedure for diagnosis of HD, the tissue specimen is taken after grasping the posterior rectal wall by biopsy forceps, it is easy to obtain appropriate specimen.[14] A biopsy forceps is used, it doesn't require a wide operative field. The identification of ganglionic cells and find out the presence or absence of hypertrophic nerves in Auerbach's plexus is studied. Hirose et al,

also described the safety of rectal mucosal punch biopsies where study shows no major complications. The present study also revealed the safety, efficacy and versatility of punch biopsy without major complication and 4% of inappropriate sample rates.

Age ranges from 1 day to 10 years with infant 1-12 months most common age group 26 (43.3%), a mean age of (16.72 ± 22.33) months. Yoshimaru, at al. study showed age ranging was 1 day to 13 years.[19] Henna et al., study shows mean age was 1.9 years.[15] Haricharan and Geogeson, study shows mean age was 4.4 years.16 Pease et al., study age ranges was 0 to 12 years.[13] This is cause of cultural differences, neglect of initial symptoms, poverty and prolonged treatment with enema and laxatives. Age distribution in this study is more or less consistent with other studies.
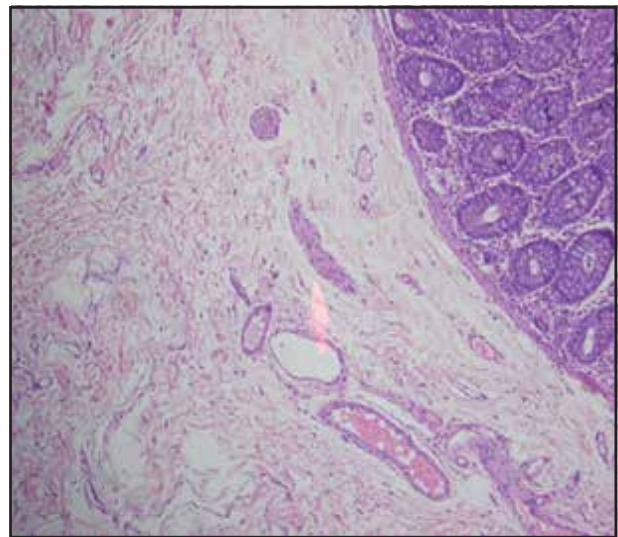
Figure 1: Photomicrograph shows bundle of hypertrophic nerves in submucosal layer. (H&E stain 400x)
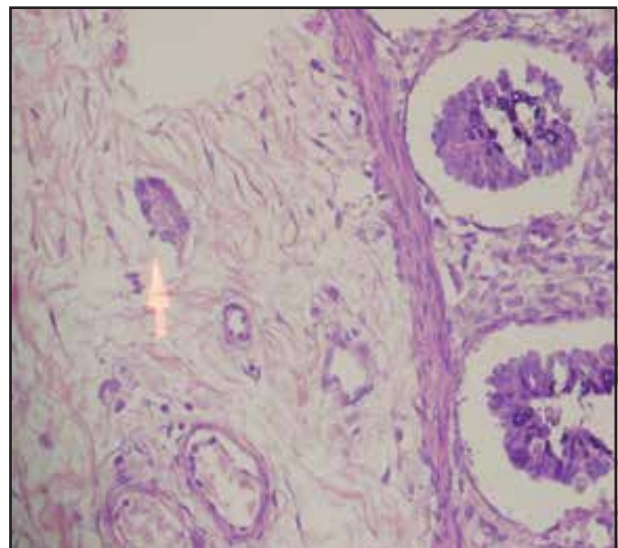
Figure 2: Photomicrograph shows ganglion cells in submucosal layer. (H&E stain 400x)

Male: Female ratio was 2:1 in this study. Henna et al., study shows M:F ratio was 4:1.[15] Muise and Cowls, studies showed male predominance.[17] Male predominance in the sex ratio in the present study were also consistent with other studies. Punch biopsy obtaining tissue in this study was adequate in 88.3%, inadequate 11.7%. In Hirose et al., study inadequate was 11.2% which is more in older children due to difficult in obtaining an accurate biopsy specimen, because of morphological change such as an increase in the thickness of the rectal wall, mucosal oedema and fibrous tissue; who received chronic enema. This is consistent with my study.

In this study, the accuracy of punch biopsy were measured by sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and efficiency of the test. Sensitivity, specificity, PPV, NPV and efficiency of the test were 92.3%, 81.8%, 94.7%, 75.0% and 90.0% respectively. Sensitivity, specificity, PPV, NPV and efficiency of Towhidul, study were 100%, 92.85%, 96.9%, 100% and 97.8% respectively.[18] This is more or less consistent with this study.

Conclusion

Considering simplicity, feasibility, absence of complication and high accuracy of rectal punch biopsy in this study, it may be the procedure of choice in the tissue diagnosis of Hirschsprung's disease.

Acknowledgement

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