Original Article

Pathological Review of Modified Rectal Biopsy for Diagnosis of Hirschsprung's Disease.

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Abstract

Background: The diagnosis of Hirschsprung's disease (HD) is based on histological confirmation of the absence of normal ganglion cells in the colonic wall utilising full-thickness rectal biopsies (FRB) or suction rectal mucosal biopsies. The attendant risks in the former and cost of consumables in the later resulted in the development of a modified rectal biopsy using instruments (MRB). Reports of how this method compares to the gold standard differ in the literature. This study evaluated the adequacy of modified rectal mucosal biopsy histology in the definitive diagnosis of Hirschsprung's disease compared to traditional biopsy techniques. **Method:** A histological review of 438 MRBs, and 119 Pull-through specimens and FRBs performed over an 18-year period in a single institution. Specimens were formalin fixed, paraffin processed and stained with hematoxylin & eosin. A developed set of criteria was used to assess diagnostic histological accuracy for the MRBs. Data analysis was done. **Results:** 321 males and 117 females with median age of 10 months were studied. 84.2% of which were diagnosed to be HD upon review of their MRBs; MRB inadequacy in 9.5%, and 6.2% had other diagnosis. Accuracy analysis was carried out on 107 patients who had both MRB and either definitive Pull-through or FRB. Sensitivity and specificity of 100% and 80% respectively, and positive and negative predictive values of 99% and 100% were derived. **Conclusion:** A single MRB specimen obtained by instrument can effectively diagnose Hirschsprung's disease but requires interpretation by experienced pathologists utilising standardized reporting formats guided by best practices. This will positively impact patient management.

Keywords: Hirschsprung's disease, Modified Rectal Biopsy, Sensitivity and Specificity

INTRODUCTION

Hirschsprung's disease (HD) is a congenital anomaly characterised by the absence of parasympathetic ganglia in the bowel resulting in functional constipation and its sequelae. Its presentation was first described by a Danish pediatrician, Harald Hirschsprung in 1886. The absence of ganglia in the bowel was postulated by Tittel in 1901 and demonstrated by Alberto Dalla Valle in 1920. [1] Development in the diagnosis of HD was made by Swenson in his groundbreaking work expounding the full-thickness rectal biopsy (FRB). Helen Noblett in 1969 developed a tube to perform a suction biopsy of the rectal wall for the diagnosis of HD. These have remained the preferred method to

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demonstrate the absence of ganglion cells in both parasympathetic plexuses.

Rectal biopsy for diagnosis of HD traditionally relied on FRB involving the whole bowel wall. Improved knowledge of diagnostic methods has led to the successful adoption of the use of rectal suction biopsy (RSB). Instrument biopsy with varying thicknesses of bowel wall has been explored with some successes.[2-4] An adequate rectal sample for pathological examination is essential and when this is provided, the pathologist needs to be conversant with the detection of ganglion cells in a submucosal level specimen.

The adequacy of the MRB, a grasp/instrument biopsy compared to full thickness biopsy (FRB) or RSB has been a topic of discussion for decades. [2] [3] [5] The use of RSB and MRB are gradually replacing FRB and the identification of a single normal ganglion cell body rules out the diagnosis of HD.[2,3,5-7]

Also, routine histopathological reporting can be augmented by enzyme histochemistry or immunohistochemistry targeting acetylcholinesterase and/or calretinin.[3,6] This has helped to achieve over 90% accuracy in the diagnosis of HD particularly when repeated biopsies or multiple samplings have been done. [2,3,7] However, facilities and resources for enzyme histochemistry and immunohistochemistry are expensive and are not readily available in sub-Saharan Africa.

Early diagnosis and appropriate treatment give acceptable quality of life in HD.[4] The sequelae of HD include neonatal intestinal obstruction, chronic constipation, enterocolitis associated with high mortality, and failure to thrive. This is more so when associated with major anomalies or disorders in syndromic presentations. [8] Our objective in this study is to compare the adequacy of MRB using instruments in the identification of ganglion cells in hematoxylin and eosin-stained tissues with traditional methods.

PATIENTS AND METHODS

This was a consecutive histopathological analysis of all patients less than 18years of age with clinical diagnosis of HD, who had MRB, with or without definitive Pull-through surgery (Transanal Endorectal Pull-Through) during the study period of January 2004 to December 2021 (18 years), in a tertiary health center in Zaria, Nigeria.

Relevant information was retrieved from patients' case files, procedure registers, and pathology registers. All initial histopathological diagnoses were reviewed using a set of modified criteria (Table 1) developed by the investigators to assess the adequacy of specimens submitted for analysis and diagnosis of HD in order to answer our research questions of whether the samples obtained from the modified rectal biopsy are adequate for histological analysis and accurate in the diagnosis of HD.

The initial diagnosis from MRBs were compared with the new diagnosis obtained using modified criteria for specificity and accuracy. The results were then compared with the diagnosis from FRBs which is the gold standard. Patients with diagnosis of HD using the partial thickness biopsy without definitive pull through surgery and, histologically crushed tissue were excluded from accuracy analysis. Inadequate cases were reviewed using the modified set criteria.

The sensitivity, specificity and accuracy of modified rectal biopsy in diagnosis of Hirschsprung's disease were calculated using standard formulae:

- Sensitivity=true positive/ (true positive+ false negative)
- Specificity= true negative/ (true negative+ false positive)
- Positive predictive value= true positive/ (true positive+ false positive)
- Negative predictive value = true negative/ (true negative +false negative)

These are presented in text. Categorical data were presented in values, and percentages. Histological micrographs of findings are presented and discussed.

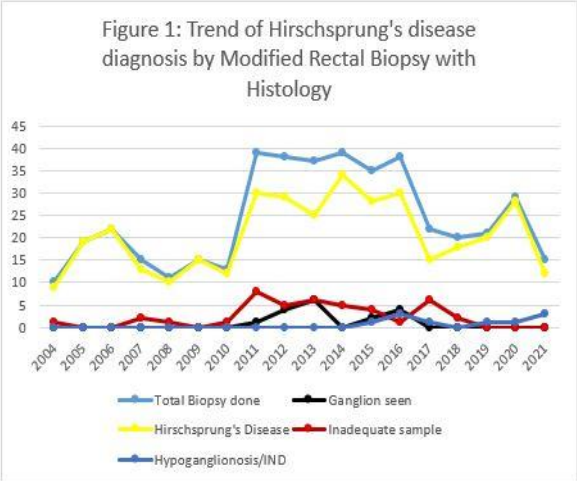
RESULTS

Four hundred and thirty-eight patients with clinical diagnosis of HD who had MRBs sent for histopathological analysis were studied. There were 321 males and 117 females (M: F= 3:1) with an age range of 3 days to 17years (median=10months). The trend of the number of biopsies over the period is shown in figure 1. The relationship of age with histological findings are presented in the table.

Study Adequacy Assessment Criteria modified from published literature [7] [9] [10]

Adequate and diagnostic	Inadequate and non-diagnostic
At least a 3mm wide mucosa biopsy	Less than 3mm wide
Rectal mucosa and submucosa present	Only rectal mucosa, no submucosa present
Submucosa is greater or equal to a quarter of the histological section cut perpendicular to the mucosa	Tissue crushing affecting more than one sixth of the histological section
Neurovascular structures present	Anal mucosa
No ganglion cells seen in serial histological sections	Inflamed granulation tissue with fibrosis of the submucosa

Histological review carried out on stained modified rectal biopsy sections showed HD (no ganglion cells seen) in 369 (84.2%), No HD (normal ganglion cells seen) in 17 (3.9%), rectal mucosa only in 22 (5.0%),



anal mucosa only in 12 (2.7%), extensive crushed tissue in 8 (1.8%), Intestinal Neuronal Dysplasia (IND) in 6 (1.4%), and Hypoganglionosis in 4 (0.9%). Cross-tabular comparison to initial diagnoses is presented in the table. Fifty-six (12.8%) biopsies deemed inadequate following initial evaluation either had repeat modified rectal biopsies or full-thickness rectal biopsies. The repeat modified rectal biopsies were included in the histological review and these findings documented as Review diagnoses in the table.

Of the ten cases of Hypoganglionosis and IND, only in one case of IND was its diagnosis made by the examination of both MRB and resection (Transanal Endorectal Pull-through) specimens and this was included in the accuracy analyses. Inadequate specimens (42, 9.5%) which included rectal mucosa only, anal mucosa only, and extensive crushed tissue fulfilled the criteria for inadequacy and were excluded from the accuracy analyses. 26 (5.9%) patients had multiple biopsies to enhance diagnosis. In one patient who had two samples taken at different levels, one showed aganglionosis while the other showed ganglion cells present. In the remaining 25 patients' samples, the multiple biopsies had similar findings.

Among 35 biopsies initially reported to be "Not HD" (normal ganglion cells present), 12 (34.3%) were without ganglion cells (HD), 3 (8.6%) showed Hypoganglionosis and 3 (8.6%) had IND. The discordance rates were 51.4% in "Not HD" (normal ganglion cells present), 0.6% in HD (absent ganglion), and 26.0% inadequate for diagnosis (all inadequate biopsies). Of the 53 biopsies initially reported to be 'Inadequate', 10 were reviewed to be 'Adequate' by the study criteria and reported as HD.

There were 119 patients who had diagnostic modified rectal biopsies and had subsequent full-thickness diagnostic biopsies (14) or definitive (Pull-through) surgeries (105); 107 of which provided avenue for accuracy analyses utilizing the gold standard. The table shows the results for true positive, true negative, false positive, and false negative. Accuracy analyses of the Modified rectal biopsy in comparison to Full-

Table 1: Pathological Review Findings in the Diagnosis of Hirschsprung's Disease in ABUTH, Zaria

	Reviewed Histological Diagnoses							
Age groups	**HD**	**Normal Ganglion cells seen**	**Hypoganglionosis**	**IND**	**Anal mucosa**	**Rectal Mucosa only***	**Crushed tissue**	**Total**
<1yr	210	11	2	3	5	13	3	**247**
>1yr	159	6	2	3	7	9	5	**191**
Total	**369**	**17**	**4**	**6**	**12**	**22**	**8**	**438**
<5yrs	315	14	3	3	9	18	6	**368**
>5yrs	54	3	1	3	3	4	2	**70**
Total	**369**	**17**	**4**	**6**	**12**	**22**	**8**	**438**
Initial Histological Diagnoses								
HD	347	0	0	1	0	1	0	**349**
Inadequate Specimen	10	0	1	2	12	21	8	**53**
Ganglion seen	12	17	3	3	0	0	0	**35**
Anal mucosa	0	0	0	0	1	0	0	**1**
Total	**369**	**17**	**4**	**6**	**12**	**22**	**8**	**438**
Specimens available								
Biopsy & Pull-through	102	4	0	1	4	7	1	**119**
Biopsy only	267	13	4	5	8	15	7	**320**
Total	**369**	**17**	**4**	**6**	**12**	**22**	**8**	**438**

Accuracy analyses	**TP**	**FP**	**TN**	**FN**	
	102	1	0	4	107
Positive Predictive value	99%	**Sensitivity**		100%	
Negative Predictive value	100%	**Specificity**		80%	

***Two of these patients had biopsies showing mucosa with underlying fibrosis. HD – Hirschsprung's disease, IND – Intestinal Neuronal Dysplasia type b, TP – True Positive, FP – False Positive, TN – True Negative, FN – False Negative**

assessment (gold standard) revealed a

Sensitivity of 100%, Specificity of 80%, Positive Predictive Value of 99%, and Negative Predictive Value of 100%. Photomicrographs show some histology findings in figure 2.

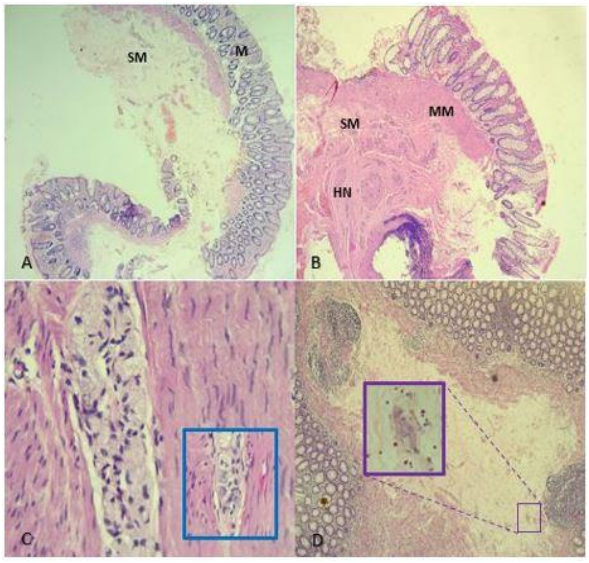


Figure 2: A&B – H&E stained low power view of sections of modified rectal biopsies from 2 patients diagnostic of HD with no ganglion cells identified in the submucosa; C – High power view of intermuscular plexus devoid of ganglion cell bodies in HD. Blue inset shows normal ganglion cells in the intermuscular plexus of a colon segment; D – Low power view of rectal mucosa and submucosa from a suspected case of HD showing hypertrophied muscularis mucosa and groups of immature ganglion cells in the submucosa typical of IND. Purple inset shows a high power magnification of one such group. M: mucosa, MM: thickened muscularis mucosae, SM: submucosa, HN: hypertrophied nerve bundles

DISCUSSION

There has been a progressive increase in the presentation of children with constipation dated back to soon after birth. There is thus a need to adequately evaluate the patients to determine the diagnosis. In our setting where enzyme histochemistry and relevant immunohistochemistry panels are not readily available, the use of haematoxylin and eosin stained rectal biopsy remains the standard tool for definitive diagnosis of Hirschsprung's disease.[4,7]

This histological examination of a full thickness rectal biopsy by pathologist with the findings of the parasympathetic aganglionosis in the enteric nervous system and hypertrophied nerve trunks are the hall mark for the diagnosis of Hirschsprung's disease.[7] This method was advocated by Swenson and Bill in 1948 as the gold standard for diagnosis of HD, currently, the use of suction biopsy for diagnosis of HD since introduction by Noblett in 1969, has been well established; [2,3] and has now overtaken the need for a full thickness biopsy with its attendant risks and the need for general anesthesia. The use of the suction biopsy has remarkably reduced delays in diagnosis and the timing for definitive surgery.[8] In the setting of limited facilities and resources that makes full thickness and/or suction rectal biopsy not readily available, a modified rectal biopsy using instruments is common practice.[4]

The histological evaluation of suction and modified rectal biopsies are similar being that both yield partial thickness biopsies. However, the pathologist needs to be familiar with the histological features and interpretation of modified rectal biopsy tissue.

Sample Adequacy

The routine biopsy in this study utilized a 'single sample', as only 5.9% of our patients had multiple biopsies which has been reported to not only increase the specimen available for histological review but has been shown to increase the adequacy of specimen for evaluation.[7] However, one out of the 26 that had multiple biopsies had a discordance, a possibility is that the patient had an ultrashort segment disease. It therefore appears that a single representative sample may be adequate for the diagnosis of HD.

A sample that includes the mucosa and submucosa is considered adequate when 50% and greater of the total volume is submucosa.[7] Although sample volume was not used in this study, the specimens greater than or equal to 3mm in length and contained submucosa of up to a quarter of a transverse section were found to be adequate for the diagnosis of HD. As adequate sampling must include the submucosa,[6,7] insufficient or inappropriate sampling resulted in inconclusive histological review as was seen in about 12 percent of our patients and necessitated repeat biopsies in one-eighth of our patients. Inadequate biopsy (excluding crushed specimen) using instrument method was 7.7%, this is similar to when RSB is used.[3]

The grasp method of rectal biopsy uses instrument that may crush samples during the procedure as was seen in this study. Other reasons for inadequate biopsy ranged from anal mucosa, rectal mucosa only, insufficient submucosal portions and in redo biopsies fibrous tissue may be biopsied.

Pathological Review and Diagnosis

Earlier studies have reported an unexplained relationship between age of patients and adequacy of biopsy in grasp biopsy.[7,11] We observe more inadequate biopsies in children below 5years compared to those above 5years (33 to 9 patients), this is similar to findings by Brady AC, et al. but contrary to findings by

Eleanor D. Muise & colleagues where ages < 1year had more inadequate biopsies.[7,11] It is essential to note that majority of our patients were under 5years and the ratio of inadequacy between the two groups was higher in those above five years. Furthermore, the inadequate biopsies in the under one year compared to those over one year was similar but the ratio of inadequacy is higher in children above one year. It appears that inadequate biopsies using grasp biopsy are more likely in patients that had biopsy later due to late presentation for diagnosis[4,8] or due to the thickness of the rectal mucosa in older age groups.

The skill level of the pathologist in the examination of suction or modified rectal biopsy specimen is very essential in the diagnosis of HD.[4] The adequacy of the specimen from rectal biopsy for the diagnosis of HD must be assessed before conclusion of histological findings. Inadequate samples that exclude or have limited submucosal component should be returned with advice for repeat biopsy stating what caused the specimen to be inadequate.

Pathologist that are developing skills may miss the diagnosis of HD.[9] In our study, there were discordances in the initial report and the review of the patients' slides. The initial report was able to identify and diagnose HD, however, misinterpretation of histological findings was observed in about half of those that ganglion cells were thought to be present but had HD; and some cases of hypoganglionosis or IND and in thirteen patients in which specimen were considered to be inadequate. Histiocytes may be identified as ganglion cells resulting in false reportage of "normal rectal biopsy" in patients with HD. The identification of immature ganglion cells may pose a challenge to the young pathologist in the diagnosis of IND.

It has been recommended that the pathology report should include information that will impact management (adequacy, ganglion cells present or absent, submucosal nerve hypertrophy present or absent, and active enterocolitis present or absent) and considerations which may impact management (results of calretinin immunohistochemistry, other immunohistochemistry, or acetylcholinesterase histochemistry).[10] The content of the reports varied in our study. Information on adequacy was usually given and most reports stated whether ganglion cells were present or absent, some included statements on nerve hypertrophy but most did not comment on presence or absence of enterocolitis except where enterocolitis was marked. Immunohistochemistry panel for diagnosis of HD is not our routine practice and was not part of the review.

Although the adequacy of the specimen was mostly commented upon, reasons for inadequacy was often not stated and those stated, ranged from "rectal mucosa only" to "anal mucosa". A clear explanation is recommended for samples deemed inconclusive or inadequate and these may include insufficient submucosa, squamous or transitional mucosa, or crush artifact. [10] In the study's review, care was taken to give reasons for inadequacy.

The bottom line of the pathology report should convey a diagnosis of the rectal biopsy and should state if the sample was adequate and diagnostic, adequate and non-diagnostic or inadequate stating the reason for inadequacy.[10] The initial reports gave inconsistent diagnoses. This study streamlined the output into uniform diagnoses that impact on patient management.

Some training programs require the pathologist to have viewed up to 100 sections and demonstrated the necessary knowledge and skills to deliver improved diagnosis and reduce the incidence of misdiagnosis.[9] Where there is doubt in sample adequacy or whether ganglion cells are present or not, intra departmental consultations should be carried out with more experienced colleagues. This brings up the need for the adoption of a standard reporting format for the diagnosis and monitoring of HD.[10]

A recent study reported unacceptably low accuracy of modified rectal biopsy/grasp biopsy in a small group of patients and recommended FRB in diagnosis of HD, despite getting only about 61% adequate biopsy for FRB[5] the authors did not however mention the skill level of the pathologist and any further staining of specimen. To determine the accuracy of modified rectal biopsy using instruments in the diagnosis of HD, the biopsy findings was compared with full thickness biopsy in same patients and pull through specimen. The sensitivity and specificity of FRB to SRB approached 100 %,[2,6] this is similar to this study where the modified rectal biopsy compared with the full thickness biopsy has a sensitivity of 100%. The slightly lower specificity may be due to inaccuracy in diagnosis of hypoganglionosis and intestinal neuronal dysplasia type b.

Minor limitations of the MRB noted were in biopsy technique related inadequacies from tissue crushing and non-representativeness, and pathologists' familiarity with interpreting MRBs on serial H&E sections. Thus, a single and adequately sampled MRB will accurately diagnose HD considering both predictive values that approached 100%. We suggest the specific training of surgeons in the performance of MRB in low resource settings; a concerted effort in the training of trainee pathologists and young pathologists in HD diagnosis and reporting; and the adoption of the protocol used in this study in pathology laboratories serving hospital communities that perform the MRB using instruments to aid in the development of practices that would impact positively on patient management.

CONCLUSION

We can conclude that the modified (partial thickness) rectal mucosal biopsy specimen obtained by instrument can effectively diagnose Hirschsprung's disease at levels similar to the suction rectal biopsy and the full thickness biopsy; and requires interpretation by experienced pathologists. Thus, there is a need for

focussed pathologist training and the standardization of reporting formats guided by best practices that will positively impact patient management.

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