

Comparing Rectal Biopsy Using Artery Forceps And Full-Thickness Rectal Biopsy In Diagnosing Hirschsprung's Disease At University Teaching Hospital, Lusaka

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Abstract

Hirschsprung's disease is a congenital disorder characterised by functional constipation whose onset is dependent on the length of the affected bowel and always involves the rectum. Rectal biopsy for histological analysis is the most definitive form of diagnosis. Full-thickness biopsy, which involves full wall laceration under general anaesthesia, continues to be commonly practiced in many African countries, including Zambia. Rectal suction biopsy, which can obtain a superficial-thickness biopsy as deep as the submucosa, has been adopted as the gold standard in the western world, leaving the invasive full-thickness biopsy with its associated risks for inconclusive cases. However, different types of forceps using the grasp and cut method have been shown to be able to obtain superficial-thickness biopsies that are as good or even superior to the rectal suction biopsy. The medium curved artery forceps was proposed to provide a simple, less invasive and hopefully cost-effective method of obtaining superficial rectal biopsies. Thirty-one (31) patients were enrolled in a prospective, single-blind study that was conducted at the University Teaching Hospital in the Department of Surgery, Paediatric Surgical unit from

2018 to 2019. Two biopsy specimens were obtained from each patient in the same sitting using the full-thickness biopsy method and artery forceps and compared the results.

Out of 31 patients, 19 (61.30%) of the full-thickness biopsy specimens were adequate for diagnosis, whereas only 4 (12.9%) of the superficial-thickness biopsy specimens using curved artery forceps were adequate. The biopsy obtained using the curved artery forceps had a high sensitivity (93.3%) and poor specificity (13.3%), and predictive value. Although not all patients required suturing hence saving on consumables, none of the patients developed any complications during or after the procedures.

Based on these results, the medium curved artery forceps is a poor choice for obtaining rectal biopsies. The traditional full-thickness biopsy procedure should be continued. Efforts should be made to acquire the necessary tools to obtain superficial thickness biopsy and reserve full-thickness biopsy for indeterminate cases.

Keywords: *Hirschsprung's disease, full-thickness biopsy, superficial-thickness biopsy, curved artery forceps*

Introduction

Hirschsprung's disease is a congenital intestinal motility disorder characterised by the absence of ganglion cells and neural cell hyperplasia in variable lengths of the distal parts of the intestine [1]. Neonates will present with a history of failure to pass meconium in the first 48 hours of life and others with chronic constipation and enterocolitis later in childhood [2].

The tests available in the diagnostic workup of Hirschsprung's disease include contrast enema, anorectal manometry and rectal biopsy for histological evaluation for the absence of ganglion cells [3]. As the rectum is almost always affected in Hirschsprung's disease, histological evaluation of biopsies obtained from the rectum provides the most definitive means of making a diagnosis of aganglionosis [4][5].

Diagnostic accuracy depends on the adequacy of the specimen, the level at which they were obtained, the number of sections studied and the skill of the pathologist [6]. The methods recorded in the literature include the traditional full-thickness rectal wall biopsy, the rectal suction biopsy and the grasp and cut biopsy. Reports indicate that up to 80 per cent of biopsies performed on constipation patients could be unnecessary, with only 12–17 per cent yielding a positive result of aganglionosis [7]. The full-thickness biopsy is associated with severe complications at a rate of 6.6 per cent [1] [8]. Rectal suction biopsy is associated with complications ranging from 0-15 per cent, with inadequate specimen being most common [8][9]. The rectal suction biopsy has been adopted as the gold standard, while the full-thickness biopsy is reserved for inconclusive cases. Most African countries commonly

perform full-thickness rectal wall biopsy due to none availability of rectal suction devices (figure 1), as demonstrated in a Nigerian survey conducted among 31 paediatric surgeons, which showed that 84 per cent performed full-thickness biopsy [10][9]. Different types of forceps have been successfully used to obtain adequate superficial rectal biopsies for the diagnosis of Hirschsprung's disease [10][11]. The grasp biopsy using a Kervorkian-Younger uterine biopsy forceps (Figure 2) provided adequate specimen in more than 90 per cent of patients in all age groups from 1 to 7 years compared to rectal suction biopsy that provided adequate specimen in 72 per cent of 1 to 3 years and reduced to 50 per cent in those above 3 years. However, subsequent studies have demonstrated that adequate rectal suction biopsies can be obtained even in older age groups for diagnosis [12]. The jumbo biopsy forceps demonstrated an overall success rate of 86 per cent based on specimen adequacy to diagnose Hirschsprung's disease on 156 patients aged 7 weeks to 20 years [13]. Using appropriate forceps, the grasp and cut method appears to be an acceptable substitute for the rectal suction biopsy and, hence, reserve the full-thickness biopsy for indeterminate cases.

Hirschsprung's disease has a global incidence of 1 in 5,000 live births. The cases seen at University Teaching Hospital are referred from all parts of the country, and it is seen more in males than in females. Full-thickness biopsy under general anaesthesia is currently the procedure of choice to diagnose Hirschsprung's disease at the University Teaching Hospital. It makes full-thickness laceration of the colon and requires stitching, and often some

minor bleeding is involved. Aside from the risks associated with anaesthesia, it can give rise to haemorrhage, infection, re-perforation and other complications associated with future surgical manoeuvres in the intestine due to scarring or fibrosis at the biopsy area [1] [13].

With reports of only 17 per cent of biopsies yielding positive results of aganglionosis in constipation patients, less invasive techniques will help reduce the number of children exposed to the risks associated with the invasive full-thickness biopsy [7]. The rectal suction biopsy is safer and can be done at the bedside but require special devices which are not readily available. Grasp and cut biopsy with appropriate forceps can perform as well as a suction rectal biopsy in providing superficial thickness biopsy. Forceps do not require specialised maintenance as a rectal suction device.

Though the University Teaching Hospital does not have rectal suction biopsy kits, the grasp and cut method using medium curved artery forceps to obtain superficial thickness biopsies is proposed in this study.

Materials and Methods

This was a prospective, single-blind comparison study conducted in the Department of Surgery, Paediatric and Neonatal surgical unit (D-Block) at the University Teaching Hospital in Lusaka. A total of 31 patients were recruited into the study after meeting the inclusion criteria. Two biopsies were obtained from each patient under general anaesthesia, the full-thickness biopsy and a superficial thickness biopsy with curved artery forceps.

The patient was placed in the lithotomy position, and sterile gauze soaked with

povidone-iodine was inserted in the rectum to avoid any soiling of the field with stools. The biopsies were obtained on the posterior rectal wall. A chromic catgut 3.0 stay suture was first placed one centimetre proximal to the dentate line to obtain a full-thickness biopsy. Using traction on the first stay suture, the second and third stay sutures were placed at two centimetres and three centimetres proximal to the dentate line. The second stay suture was then elevated, and the rectal mucosa and underlying muscle were cut using sharp scissors. Continuous haemostatic sutures were placed using the third stay suture. With traction still placed on the first suture, a superficial-thickness biopsy was obtained using the curved artery forceps and a blade to cut the rectal mucosa. If any active bleeding was observed, the stay suture was used to place simple sutures; otherwise, the stay sutures were cut and the rectum packed with Vaseline gauze. The samples were preserved in 10 per cent formalin in bottles labelled randomly as A and B and the details of the method used to collect each of the specimens were recorded. A consultant pathologist assigned to analyse the specimens was blinded by not knowing the method used to collect any of the specimens in either bottle. The pathologist was required to report on the following:

- a. adequacy of the specimen determined by the presence of submucosa;
- b. inadequacy of specimen determined by absence of submucosa; and
- c. presence of ganglion cells [11].

The results of the two methods were then compared to determine the effectiveness of the proposed method (superficial thickness using artery forceps). Data entry was done using IBM Statistical

Package for Social Science Software (SPSS) version 23. The Data collected was stored on data spreadsheet sheet in IBM SPSS Statistics Version 23. The data were summarised in frequencies, percentages, cross-tabulation tables and graphical presentations. The Sensitivity, Specificity, Positive and Negative Predictive Values were also determined.

Results

Characteristics of participants

The participants had a mean age of 2.7 years and a mean weight of 12729 grams (Table 2). The majority of the 31 participants were males, 27 (87.1%) (Table 3).

Presence of Ganglion according to sex

Full-thickness biopsy results showed that 16 out of 31 had ganglion cells meaning they had no disease. All 4 females in the study had ganglion cells hence were all normal (Table 4). Superficial-thickness biopsy results showed that 4 out of 31 had ganglion cells with only 3 out of the 4 females having ganglion cells (Table 5).

Full-thickness biopsy Vs Superficial-thickness biopsy for adequacy of biopsy specimen

Full-thickness biopsy specimen results show that 19 (61.3%) specimens were adequate for diagnosis (Table 6). Superficial-thickness biopsy results show that only 4 (12.9%) specimens were adequate for diagnosis.

Full-thickness biopsy Vs Superficial-thickness biopsy for the presence of Ganglion cells

Full-thickness biopsy revealed that 16 (51.6%) of the specimen had ganglion cells present, representing the total specimen found without the disease

(Table 7). Superficial-thickness biopsy results revealed that only 4 (12.9%) had ganglia and thus negative for the disease.

Correlation table

Table 8 shows a significant strong positive correlation between the full-thickness biopsy for adequate diagnosis and the full thickness biopsy with the presence of ganglion (0.63**) and between the full-thickness biopsy for adequate diagnosis and superficial-thickness biopsy adequate for diagnosis (0.49**).

Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value

The test gives a sensitivity of 93.3 per cent. This entails that the test detects 93.3 per cent of patients with the disease (true positives) while 6.7 per cent with the disease go undetected (false negatives) as shown in table 9. The results show a specificity of 0.133. This means that the test correctly identifies 13.3 per cent of patients as test negative (true negatives), but 86.7 per cent of patients without the disease are incorrectly identified as to test positive (false positives). Our test shows a positive predictive value of 51.8 per cent, implying that if a patient tests positive with the superficial-thickness biopsy test, then the probability of them being positive using the gold standard (full-thickness biopsy test) in this study is 51.9 per cent. The superficial-thickness biopsy test shows a negative predictive value of 66.8 per cent, which means that only 66.6 per cent of the negatives are true negatives.

Discussion

The study demonstrated that the mean age at presentation was 2.7 years while the peak age was 5 years, unlike in developed

countries where 90 per cent present as neonates [2]. This delay in presentation could be due to a lack of awareness and preference to seek traditional options before seeking medical advice. In addition, none of the females in the study was diagnosed with the disease based on the full-thickness biopsy results, which seemingly agrees with the literature that the disease occurs more in males than in females, at a ratio of four-to-one [14].

A rectal biopsy specimen is considered adequate if it at least has one-third to one-half of submucosa [15]. However, Hirsch *et al.* stated that even biopsies with a moderate amount of submucosa, or only included mucosa, do not pose a problem for diagnosis because ganglion cells typically adhere to the mucosa as the submucosa is torn away during biopsy [9]. In this study, 61.3 per cent (19) specimens obtained using full-thickness biopsy were reported to have submucosa compared to 12.9 per cent (4) obtained with the proposed method. Muise and Cowles stated that inadequate rectal biopsies with insufficient submucosa occur in up to 26 per cent. However, in this case, the proposed instrument resulted in 87.1 per cent (27) inadequate specimens compared to 38.7 per cent using the full-thickness method. Having inadequate biopsies results in diagnostic delays due to the need for repeat biopsy, and hence more extended hospital stay, increased cost of treatment, prolonged parental anxiety and unnecessary work for the pathologist [1] [13].

Specimen obtained using the full-thickness rectal biopsy showed that 48.4 per cent (15) had no ganglion implying a diagnosis of Hirschsprung's disease, and 51.6 per cent (16) were normal. For the superficial rectal biopsy, 87.1 per cent (27) had no ganglia, while

12.9 per cent (4) were normal. These results imply that specimens obtained by the superficial-thickness rectal biopsy missed 25 per cent (12) of the 16 cases found normal using the full-thickness rectal biopsy procedure. This implies that the proposed method would result in a number of normal patients being wrongly diagnosed with the disease. Furthermore, none of the specimens that were classified as inadequate based on the absence of submucosa demonstrated any ganglion cells, according to Hirsch *et al.* who stated that even biopsies with only mucosa do not pose a problem for diagnosis because ganglion cells typically adhere to the mucosa as the submucosa is torn away during biopsy [9]. Although studies have shown that the level of the performing surgeon does not impact the yield, the adequacy of the rectal biopsy is said to be related to the instruments used and the thickness of the rectal wall [8].

The correlation between full-thickness biopsy with the presence of ganglion and superficial-thickness biopsy adequate for diagnosis and between full-thickness biopsy with the presence of ganglion and a superficial-thickness biopsy with ganglion were both weak at 0.18. The most important relationships, in this case, were very weak and insignificant. This indicates that the superficial-thickness biopsy using artery forceps could not obtain adequate specimens with a ganglion in normal patients for the disease to be ruled out.

The high sensitivity (93.23%) of the proposed test implies that the test is able to detect most of the patients with the disease, but the low specificity (13.33%) implies that it is not able to detect those without the disease adequately.

This would result in high numbers of normal patients diagnosed with the disease.

The calculated positive predictive value of 51.85 per cent and negative predictive value of 66.67 per cent further demonstrate that the proposed instrument would result in more patients being wrongly diagnosed.

The complication rates for full-thickness and rectal suction biopsies have been reported at 6.6 per cent and from 0 to 15 per cent, respectively [8]. None of the patients in the study suffered any complications during or after the procedure. None of the referenced articles reported any complication rates regarding the grasp biopsy method. One of the explanations may be due to the fact that the biopsies are obtained under direct visualisation, unlike rectal suction biopsy and that the nature of the biopsy is more superficial, unlike the full-thickness biopsy. The biopsy sites were sutured or packed with Vaseline gauze in the rectum after the procedure if the bleeding was minimal.

Conclusion

The grasp and cut biopsy of obtaining superficial rectal biopsy using different forceps has been demonstrated to have good results in other centres. This study has failed to show similar results. This study demonstrates that improvisations that appear to work sometimes come to fall short of expectations. This calls for higher authorities to make available the specific tools for such procedures. Against this background, the full-thickness biopsy remains the only plausible procedure for the adequate diagnosis of Hirschsprung's disease at the University Teaching Hospital and Zambia at large.

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Tables and Figures

Table 1:

Inclusion Criteria	Exclusion Criteria
<ul style="list-style-type: none"> • Neonates presenting with delayed passage of meconium for more than 24 hours and abdominal distension. • Infants with constipation and obstipation dating back to the neonatal period or developed afterwards, early in the first few months. • Older children with long-standing history of difficult stool passage and resistant to proper and energetic laxative therapy. • Patients presenting with complications expected to occur with Hirschsprung's disease, namely enterocolitis and colonic obstruction after stabilisation. • Patients whose consent to participate in the study has been obtained from their guardians 	<ul style="list-style-type: none"> • Complicated cases requiring immediate surgical intervention i.e. cases of obstructed Hirschsprung's disease not responding to repeated colonic washouts and cases of perforated viscus, where urgent preparation and exploration is required. • Cases where an evident local cause is encountered in the perianal region, namely cases of anal fissures and cases following anorectal suppuration (perianal abscesses) and cases following operative procedures carried out in this region and readily explaining the presentation

Table 2 : Age and Weight Distribution

Statistics			
		Weight of patient in grams	Age of patient in years
N	Valid	31	31
	Missing	0	0
Mean		12729.03	2.7365
Median		12000.00	2.0000
Mode		2800 ^a	5.00
Std. Deviation		8542.724	2.35576
Range		26000	8.92
Minimum		2000	.08
Maximum		28000	9.00
a. Multiple modes exist. The smallest value is shown			

Average weight was 12.7kg and most patients presented at 5 years of age

Table 3 : Gender Distribution

Gender of Patient		Frequency	Percentage	Valid Percentage	Cumulative Percentage
Valid	Female	4	12.9%	12.9%	12.9%
	Male	27	87.1%	87.1%	100.0%
	Total	31	100.0%	100%	

There were 27 males and 4 females in the study

Table 4 : Cross Tabulation of Gender Vs Full-Thickness Biopsy with Ganglion

Gender of patient * full thickness biopsy with the presence of ganglion Cross tabulation				
Count				
		Full thickness biopsy with presence of ganglion		Total
		No	Yes	
Gender of patient	Female	0	4	4
	Male	15	12	27
Total		15	16	31

Full-thickness biopsy results showed that all the 4 females had ganglion cells and hence did not have Hirschsprung's disease. 15 males were normal while 12 demonstrated no ganglion cells implying they had the disease.

Table 5 : Cross Tabulation of Gender vs Superficial Thickness Biopsy With Ganglion

Gender of patient * Superficial thickness biopsy with ganglion Cross tabulation				
Count				
		Superficial thickness biopsy with presence of ganglion		Total
		No	Yes	
Gender of patient	Female	3	1	4
	Male	24	3	27
Total		27	4	31

Superficial-thickness biopsy results showed that only 1 female was normal and only 3 males were normal while for the rest no ganglion was reported. This implies that 3 females and 24 males had the disease based on this biopsy method using a curved artery forceps.

Table 6 : Full-Thickness Vs Superficial-Thickness for adequate specimen for diagnosis

Responses	Full-thickness biopsy for adequate diagnosis		Superficial-thickness biopsy adequate for adequate diagnosis	
	Frequency	Percentage	Frequency	Percentage
Yes	19	61.3	4	12.9
No	12	38.7	27	87.1
Total	31	100	31	100

Full-thickness biopsy results show that 19 specimens were adequate for histological diagnosis while 12 were not adequate. Superficial-thickness biopsy results show that only 4 specimens were adequate while the rest were inadequate.

Table 7 : Full-thickness vs Superficial-thickness biopsy for presence of ganglion

Responses	Full-thickness biopsy with presence of ganglion		Superficial-thickness biopsy with ganglion	
	Frequency	Percentage	Frequency	Percentage
Yes	16	51.6%	4	12.9%
No	15	48.4%	27	87.1%
Total	31	100%	31	100%

Full-thickness biopsy results showed that 16 had ganglion cells present and the rest did not, while the superficial-thickness biopsy results show that only 4 had ganglion cells present.

Table 8 : Correlation Table**Correlations**

VVVV Variables	full thickness biopsy for adequate diagnosis	full thickness biopsy with presence of ganglion	Superficial thickness biopsy adequate for diagnosis	Superficial thickness biopsy with ganglion	
full thickness biopsy for adequate diagnosis	Pearson Correlation Sig. (2-tailed) N	1 .626** 31	.626** .000 31	.494** .005 31	.494** .005 31
full thickness biopsy with presence of ganglion	Pearson Correlation Sig. (2-tailed) N	.626** .000 31	1 .319 31	.185 .319 31	.185 .319 31
Superficial thickness biopsy adequate for diagnosis	Pearson Correlation Sig. (2-tailed) N	.494** .005 31	.185 .319 31	1 .000 31	1.000** .000 31
Superficial thickness biopsy with ganglion	Pearson Correlation Sig. (2-tailed) N	.494** .005 31	.185 .319 31	1.000** .000 31	1 31

** . Correlation is significant at the 0.01 level (2-tailed).

There is a significant strong positive correlation between the full-thickness biopsy for adequate diagnosis and the full thickness biopsy with presence of ganglion presented by 0.63**. The table also shows that there is a significant positive correlation of 0.49** between the full-thickness biopsy for adequate diagnosis and superficial-thickness biopsy adequate for diagnosis.

Table 9 - Sensitivity, Specificity and Predictive values

	Disease Present	Disease Absent
Test Positive	TRUE POSITIVE (TP) a = 14	FALSE POSITIVE (FP) b = 13
Test negative	FALSE NEGATIVE (FN) c = 1	TRUE NEGATIVE (TN) d = 3
Totals	(a+c) = 15	(b+d) = 16
	Sensitivity $a/(a+c) = 0.933 = 93.33\%$	Specificity $d/(b+d) = 0.1333 = 13.33\%$
	Positive Predictive Value $a/(a + b)$ $= 0.5185 = 51.85\%$	Negative Predictive Value $d/(c + d)$ $= 0.6667 = 66.67\%$



Figure 1: Rectal Suction Biopsy Device [16]

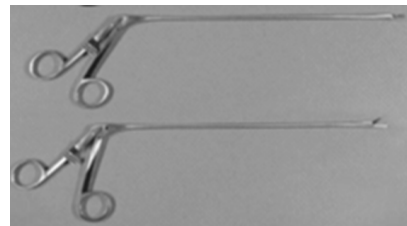


Figure 2: Kervorkian-Younger uterine biopsy forceps (closed [top] and open [bottom]) [11]



Figure 3: Medium curved artery forceps [17]