ORIGINAL ARTICLE

Suction rectal biopsy (SRB) in Hirschsprung's Disease: Is a single macroscopically adequate sample sufficient?

Pavin Kaur BAL[1], Shireen Anne NAH[2], Wan Mohd Ruzaimie WAN MOHAMAD NOOR[3], Mohd Tarmizi MD NOR[3], Srihari SINGARAVEL[2], Wei Sheng TAN[2], Yew Wei TAN[2], Anand SANMUGAM[2]*

[1]Hospital Raja Permaisuri Bainun, Ipoh; [2]Division of Paediatric And Neonatal Surgery, Department of Surgery, Faculty of Medicine, University of Malaya; [3]Department of Surgery, Hospital Raja Permaisuri Zainab II.

Abstract

Hirschsprung's Disease (HD) is a congenital disorder causing severe constipation in infants and children. Suction rectal biopsy (SRB) is the preferred technique for obtaining tissue samples for histopathological evaluation. In low-resource settings like Malaysia, cost-effective diagnostic approaches are necessary, making single sample SRB valuable. This study evaluates the diagnostic accuracy and sufficiency of a single macroscopically adequate sample in suction rectal biopsies for the histopathological confirmation of HD. We conducted a retrospective study of children who underwent suction rectal biopsies for the diagnosis of HD at Hospital Raja Perempuan Zainab II (HRPZII), Kota Bharu, Kelantan. A total of 68 patients were included in the study. The inadequacy rate for bedside SRB was 14%, comparable to current literature. Our study found no statistically significant association between sample inadequacy and gestational age, gender, birth weight, or weight at biopsy. Complication rates were 0%, consistent with literature reports. Calretinin staining, an additional technique, was performed in 23 biopsy episodes, with a 4.3% inadequacy rate, compared to 20% in specimens not subjected to calretinin staining. The cost of SRB almost doubled with each additional sample taken, significant in low-resource environments. In conclusion, single sample SRBs can be adequately diagnostic and cost-effective in low-resource settings, providing valuable insights for healthcare facilities in Malaysia and other developing countries. The use of adjunctive techniques such as calretinin staining may improve diagnostic accuracy while maintaining cost-effectiveness. Further prospective studies with larger sample sizes are needed to validate these findings.

Keywords: Hirschsprung disease, rectal biopsy, suction rectal biopsy, Rbi2, calretinin

INTRODUCTION

Hirschsprung's Disease (HD) is a congenital disorder characterised by the absence of ganglion cells in the intestinal submucosal and myenteric plexuses, leading to functional obstruction and severe constipation in affected infants and children. Histopathological confirmation of HD is a crucial step in the diagnostic process and subsequent management of patients. Suction rectal biopsy (SRB), first introduced by Dobbins and Bills in 1965, has become the preferred technique for obtaining tissue samples for histopathological evaluation.[1] Modifications to the initial technique, such as the introduction of Noblett's Forceps in 1969 and the more recent Rbi2 suction rectal biopsy forceps, have sought to improve efficacy and cost-effectiveness.[2,3]

The quality of the biopsy sample is of paramount importance due to the lower concentration of ganglion cells in the submucosal plexus.[4-6] As a result, most centres routinely obtain at least two samples to ensure adequate tissue for interpretation.[1-5,7,8] However, single-use biopsy cartridges can be expensive, posing a challenge for resource-limited settings and public healthcare facilities in developing countries like Malaysia, where paediatric patients often lack insurance coverage for congenital diseases. Consequently, cost-effective diagnostic approaches, such as single sample SRB, hold great value in these settings.

In this study, we aim to evaluate the diagnostic accuracy and sufficiency of a single

Address for correspondence: Associate Professor Dr Anand SANMUGAM, Division of Paediatric and Neonatal Surgery, Department of Surgery, Faculty of Medicine, University of Malaya. Tel: 03-79494422. Email: *Anandshan@um.edu.my*

macroscopically adequate sample in suction rectal biopsies for the histopathological confirmation of Hirschsprung's Disease. Our findings may provide valuable insights for healthcare facilities in Malaysia and other developing countries, enabling them to optimise their diagnostic strategies while maintaining cost-effectiveness and ensuring the timely identification and management of patients with HD.

MATERIAL AND METHODS

Study Design and Setting
We conducted a retrospective study by reviewing the medical records of children who underwent suction rectal biopsies for the diagnosis of HD at Hospital Raja Perempuan Zainab II (HRPZII), Kota Bharu, Kelantan. The research proposal was approved by the Medical Research and Ethics Committee board, Ministry of Health Malaysia (NMRR.ID: NMRR-18-3996-44728).

Definitions
A biopsy attempt was considered the number of times a new capsule was used to obtain a macroscopically adequate specimen A biopsy episode was defined as an episode resulting in a SRB specimen sent to the lab for analysis. A biopsy episode was deemed non-diagnostic when all specimens taken within the episode could neither confirm nor exclude HD, and a repeat biopsy was recommended. Complications included evidence of post-procedure bleeding requiring transfusion or suturing under general anaesthesia and signs or symptoms of rectal perforation.

Inclusion and Exclusion Criteria
We included children less than one year of age, suspected of having HD, and subjected to a single sample bedside suction rectal biopsy procedure at HRPZII between January 2016 and July 2019. Patients older than one year and those who had more than one suction rectal biopsy sample sent per biopsy episode were excluded from the study.

Suction Rectal Biopsy Technique
The procedure was performed at the bedside without sedation, either by or under the supervision of a senior medical officer with at least six months of experience in the department. The child was placed in a lateral position, and a lubricated biopsy forceps was inserted along the posterior wall of the rectum, with the cutting blade aimed at least 3 cm above the dentate line. Suction was applied using a 10cc syringe, and the cutting blade was triggered to obtain the specimen. Macroscopically adequate specimens, defined as those with equal amounts of pinkish mucosa and white glistening submucosal tissue, were immediately sent to the histopathology lab for processing. The patient was observed for one day to monitor for any signs or symptoms of bleeding or perforation before being discharged.

Histopathological Analysis
The specimens were routinely stained with Hematoxylin and Eosin (H&E). Additional tests, such as acetylcholinesterase (AChE) study and calretinin immunohistochemical staining, were performed at the pathologist's discretion.

Data Collection and Statistical Analysis
We collected demographic data, anthropometric data, procedure-related data, complications, cost of the procedure, and histopathological results.

Data were analysed using IBM SPSS Statistics 23 software. Continuous data were presented as medians with standard deviations, and categorical data were presented as numbers and frequencies (%). Descriptive statistics were used to summarise the data. Fisher's Exact test was employed to evaluate categorical data such as gestational age, age at SRB, gender, and weight at SRB, with a p-value of less than 0.05 considered statistically significant. Logistic regression was used to study independent variables, and variables with $p > 0.25$ were subjected to multiple logistic regression, with significance taken at $p < 0.05$.

RESULTS

A total of 77 patients who had a bedside suction rectal biopsy were identified from the SRB Registry of the Paediatric Surgery Department in HRPZ II from January 2016 to July 2019. A total of 68 patients who met the criteria were finally included in this study.

Demographics, Anthropometrics, Procedural Details and Outcome
The demographics, procedural details and outcomes are summarised in Table 1. The mean age at biopsy was 46.3 days of life. Mean birth weight was 2.9kg where as the mean weight at biopsy was 3.5 kg. In the 68 patients who had a single sample SRB, 60 were born term (88.2%) and 8 (11.8%) were preterm.

In 65 (95.6%) biopsy episodes, a single attempt produced a macroscopically adequate

Table 1: Demographic details, procedural details and outcomes

	Frequency (%)
Demographic and Anthropometric Data	
Gestational Age	
Preterm	8 (11.8)
Term	60(88.2)
Gender	
Boy	36 (52.9)
Girl	32 (47.1)
Birth weight (kg)*	2.9 (0.58)
Age at diagnosis (Days) *	31.1 (30.95)
Age at Suction Rectal Biopsy (Days)*	46.3 (36.04)
Weight of Suction Rectal Biopsy (kg)*	3.5 (1.38)
Histopathological Examination	
Number of attempts	
(only one macroscopically adequate sample sent)	
1	65 (95.6)
2	3(4.4)
Ganglion cells	28 (41.2)
Hypertrophic nerve	25 (36.8)
Acetylcholinesterase	
Performed	65 (95.6)
Not performed	3 (4.4)
Calretinin test	
Performed	23 (33.8)
Not performed	45 (66.2)
Final report of Suction Rectal Biopsy	
Conclusive (Diagnostic)	58 (85.3)
Inconclusive (Non-diagnostic)	10 (14.7)
Clinical Outcomes	
Complications	0

*Mean (standard deviation)

sample. In the remaining 3 (4.4%), only the macroscopically adequate sample obtained from the subsequent attempt were sent to the pathologist. All the biopsy specimens were subjected to H&E staining, 95.6% of specimens were subjected to AChE staining and 33.8% were subjected to calretinin staining at the discretion of the pathologist.

The inadequacy rate for bedside SRB in this study was 14%. where 10 out of the 68 biopsy episodes were reported as non-diagnostic.

Factors associated with non-diagnostic samples
There was no association that was statistically significant between sample inadequacy and gestational age, gender, birth weight, or weight at biopsy (Table 2).

The use of calretinin as an adjunct
In this study, calretinin staining was performed in 23 biopsy episodes. Only 1 of 23 (4.3%) of these biopsy episodes was deemed inadequate by the pathologist. In 45 biopsy episodes, calretinin staining was not performed. In this group, 9(20%) biopsy episodes were reported as inadequate (diagnostic).

There was no statistically significant difference seen between the diagnostic rate in the biopsy specimens stained with calretinin and the biopsy specimens not stained with calretinin (p value=0.147)

Complications
There were no complications seen in our study.

TABLE 2: Factors associated with the adequacy of suction rectal biopsy using simple logistics regression

	Crude (95% CI)	OR (p value)
Gestational Age		
Preterm	1	
Term	0.81 (0.09,7.39)	0.851
Gender		
Girl	1	
Boy	1.85 (0.47,7.24)	0.379
Birth weight (kg)	0.84 (0.24,2.97)	0.836
Age at Suction Rectal Biopsy (days)		
0-30	1	
Above 30	2.87 (0.68,12.22)	0.153
Weight at SRB (kg)	0.87 (0.49,1.54)	0.634

Cost of SRB

In our institution, the cost of processing a single sample SRB is Malaysian Ringgit (MYR) 165.13 (approximately 28.7 pounds sterling (GBP)). There is a 98% increase in cost if 2 samples are sent (Table 3).

DISCUSSION

Our study focused on the adequacy of single sample suction rectal biopsies (SRBs) for the diagnosis of Hirschsprung's Disease (HD) and found an inadequacy rate of 14%. This rate is comparable to those reported in the current literature, where most studies took between 1-5 samples per biopsy episode.[9-11,13-16,19] For instance, Dobbins and Bills recommended 3-5 biopsies per biopsy episode and had a non-diagnostic rate of 9.3%[2], while Noblett's study with 116 biopsy specimens reported no inadequacy.[3] A study by Athow *et al.* with 1-3 biopsy specimens per episode had a 16% non-diagnostic rate.[11]

The cost of diagnostic procedures is an important consideration, especially in low-resource settings. In the context of a publicly funded healthcare centre in Malaysia, the cost-effectiveness of a single sample SRB is of great value. Our study showed that the cost of SRB almost doubles with each additional sample taken, which is particularly significant in low-resource environments. Hall *et al.* compared the cost-effectiveness of two different biopsy forceps: the Rbi2 forceps, which use disposable single-use capsules with a sharp cutting blade, and the Noblett's forceps, which are reusable.[12] The study found that although the Rbi2 forceps has slightly higher cost due to the disposable biopsy capsules, the additional cost is likely lower compared to the cost of repeating a biopsy. This is particularly relevant when considering prolonged hospital stays and additional admission episodes associated with inadequate biopsy specimens. The use of Rbi2 forceps in their study led to increased sample adequacy, further supporting their cost-effectiveness. Alizai *et al.* discussed the cost-effectiveness of taking multiple biopsy specimens per biopsy episode, recommending 2-3 biopsy specimens.[10]

TABLE 3: Cost of suction rectal biopsy

Test/Equipment	Cost	
	1 Sample	2 Samples
Hematoxylin and Eosin Staining	RM10.06	RM16.90
Acetylcholinesterase Staining	RM5.07	RM10.14
Rbi2 Bullets	RM150.00	RM300.00
Total	RM165.13	RM327.04

Although multiple specimens might increase the likelihood of obtaining an adequate sample, it also raises the cost of the procedure, which may be a significant consideration in low-resource settings. In a study by Hayes *et al*.[15], the authors recommended 2-3 biopsy specimens per biopsy episode and reported a non-diagnostic rate of 23%. This study highlights the need to balance the cost of additional biopsy specimens with the risk of obtaining inadequate samples, which may require further diagnostic tests or even a repeat biopsy. These findings suggest that single sample SRBs can potentially provide sufficient diagnostic information, making it a cost-effective option in low-resource settings.

Our study also identified no statistical significant association between non-diagnostic rates of single sample SRBs with age at suction rectal biopsy. In a study by A study by De Lorijn *et al*., non-diagnostic rate was higher in neonates compared to infants.[17] Possible reasons for non-diagnostic biopsy episodes include variability in the biopsy site, superficial biopsy, and immaturity of the enzyme system.[8]

The complication rates from SRB in our study were 0%, which is consistent with literature reports ranging from 0-2%.[14,15,20,21] This could be attributed to the use of a single sample per biopsy episode and the Rbi2 forceps with a single-use capsule and sharp blade, ensuring a clean cut and consistent pressure application, as suggested by Noblett.

Although literature on the cost comparison of suction rectal biopsy is scarce, NJ Hall *et al*. suggested that the higher cost of Rbi2 forceps is likely lower compared to the cost of repeating a biopsy, considering prolonged hospital stays and additional admission episodes.[9]

The use of calretinin as an alternative to acetylcholinesterase staining has been supported in the literature.[16-22] In our study, the non-diagnostic rate of samples subjected to calretinin staining was 4.3%, compared to 20% in specimens not subjected to calretinin staining. The use of adjunctive techniques, such as calretinin immunohistochemical staining, has been shown to improve the accuracy of SRB for diagnosing HD.[19] While this additional staining technique may increase the initial cost of the biopsy, it has the potential to reduce the need for repeat biopsies or more invasive diagnostic procedures, ultimately reducing overall healthcare costs. In this study, the determination to utilise calretinin staining was based on the discretion and clinical judgment of the pathologist, emphasising the necessity for judicious decision-making within a resource-constrained environment. This selective approach ensures that the valuable resources available are allocated efficiently and optimally, while maintaining the quality of diagnosis and patient care in such circumstances.

The use of a retrospective study design is justified in this context as it allows for a real-world evaluation of the constraints faced by resource-challenged healthcare setups. Utilising data from actual clinical practice has provided us valuable insights into the effectiveness, safety, and cost implications of implementing a single sample SRB for the diagnosis of HD. The limitations of this study includes its retrospective nature and a relatively small sample size, which may affect the accuracy of the results and the statistical significance of multivariate analysis. A prospective study with a larger sample size, comparing single sample SRBs to multiple sample SRBs at different centres, would provide more conclusive evidence.

CONCLUSION

In conclusion, our study indicates that single sample SRBs can be an adequately diagnostic and cost-effective diagnostic tool for Hirschsprung's Disease, particularly in low-resource settings.

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