COMPARING RECTAL BIOPSY USING ARTERY FORCEPS AND FULL-THICKNESS RECTAL BIOPSY IN DIAGNOSING HIRSCHSPRUNG'S DISEASE AT UNIVER-SITY TEACHING HOSPITAL, LUSAKA

BY

CHIZOMA GRAINER

A dissertation submitted to the University of Zambia in partial fulfillment of the requirements for the award of Master of Medicine in Pediatric Surgery

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DECLARATION

I, **Dr Chizoma Grainer,** hereby declare that this dissertation entitled "Comparing Rectal Biopsy Using Artery Forceps and Full-Thickness Rectal Biopsy in Diagnosing Hirschsprung's Disease at University Teaching Hospital, Lusaka" represents my own work and has not been presented either wholly or in part for a diploma or degree at the University of Zambia or any other institution elsewhere.

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APPROVAL

This dissertation of Dr Chizoma Grainer has been approved as fulfilling the requirements for the award of the degree of Master of Medicine in Pediatric Surgery by the University of Zambia.

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Examiner 2	Signature	Date
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ABSTRACT

Hirschprung's disease is a congenital disorder that is characterised by functional constipation whose onset is dependent on the length of affected bowel and always involves the rectum. Despite having a diagnostic challenge, rectal biopsy for histological analysis is the most definitive form of diagnosis. The average number of patients seen at University Teaching Hospital in Zambia, generally come as referrals. Superficial thickness biopsy using the rectal suction method has been adopted as the gold standard for obtaining rectal biopsy in the western world leaving the invasive full thickness biopsy for inconclusive cases. However, superficial thickness biopsy using special types of forceps have been quoted to be as good or even superior to the rectal suction biopsy. Superficial thickness biopsy using curved artery forceps is proposed in this study to provide a simple cost-effective method of obtaining adequate rectal biopsy sample for diagnosis.

The aim of this study was to compare superficial rectal biopsy using an artery forceps and full thickness rectal biopsy in the diagnosis of Hirschsprung's disease in a resource limited environment at University Teaching Hospital, Lusaka

This was a prospective single-blind study conducted at University Teaching Hospital in the department of Surgery, paediatric unit from June 2018 to March 2019. Thirty-one patients, who presented with chronic constipation and delayed passage of meconium, were enrolled in the study. A structured questionnaire was used to gather information from the patient and record files. Two rectal biopsies using two different procedures under study were obtained from each patient and submitted for histopathological evaluation to the histopathology department. The results of the two methods were then compared.

All patients had chronic constipation and some had a history of delayed passage of meconium. 19 out of 31 full thickness biopsies had adequate biopsy whereas only four out of 31 had adequate biopsy with superficial thickness method using curved artery forceps. The biopsy obtained using the curved artery forceps had a high sensitivity and poor specificity and predictive value. This meant that too many patients would require a re-biopsy. 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, this study recommends that the traditional full-thickness biopsy procedure should continue to be used until such a time when rectal suction biopsy sets for superficial-thickness biopsy can be made available.

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

DEDICATION

I dedicate this work to my mother, Anna Lole Chizoma, my son, Himalumba Speed Chizoma and the entire family whose love and support helped me push forward to make this possible.

ACKNOWLEDGEMENT

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DEFINITION OF TERMS

Adequate rectal biopsy: Biopsy specimen with at least the presence of the submucosa and submucosal plexus for histological diagnosis

Aganglionosis: Absence of ganglion cells

Full-thickness rectal biopsy (or full wall biopsy): Biopsy specimen containing muscularis mucosa and muscularis externa including meissner's and auerbach's plexuses

Hypertrophic nerve fibres: Abnormally enlarged nerves

Inadequate rectal biopsy: Biopsy specimen without the presence of submucosa and therefore no submucosal plexus for histological diagnosis

Superficial-thickness rectal biopsy: Biopsy specimen contains only muscularis mucosa including meissner's plexus

CHAPTER ONE: INTRODUCTION

1.1 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]. The absence of peristalsis in the affected bowel results in a functional intestinal obstruction. It is also referred to as congenital megacolon or congenital colonic aganglionosis [2]. Literature showed that the aganglionosis is confined to rectosigmoid in 75 percent of patients; sigmoid, splenic flexure or transverse colon in 17 percent, total colon in 17 percent and total colon along with a short segment of the terminal ileum in 8 percent [3]. 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 which demonstrates the presence of a transitional zone as the critical feature to suspect Hirschsprung's disease, anorectal manometry used to assess the rectoanal inhibition reflex which if absent indicates Hirschsprung's disease 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 definite means of making a diagnosis of aganglionosis [4][5].

Diagnostic accuracy depends on the adequacy of the specimen, level at which they were obtained, number of sections studied and the skill of the pathologist [6]. From the time rectal biopsy was first introduced by Swenson, it has evolved from the traditional method

of obtaining full-thickness biopsy under general anaesthesia to rectal suction biopsy that can be performed as a bedside procedure [7]. The suction devices which are less invasive, are not readily available in most African countries, hence full-thickness rectal wall biopsy continues to be commonly performed [8]. Attempts have been made to come up with methods that are as effective in obtaining tissue samples less invasively in the cheapest way possible by using readily available tools at various centres. Varied and simple techniques using different types of forceps to obtain rectal biopsies have shown that Hirschsprung's disease can be diagnosed or ruled out confidently [8][9]. This study is directed at trying to determine if readily available forceps in a poor resource setting can be used to obtain adequate rectal tissue samples that can be used to make a diagnosis of Hirschsprung's disease.

1.2 Statement of the Problem

Hirschsprung's disease has a global incidence of one in 5000 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. Data collected from D-Block theatre register of patients who underwent full-thickness rectal biopsy in 2015 were thirty of which twenty four were males and six were females. The youngest and oldest patients were aged three months and seven years respectively.

Full-thickness biopsy is currently the procedure of choice for the diagnosis of Hirschsprung's disease at the University Teaching Hospital. It makes full-thickness laceration of the colon requiring stitching and often some minor bleeding is involved. In addition to risks associated with general anesthesia, it can give rise to infection, re-

perforation and other complications associated with future surgical manoeuvres in the intestine due to scarring or fibrosis at the biopsy area [1][10].

Reports indicate that up to 80 percent of biopsies performed on constipation patients could be unnecessary, with only 12–17 percent yielding a positive result of aganglionosis [11]. Appropriate screening and use of a less invasive procedure that equally provides enough specimen for a diagnosis may help reduce the number of children exposed to the risks associated with full-thickness rectal biopsy procedure [10][11].

Contrast enema can only suggest the diagnosis and it has been shown to have the lowest sensitivity (65–80%) and specificity (66–100%), while rectal suction biopsy has the highest sensitivity (91–100%) and specificity (97–100%) [12]. Rectal suction biopsy technique has been adopted as the gold standard while the more invasive full-thickness biopsy technique has been reserved for indeterminate cases that require a repeat biopsy. Grasp and cut method using different forceps has demonstrated to be as good as rectal suction biopsy. This method is able to obtain adequate superficial-thickness rectal biopsy for diagnosis in over 90 percent of cases which is comparable to rectal suction biopsy and full-thickness biopsy [10].

Though the University Teaching Hospital does not have rectal suction biopsy kits, the grasp and cut method using readily available forceps to obtain superficial-thickness rectal biopsy may be an alternative to rectal suction biopsy and reserve full-thickness biopsy for unequivocal cases.

1.3 Study Justification

Rectal suction biopsy was adopted in 2004 as the current gold standard to replace full-thickness rectal biopsy at the fourth international meeting on Hirschsprung's disease and related neurocristopathies' [13]. However this procedure is not available in poor resource settings like Zambia because rectal suction biopsy kits are not available. In Africa, full-thickness biopsy is widely practiced because rectal suction devices are not readily available and are reported to be expensive and require maintenance [2][8]. The full-thickness biopsy requires to be done under general anaesthesia in theatre when compared to the rectal suction biopsy.

A cheaper alternative available to rectal suction biopsy appears to be the use of grasp biopsy method. Forceps for biopsy do not require specialised maintenance except for disinfection and sterilisation. The grasp biopsy is associated with good specimen yields for diagnosis ranging from 90 to 96 percent and very few complications when compared to rectal suction biopsy. In addition, studies have shown that the caliber of the surgeon performing the procedure has no effect on the specimen yield. The full-thickness biopsy method can therefore be reserved for indeterminate cases that require repeat biopsy.

Grasp biopsy can readily be done in poor resource settings like Zambia because the instruments required to conduct the procedure are readily available. A medium curved artery forceps is one such instrument that is suggested for this purpose. Obtaining adequate specimen with appropriate and readily available tools would mean that the duration of the procedure can be reduced as the technique can be much simpler and would not always require any suturing afterwards. This would further mean that the idea of offering this method of taking a rectal biopsy using forceps as an out-patient procedure

may be entertained in future especially in older children. As long as there are no contraindications to conduct grasp biopsy as an out-patient procedure it would significantly reduce on cost and time compared to full-thickness biopsy as the need for general anesthesia and an operating theater with its staff would be eliminated. However there is need to compare if results for grasp biopsy using a medium curved artery forceps can be as reliable as those obtained using the full-thickness biopsy hence the need for this research to be conducted.

1.4 Research Question

Can the diagnosis of Hirschsprung's disease be made from superficial-thickness rectal biopsy obtained using a medium curved artery forceps which can be comparable to the use of the traditional full-thickness rectal biopsy?

1.5 Objectives

1.5.1 General Objective

To compare the outcome and adequacy of superficial-thickness rectal biopsy using an artery forceps with full-thickness rectal biopsy in the diagnosis of Hirschsprung's disease at University Teaching Hospital, Lusaka.

1.5.1 Specific Objectives

1. To determine the adequacy of specimen obtained by artery forceps (grasp biopsy) in the diagnosis of Hirschsprung's disease by presence of submucosa.
2. To establish the specificity and sensitivity of diagnosing Hirschsprung's disease using artery forceps compared with full thickness biopsy
3. To evaluate the general complication rate of rectal biopsy.
4. To determine if grasp biopsy using artery forceps can replace full-thickness biopsy in the diagnosis of Hirschsprung's disease.

CHAPTER TWO: LITERATURE REVIEW

Embryology

In the third week of gestation, gastrulation establishes the three germ layers, endoderm, mesoderm, and ectoderm in the embryo. The folding of the embryo results in the formation of the primitive gut [14]. Neural crest cells derived from the ectoderm migrate and populate the gut to form the myenteric plexus and the submucosal plexus [15]. Normal ganglion cell distribution is present at twenty four weeks of gestation in humans and continues to mature on into childhood [16].

Hirschsprung's disease is due to the incomplete migration of the neural crest cells along with cellular and molecular abnormalities in the development of the enteric nervous system. Therefore children with Hirschsprung's disease will present with obstruction caused by spasm and a lack of propagation of the peristaltic wave in the aganglionic bowel segment [14].

Pathology

Normal intestinal motility is primarily under the control of intrinsic neurons that control both contraction and relaxation of smooth muscle, with relaxation predominating. Extrinsic control is mainly through the cholinergic and adrenergic fibres [2]. In Hirschsprung's disease, ganglion cells are absent, leading to a marked increase in extrinsic innervation by 2-3 times that of normal innervation with the adrenergic (excitatory) system thought to predominate over the cholinergic (inhibitory) system, thus leading to increase in smooth muscle tone[16]. Absence of the intrinsic enteric inhibitory nerves with increase in smooth muscle tone leads to an imbalance of smooth muscle contractility, uncoordinated peristalsis, and a functional obstruction [2][17]. The gross

appearance of a bowel segment affected by Hirschsprung's disease is an aganglionic distal spastic and narrow segment with a proximal hypertrophic and dilated bowel separated by a five centimetre to 10 centimetre transition zone [2].

Histologically, the absence of ganglion cells in the distal intestine is the hallmark of the disease. Ganglion cells are absent in both the submucosal (Meissner's) plexus and the myenteric (Auerbach's) plexus. There is usually a marked hypertrophy of nerve fibres that extend into the submucosa that may be seen on routine hematoxylin-eosin stained slides but are more easily seen using an acetylcholinesterase stain. Cases with long segment or total colon Hirschsprung's disease may not have nerve hypertrophy on rectal biopsy. Aganglionosis is always present in the rectum and progresses proximally and continuously for a varying distance. The transition zone has a five centimetre to 10 centimetre progressive decrease of ganglion cells until the aganglionosis level is reached. The transition zone may not be symmetric circumferentially, which has implications in deciding how much bowel to remove during pull through, the definitive surgical treatment [2].

Presentation

Delayed passage of meconium has been emphasised as an early sign indicating the presence of Hirschsprung's disease in new-born infants. Neonates normally pass meconium within twenty four hours of life but 60 percent to 90 percent of neonates with Hirschsprung's disease fail to pass meconium within that period. In a study conducted by Lee et al, it was noted that 30 percent of their patients with Hirschsprung's disease had a history of delayed passage of meconium [18]. Most patients in the African series presented with intestinal obstruction while about 30 percent presented with constipation, 11 percent with enterocolitis, and two percent with intestinal perforation [2].

Hirschsprung's disease characteristically presents in any of the three following ways: neonatal bowel obstruction, chronic constipation and enterocolitis. Obstruction in the neonatal period will occur in approximately 50 percent to 90 percent of children with Hirschsprung's disease. A study of normal African new-borns found that 75 percent passed meconium within 24 hours of birth, 92 percent within 48 hours and 98 percent within 72 hours [19].

In Nigeria, however, 30-40 percent of cases present as neonates. Ninety percent of children with Hirschsprung's disease will have delayed passage of meconium beyond 24 hours which is characteristic [20]. Symptoms will include abdominal distension, bilious vomiting, and feeding intolerance suggestive of distal intestinal obstruction. Chronic constipation is the common presentation in patients who present later in childhood. This is most common among breast-fed infants, who typically develop constipation around the time of weaning [21]. Clinical features that are suggestive of Hirschsprung's disease include gross abdominal distention, dependence on enemas without significant encopresis and failure to thrive [2].

Diagnosis

Different investigation modalities are used in the diagnosis of Hirschsprung's disease and these vary in their diagnostic accuracy. Rectal biopsy is a definitive method for the diagnosis of Hirschsprung's disease. Histological examination of rectal wall biopsy remains the gold standard for the diagnosis of Hirschsprung's disease.

Rectal Biopsy Specimen Criteria

Other than radiology which basically suggests the presence of Hirschsprung's disease, rectal biopsy provides the most definite means of making a diagnosis of Hirschsprung's disease. According to a recent consensus article by the Gastro 2009 International Working Group, diagnostic rectal biopsies should be taken one to 2.5 centimetres proximal to anorectal squamocolumnar junction, should measure at least three millimetres, and should include at least one third of the submucosa [22]. However, even the presence of a moderate amount of submucosa is considered adequate to make a diagnosis. In addition, at least two or three tissue samples have to be obtained to increase the yield [8]. Illustrations in figures 1 and 2 show the submucosal and myenteric ganglia as well as the site for obtaining a rectal biopsy.

Rectal biopsy methods and the proposed method

Histological examination of rectal wall biopsy remains the gold standard for the diagnosis of Hirschsprung's disease. Although use of radiological investigations with contrast enema and manometric studies may be highly suggestive, the most definitive diagnosis of Hirschsprung's disease requires a rectal biopsy. The methods recorded in literature include the traditional full-thickness rectal wall biopsy, the rectal suction biopsy and the grasp and cut biopsy.

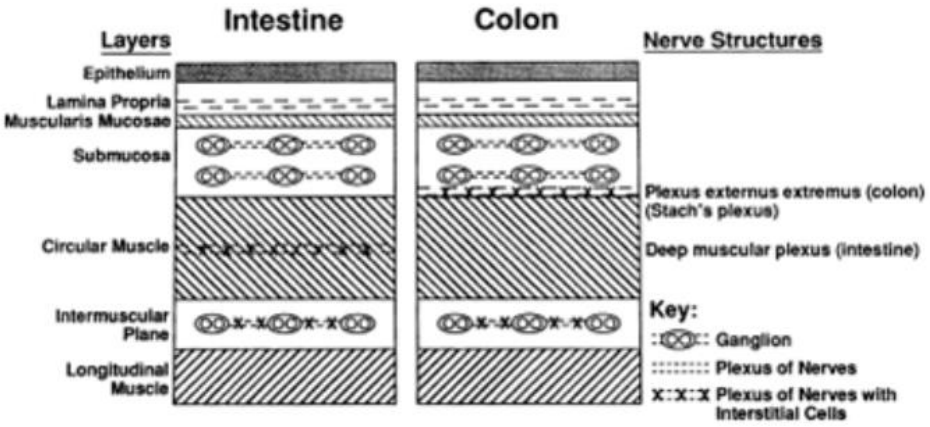


Figure 1: Diagrammatic Cross-sections of the Wall of the Small Intestine and the Large Intestine showing Ganglia in the Submucosa and Myenteric Plexus (Intermuscular Plane) [23]

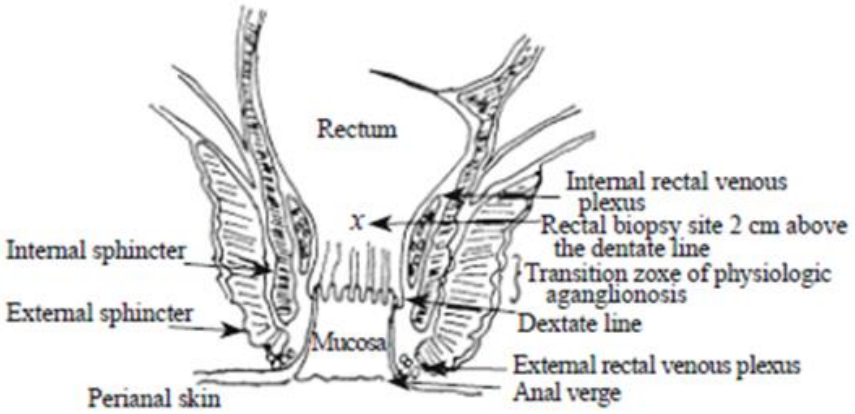


Figure 2: Illustration of the Rectum and the site Marked X for a Rectal Biopsy [24]

Full-thickness biopsy provides abundant specimen for histological diagnosis where both the muscularis mucosae and muscularis propria are visible. The drawbacks are that the procedure is invasive and the patient must undergo general anaesthesia. It makes a full-thickness laceration of the colon wall, requires stitching and often some minor bleeding is involved. It can give rise to infection, re-perforation and other complications associated with surgical manoeuvres in the intestine [1]. The tissue trauma and associated inflammation and scarring as well as the suture closure of the larger and deeper defect

associated with full-thickness rectal biopsy may complicate the pull-through procedure during dissection and development of a submucosal plane in the biopsy region which lies in the field.[25]

Rectal suction biopsy was introduced after it was demonstrated by Gherhardi in 1960 that the level of aganglionosis was identical in the submucosa plexus and myenteric plexus [26].Aldridge and Campbell in 1968 further demonstrated that the density of ganglion cells in the submucosal plexus was sufficient above the hypoganglionic zone within 1–2 cm of the anal verge [26]. The rectal suction biopsies obtained contain the same depth of submucosa as the depth of mucosa above it and, therefore, only allow for investigation of the submucosal plexus. A Rectal Suction device (figure 3) can be used to obtain a rectal biopsy as a bedside procedure and requires no general anaesthesia or suturing. A rectal suction biopsy is performed via a catheter inserted into the rectum that blindly clutches and cuts a small piece of the rectal wall via a small vacuum created at its tip (figure 4) [1]. The rectal suction biopsy is a well-documented and widespread method that shortens hospital stay and reduces patient suffering [1].

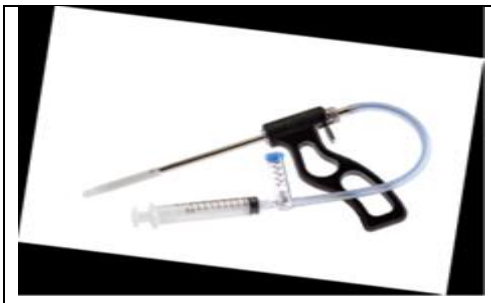


Figure 3 - Rectal Suction Biopsy Device [27]

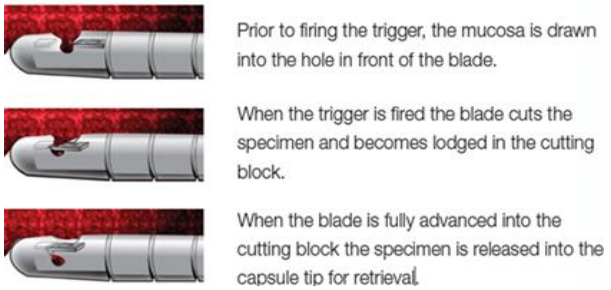


Figure 4 - Obtaining a Rectal Suction Biopsy Specimen [27]

The rectal grasp biopsy technique is a method that was introduced by Shandling in 1961 from the traditional full-thickness biopsy [9]. It provides an alternative way of obtaining

superficial-thickness biopsy to rectal suction method. The technique involves placing the forceps in position, 2 to 3 cm above the dentate line, and a bite of mucosa and submucosa is taken under direct vision which is an added advantage. A rectal biopsy tube maybe used to localise the biopsy site (figure 7). While keeping the jaws firmly closed the instrument is sharply withdrawn to obtain the specimen.

Most African countries commonly practice full-thickness biopsy, and Zambia is not an exception [8]. A Nigerian survey conducted among 31 paediatric surgeons demonstrated that 84 percent performed full-thickness biopsy, 10 percent did grasp and cut biopsy and 7 percent did suction biopsy which was in contrast with an Australian series where rectal suction biopsy was done in all 126 children with 101 positive results [28]. Despite rectal suction devices not being readily available in Nigeria, which was attributed to resource crunch and lack of technology, a recommendation to make efforts to increase their use was made because of the benefits associated with the procedure [28]. However, the grasp and cut biopsy using appropriate forceps can equally be used as a substitute and reserve full-thickness biopsy for indeterminate cases.

The diagnostic yield of the grasp and cut biopsy has been shown to be comparable to rectal suction biopsy. In one such study that was done to evaluate the effectiveness of using the grasp and cut biopsy specimen from the rectum in suspected Hirschsprung's disease patients yielded promising results across all age groups [8]. The grasp biopsy on patients aged one to 17 years using a Kervorkian-Younge uterine biopsy forceps (figure 5) provided a sample with some submucosa and yielded submucosal ganglion cells in more than 90 percent of patients in all age groups. Thus, the single grasp biopsy excluded Hirschsprung disease in at least 92 percent of all patients whereas the single suction

biopsy, obtained at the same time, excluded Hirschsprung disease in 73 percent of patients 1 to 3 years old and only 50 percent of patients older than 3 years [9]. However, a recent study demonstrated that adequate rectal suction biopsies can be obtained even in older age groups for diagnosis [29].

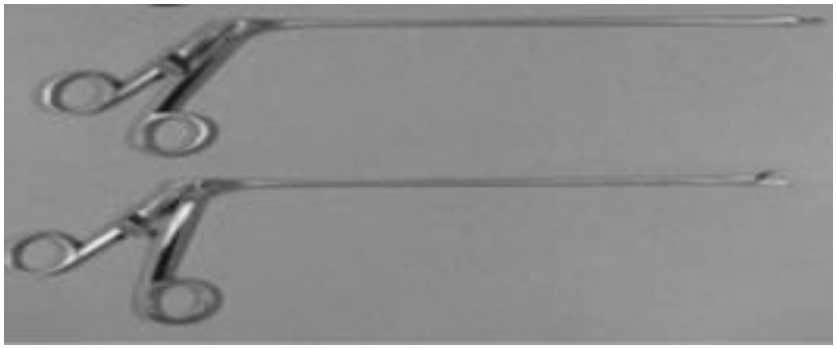


Figure 5 - Kervorkian-Younge Uterine Biopsy Forceps (closed [top] and open [bottom]) [9]

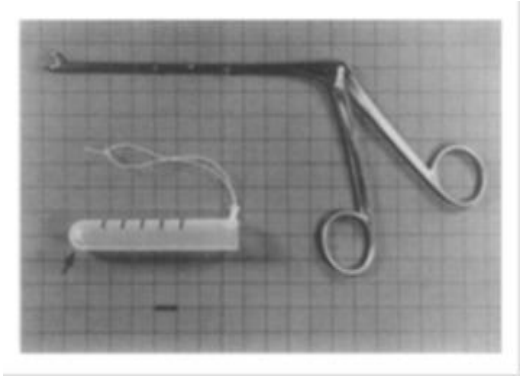


Figure 6 – Laryngeal Biopsy Forceps with a Rectal Biopsy tube with side Hole (arrow) [30]

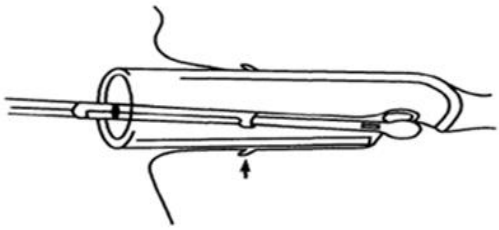


Figure 7 – A Schematic representation of Rectal Biopsy Tube. The Protruded Rectal Mucosa into the side Hole of the Tube is pulled off with Biopsy Forceps. Arrow indicates Dentate Line [30]

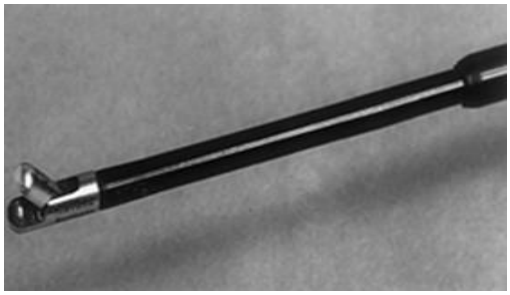


Figure 8 - The Storz Rectal Cup Biopsy Forceps [31]

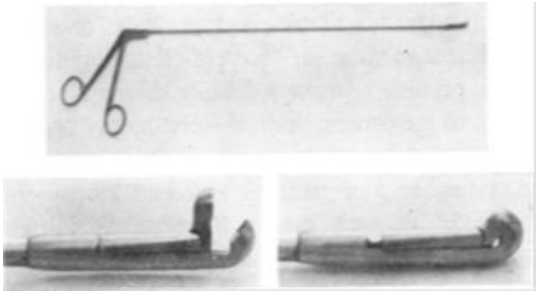


Figure 9- Chevalier-Jackson Biopsy Forceps [32]

Other forceps that have been used and recorded in literature include the Gruenwald nasal cutting forceps, laryngeal biopsy forceps (figure 6), the Storz rectal cup biopsy (Figure 8) and Chevalier-Jackson biopsy forceps (figure 9).

In a retrospective study where a jumbo biopsy forceps was used to obtain superficial rectal biopsies on 156 patients aged between seven weeks and 20 years (average age 6.8 years) demonstrated an overall success rate of 86 percent based on specimen adequacy to diagnose Hirschsprung's disease [10]. The yield, though not statistically significant, appears to be better where more than one sample was obtained [8]. The above studies demonstrate that some forceps provide an alternative method to rectal suction biopsy for obtaining superficial-thickness biopsy with good results.

The complication rates following rectal biopsy for Hirschsprung's disease varies in the literature and the described interventions during or immediately after the procedure included blood transfusion, diathermy, packing, stitching, or nothing at all [33][34]. Full-thickness rectal biopsy is associated with serious complications and the reported complication rate was 6.6 percent in a study involving 593 full-thickness biopsies [33][1]. Rectal suction biopsy which is considered to be safe, reliable and less invasive

has an overall complication rate in the range 0–15 percent, with inadequacy of specimen being the most frequently encountered [33][28]. Bowel perforation and rectal haemorrhage are rare [1]. In a study series of 1340 rectal suction biopsies, 6 complications were reported of which 3 were bowel perforations [1]. Haemorrhage has a reported incidence of less than one percent [33][34]. The grasp biopsy method, an alternative to rectal suction biopsy, reported no serious complications in the referred studies except for minimal post-biopsy bleeding. The safety profile of this method is increased because the biopsy is done under direct visualization of the mucosa which allows identification of any abnormal vascular structures and any excessive bleeding may be rapidly identified and treated immediately [10].

A grasp and cut rectal biopsy has therefore been demonstrated to be able to provide adequate superficial-thickness biopsy specimen comparable to rectal suction biopsy and with seemingly reduced complications when compared to the full-thickness biopsy method. For the purposes of this study a medium curved artery forceps (figure 10) was proposed and used to obtain rectal biopsies.

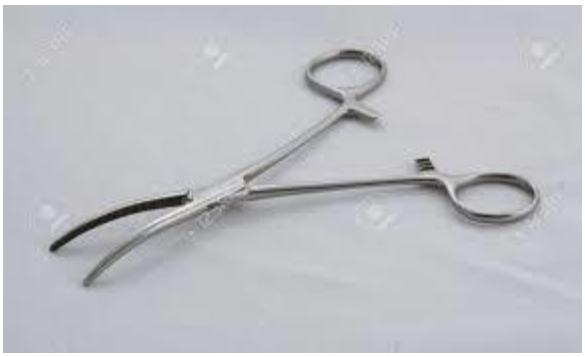


Figure 10 – Medium Curved Artery Forceps [35]

CHAPTER THREE: RESEARCH METHODS

This study was conducted in the Department of Surgery, in the unit of Paediatric and Neonatal Surgery (D-Block) at the University Teaching Hospital which is a University of Zambia affiliated tertiary centre. Patients were recruited from D-block outpatient department.

3.1. Study design:

This was a prospective single-blind comparison study. The selected suspected patients were subjected to the two procedures of collecting tissue samples which were then submitted to a consultant pathologist for histopathological analysis. In this study, the pathologist was blinded from knowing which procedure was used for the collection of full-thickness and superficial-thickness specimen. The study was aimed at comparing the effectiveness of the proposed method (superficial-thickness using artery forceps) and the recommended method (full-thickness) in the diagnosis of Hirschsprung's disease.

3.2. Study population:

The target population in the study included those children presenting with signs and symptoms of Hirschsprung's disease.

3.3. Inclusion and Exclusion Criteria

3.3.1. Inclusion Criteria

• 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 stabilization.

• Patients whose consent to participate in the study has been obtained from their guardians.

3.3.2 Exclusion Criteria

• 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

3.4. Sampling

3.4.1. Sampling Technique

In this study, convenient sampling technique was used to select the participants of the study. This sampling method involves getting participants who are conveniently available to participate in the study. The research depended on the available patients to be part of the study. Convenience sampling was also used in the study due to the infrequent manner that patients present to the University Teaching Hospital as referrals and self-referrals and due to their accessibility to the researcher.

3.4.2. Sample Size

In this case, a similar study by Sahu RK et al, 2017, to evaluate radiological investigation methods against full-thickness rectal biopsy to diagnose Hirschsprung's disease used a sample size of 19 cases [36]. The sample size in this study, n, was calculated for a population N to give a 95 percent level of confidence at 0.05 margin of error using the Yamane formula. The estimated target population was 35 patients that were to be attended in the period of this study. $n = \frac{N}{1+N(e^2)}$

The calculated sample size was 32 patients.

n = sample size

N = Number of operations in a year (Population)

e = margin of error

3.5 Study Procedures

An informed consent including all possible benefits and hazards was described to the responsible parent(s) as simply as possible. Once consent was given, a standard clinical data collection sheet was used to take and record the history and examination findings before conducting a rectal biopsy.

3.5.1 Biopsy Technique

The biopsies were obtained by the researcher, a registrar, with the help of an assistant and a theatre nurse under general anaesthesia. The patient was first placed in the lithotomy position under general anaesthesia. Sterile gauze soaked with povidone iodine was inserted in the rectum to avoid any soiling of the field with stools. The target site for all the biopsies was on the posterior rectal wall. Two biopsies were obtained from each patient, one using the full-thickness biopsy method and the other using the superficial

thickness biopsy with a curved artery forceps. A total number of 62 biopsy specimens were obtained in the study.

(i) Obtaining a full-thickness Rectal Biopsy (Specimen one)

A chromic catgut 3.0 stay suture was first placed one centimetre proximal to the dentate line. Using retraction on the first stay suture, the second and third stay sutures were placed two centimetres and three centimetres proximal to the dentate line respectively, the long limb of the third suture was left and not cut. The second stay suture is elevated and the rectal mucosa and underlying muscle are cut using a sharp scissors. Running continuous haemostatic sutures from the third to the first stay sutures was performed.

(ii) Obtaining Superficial-rectal Biopsy with Artery Forceps (Specimen two)

A curved artery forceps was used. With the forceps positioned above 1.5 centimetres from the dentate line [37], a bite of mucosa and submucosa was taken under direct vision. The specimen was then obtained using a blade cutting close to the tip of the holding forceps. The site was sutured with chromic 3.0 if bleeding was observed; otherwise the rectum was packed with Vaseline gauze if there was no bleeding.

3.5.2 Specimen Preservation for Histopathology

Once the specimens were obtained, they were delivered to the scrub nurse. The samples were preserved in separate labelled bottles in 10 percent formalin. The bottles were labelled randomly as A and B and the details of the method used to collect each of the specimens were recorded accordingly on data collection sheet before being submitted to the histopathology department for analysis. A consultant pathologist was assigned to analyse the specimens that were submitted. The pathologist was blinded in that he did not know the method that was used to collect any of the specimens in either bottle. The

pathologist was required to report on the adequacy of the specimen and status of the ganglion cells. A specimen is considered adequate if it includes submucosa, which is necessary for reliable interpretation by a pathologist [9].

3.6. Variables

Our dataset is a collection of information based on the variables that were identified to be most relevant. Appendix I shows a classification of the variables into Categorical and Non-categorical variables.

3.7. Data Management

3.7.1. Data Collection

In this study primary data collection was done using questionnaires with close ended questions which made the study highly quantitative (Appendix II). The secondary data collection was collected from various relevant sources which have been presented in the literature review and references.

3.7.2. Data Analysis

Data entry was done using IBM Statistical Package for Social Science Soft Ware (SPSS) version 23. The Data collected was stored on data spreadsheet sheet in IBM SPSS Statistics Version 23, a statistical computer package. Using SPSS version 23.0 software, quantitative data was summarised in frequencies, percentages, cross-tabulation tables and graphical presentations. The Sensitivity, Specificity, Positive and Negative Predictive Values were also determined.

3.7.3. Descriptive Analysis

(i) Continuous variables were presented as means and / or medians, and percentages.

(ii) Categorical variables were presented as percentages or proportions**.**

3.8. Ethical Considerations

Permission was obtained from the UTH management and Pathology laboratories. Ethical approval was obtained from the University of Zambia Biomedical Research and Ethics Committee (UNZABREC).

This study did not affect patients' management during the period of study. Patients were not remunerated. All information obtained was kept confidential.

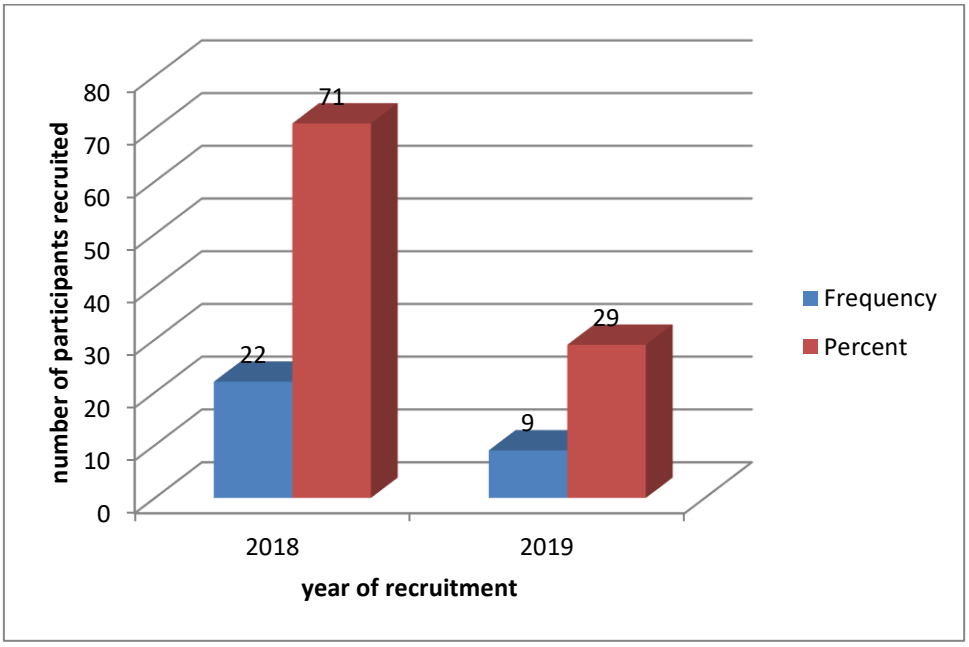
All the investigations were done by qualified personnel. The number of specimens obtained from each patient was within the acceptable guideline of obtaining at least two or three samples to increase the yield in diagnosing Hirschsprung's disease [8] [38]. The superficial-thickness biopsy procedure using an artery forceps was a minimally invasive procedure compared to the full-thickness biopsy procedure and the anticipated risks to patients included bleeding which was avoided by suturing. Further, the risks were mitigated with local anesthesia and application of pressure at puncture site respectively. A written consent was obtained from every patient.

CHAPTER FOUR: RESULTS

4.1 Year of Recruitment Distribution

Twenty-two of the participants were recruited into the study in 2018 while nine were recruited in the year 2019 representing 71 percent and 29 percent respectively (Figure 11).

Figure: 11: Year of Patient Recruitment Distribution



4.2 Characteristics of the Participants

The data was collected from the University Teaching Hospital in the department of Surgery, Paediatric unit and was analysed and processed. 31 patients were seen in the study period from the calculated sample size of 32. Therefore, the study enrolled 31 participants which represented 97 percent of the desired study sample.

Each of the participants was subjected to both procedures:

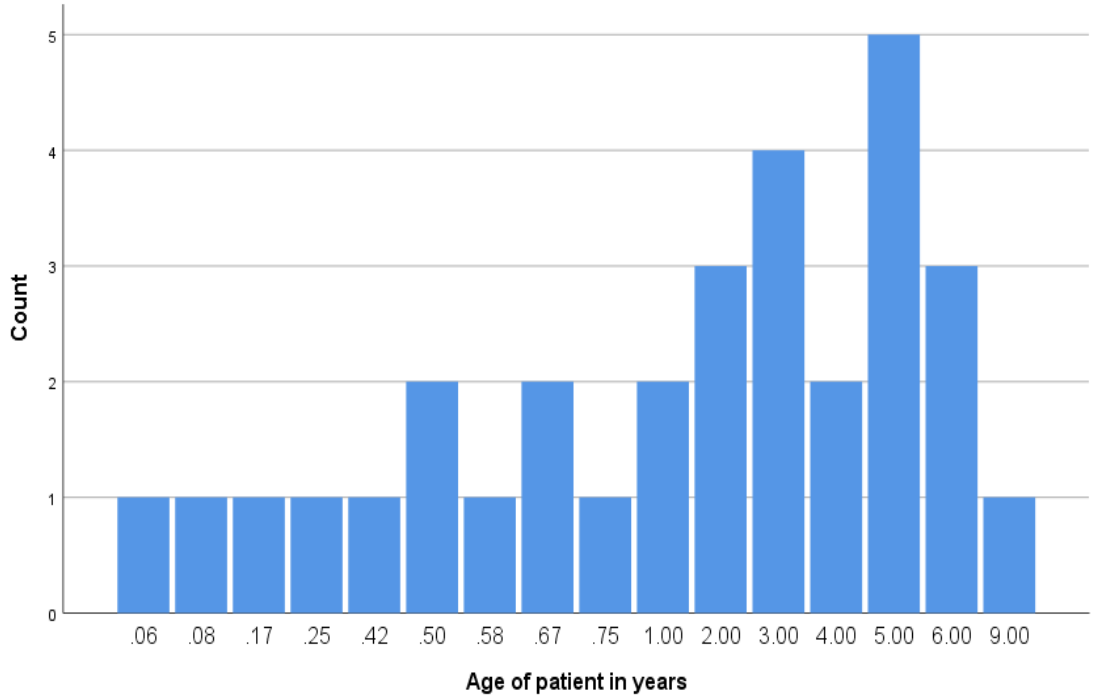
- The full-thickness rectal biopsy and

- The superficial thickness rectal biopsy using a medium curved artery forceps.

4.3 Age and Weight Distribution

The age range of the participants was between 0.06years and 9years with a mean age of 2.7 years. Check Table 4.1 and Figure 12.

Figure 12 Age Distribution



Meanwhile the weight distribution of the participants ranged from 2000grams to 28000grams, with a mean of 12729.03grams. (Table 4.1)

Table 4.1 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			

4.4. Did Patient Pass Meconium within 24 hours?

The participants under study suffered from various degrees of constipation. Of the 31 participants in the study, 10 (32.3%) had a delay in passing meconium within 24hours of birth whereas 21 (67.7%) developed constipation afterwards.

Table 4.2. Did Patient Pass Meconium within 24 hours?

Did Patient Pass Meconium within 24 hours?					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	21	67.7	67.7	67.7
	No	10	32.3	32.3	100.0
	Total	31	100.0	100.0	

4.5 Gender Distribution

In this study the participants sex distribution was 4 females and 27 males representing 12.9 percent and 87.15 percent respectively. Males made up the majority of the patients sampled.

Table 4.3 Gender Distribution

Gender of Patient					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Female	4	12.9	12.9	12.9
	Male	27	87.1	87.1	100.0
	Total	31	100.0	100.0	

Table 4.4 below shows a cross tabulation of gender against full thickness biopsy with presence of ganglion. The results show that all the females in our data set were found with ganglia and therefore are negative for the disease. Of the males in the study, 15 were positive for the disease whereas 12 where negative.

Table 4.4 Cross Tabulation of Gender Vs Full-Thickness Biopsy with Ganglion

Gender of patient * full thickness biopsy with presence of ganglion Crosstabulation				
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

From the Crosstabulation of Gender Vs. Superficial-Thickness Biopsy (Table 4.5, using the superficial-thickness biopsy procedure), specimen from 27 patients did not

demonstrate ganglion whereas four did. Table 4.5 therefore shows that 27 patients have Hirschsprung's disease. Of the 27 that were positive three were female and 24 were male.

Table 4.5 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		
		No	Yes	Total
Gender of patient	Female	3	1	4
	Male	24	3	27
Total		27	4	31

4.6 Comparing Full-thickness and Superficial-thickness Biopsy Procedure

4.6.1 Full-thickness Biopsy Vs. Superficial-thickness Biopsy Procedure for Adequate Diagnosis

The study resulted in 19 (61.30%) of the specimen collected for the full-thickness biopsy test being adequate for the test compared to 12 (38.7%) which were found inadequate (Table 4.6).

The specimen collected for the superficial-thickness biopsy using artery forceps shows that a large number, 27 (87.1%), were inadequate for making a diagnosis (Table 4.7). This is in contrast to the smaller portion of inadequate specimen found for the full-thickness biopsy procedure of 12 (38.7%). The high turnover of inadequate specimen for the superficial-thickness biopsy may be attributable to either the lack of sufficient skill in the process of collecting the specimen from the patients, or, it may point to the

inefficacy of the procedure in availing predictably adequate specimen. The former reason may require exposing the collectors of specimen to skills training whereas the latter may reveal weakness of the superficial thickness biopsy test using the artery forceps altogether.

4.6.2 Full-thickness Vs Superficial-thickness Biopsy with Presence of Ganglion

Histological analysis of the full-thickness biopsy revealed that 16 of the specimen had ganglion present which represents 51.6 percent of the total specimen found without the disease. On the other hand, 15 of the specimen ganglion were not seen implying the presence of the disease. Since only 19 of the specimen collected were adequate for the full-thickness biopsy, and since 15 were found with the disease, we conclude that 78.95 percent of the adequate specimens were positive for the disease (Table 4.9).

Table 4.6 Full-thickness Biopsy for Adequate Specimen for Diagnosis

Full-thickness Biopsy for Adequate Diagnosis					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No	12	38.7	38.7	38.7
	Yes	19	61.3	61.3	100.0
	Total	31	100.0	100.0	

Table 4.7 Superficial-thickness Biopsy for Adequate Specimen for Diagnosis

Superficial-thickness Biopsy Adequate for Adequate Diagnosis					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No	27	87.1	87.1	87.1
	Yes	4	12.9	12.9	100.0
	Total	31	100.0	100.0	

Table 4.8 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	Percent	Frequency	Percent
Yes	19	61.3	4	12.9
No	12	38.7	27	87.1
Total	31	100	31	100

Table 4.9 Full-thickness Biopsy with Presence of Ganglion

Full-thickness Biopsy with Presence of Ganglion					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No	15	48.4	48.4	48.4
	Yes	16	51.6	51.6	100.0
	Total	31	100.0	100.0	

Table 4.10 shows that 27 (87.1%) of the specimen collected using the superficial-thickness biopsy procedure with artery forceps did not demonstrate ganglia as opposed to only four (12.9%) that had ganglia and thus negative for the disease.

Table 4.10 Superficial-thickness Biopsy with Ganglion

Superficial-thickness Biopsy with Ganglion					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No	27	87.1	87.1	87.1
	Yes	4	12.9	12.9	100.0
	Total	31	100.0	100.0	

Table 4.11 Full-thickness Vs Superficial-thickness Biopsy for Presence of Ganglion

Responses	Full-thickness Biopsy with Presence of Ganglion		Superficial-thickness Biopsy with Ganglion	
	Frequency	Percent	Frequency	Percent
Yes	16	51.6	4	12.9
No	15	48.4	27	87.1
Total	31	100	31	100

4.6.3 Correlations

Table 4.12 Correlation Table

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	1	.626**	.494**	.494**
	Sig. (2-tailed)		.000	.005	.005
	N	31	31	31	31
Full Thickness Biopsy with Presence of Ganglion	Pearson Correlation	.626**	1	.185	.185
	Sig. (2-tailed)	.000		.319	.319
	N	31	31	31	31
Superficial Thickness Biopsy Adequate for Diagnosis	Pearson Correlation	.494**	.185	1	1.000**
	Sig. (2-tailed)	.005	.319		.000
	N	31	31	31	31
Superficial Thickness Biopsy with Ganglion	Pearson Correlation	.494**	.185	1.000**	1
	Sig. (2-tailed)	.005	.319	.000	
	N	31	31	31	31

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

The table above shows that 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. Comparing the full-thickness biopsy with presence of ganglion and superficial-thickness biopsy adequate for diagnosis there was an insignificant very weak positive relationship of 0.18. The other insignificant weak positive correlation of 0.18 was noticed between a full-thickness biopsy with presence of ganglion and a superficial-thickness biopsy with ganglion. The most important relationships in this case were very weak and insignificant. This also indicates that the superficial-thickness biopsy adequate for diagnosis and superficial-thickness biopsy with ganglion are not adequate and effective for adequate diagnosis and the presence of ganglion.

4.7 Specificity and Sensitivity Test for Detecting Ganglion

4.7.1 Sensitivity

The sensitivity of a clinical test refers to the ability of the test to correctly identify those patients with the disease. The test gives a sensitivity of 93.3 percent. This entails that the test detects 93.3 percent of patients with the disease (true positives) while 6.67 percent with the disease go undetected (false negatives).

4.7.2 Specificity

The specificity of a clinical test refers to the ability of the test to correctly identify those patients without the disease. The results show a specificity of 0.133. This means that the

test correctly identifies 13.3 percent patients without the disease i.e., 13.3 percent specificity correctly reports 13.3 percent of patients without the disease as test negative (true negatives) but 86.67 percent patients without the disease are incorrectly identified as test positive (false positives).

Table 4.13 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%

4.7.3 Positive Predictive Value

The positive predictive value of a test is a proportion that is useful to clinicians since it answers the question: 'How likely is it that this patient has the disease given that the test result is positive?' In other words, the positive predictive value tells you how likely it is for someone who tests positive (screen positive) to actually having the disease (true positive). It answers the question, "I tested positive. Does this mean I definitely have the disease? Our test shows a positive predictive value of 51.85 percent, 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) is 51.85 percent.

4.7.4. Negative Predictive Value

The negative predictive value answers the question, ‘If a test subject has a negative screening test, what is the probability that the subject really does not have the disease?’ negative predictive value tells us how many of test negatives are true negatives; and if this number is higher, then it suggests that this new test is doing as good as ′gold standard.′ The superficial-thickness biopsy test shows an negative predictive value of 66.67 percent which means that only 66.67 percent of the negatives are indeed true negatives.

CHAPTER FIVE: DISCUSSION

Chronic constipation is a common problem seen in children and those suspected to have Hirschsprung's disease require a rectal biopsy to make a diagnosis [9]. At University Teaching Hospital, the clinical picture aided by contrast enema help to suggest the diagnosis but a definitive diagnosis is made by histological evaluation of a rectal biopsy.

Full-thickness biopsy, which is widely practiced in Africa, includes the muscle layers of the rectal wall. The discovery that the ganglionic-aganglionic junction was at the same level for both the submucosal (Meissner's) plexus and the myenteric (Auerbach) plexus in patients with Hirschsprung's disease meant that the superficial biopsy of mucosa and submucosa alone was sufficient to make a diagnosis [9]. The rectal suction biopsy and the grasp biopsy methods were developed on this premise [9] [31]. The rectal suction biopsy method has gone on to be adopted as a gold standard for obtaining rectal biopsies in the developed world [13]. Results of studies to compare specimens obtained using grasp biopsy with different forceps and rectal suction biopsy have been shown to be similar.

The aim of this study is to determine whether the grasp method using a curved artery forceps to obtain superficial rectal biopsy is likely to provide adequate tissue to make a diagnosis.

The total number of patients enrolled was 31 out of the calculated 32, representing 97 percent of the population. The patients were enrolled from 2018 to 2019 (Fig 11).

Pankaj Gupta et al (2017) stated that although the exact worldwide incidence is unknown, international studies have reported rates ranging from approximately 1 case per 1500 –

7000 new-borns. They further stated that the disease occurs more in males than in females, with a male-to-female ratio of four-to-one [39]. However, Naima and Jamshed (2014) state that the incidence occurs five times more commonly in males than females [40]. Table 4.3 shows that most of the patients in the study were males at 87.1 percent (27). The females were at 12.9 percent (4) and thus giving the male-to-female ratio of 6.75: 1 which is similar to what is quoted in the literature.

In Africa, 20 – 40 percent present as neonates, compared to more than 90 percent in developed countries [2]. A wide variation in age distribution was noted at the time of presentation to our hospital. The ages of the patients in the study ranged from three weeks to nine years. The mean age at presentation was at 2.7 years while the peak age was at five years (Fig 12). This shows that most of the children presented beyond the neonatal period thus delaying the diagnosis of the disease. This delay in presentation in our study could be due to lack of awareness and absence of proper referral systems from primary care clinics, patients living in remote isolated areas with difficult to reach health centres and preference to seek traditional options before seeking medical advice.

The weight distribution ranged from two kilogrammes to 28 kilogrammes. The mean weight was 12.7 kilograms. (Table 4.1). This implies that most of the patients were generally of the right weight for age. However it is noted in literature that most of patients who suffer from Hirschsprung's disease generally have a poor nutritional status as the child grows.

The symptoms in the study included presence or history of delayed passage of meconium and abdominal distention in neonates whereas for the older children included chronic

constipation in addition. Rectal biopsies should be performed on all patients suspected to have Hirschsprung's disease as there is no clinical parameter that can adequately predict which patients do not require biopsy. [8]. Although studies have been done to try and reduce on unnecessary biopsies, other studies have suggested that selecting patients for rectal biopsy on +othe basis of clinical criteria would lead to many patients with Hirschsprung's disease (in one study up to 10%) being missed or a delay in diagnosis [8][41].

This study compared two procedures of obtaining rectal biopsies, the full-thickness rectal biopsy considered as the gold standard and the superficial rectal biopsy using an artery forceps as the proposed instrument.

A specimen rectal biopsy is considered to be adequate if it at least has one-third to one-half of submucosa [24]. Hirsch et al (2011) however, stated that even biopsies with a moderate amount of submucosa, or only included mucosa, this does not pose a problem for diagnosis because ganglion cells typically adhere to the mucosa as the submucosa is torn away during biopsy [28]. In this study, 61.3 percent (19) specimen obtained using the gold standard were reported to have submucosa compared to 12.9 percent (4) obtained with the proposed method (Tables 4.6, 4.7, 4.8). This implies that the proposed procedure lags behind by a very wide margin in obtaining adequate samples. Muise and Cowles (2016) stated that inadequate rectal biopsies with insufficient submucosa occur in up to 26 percent. However in this case, the proposed instrument resulted in 87.1% (27) inadequate specimens compared to 38.7% using the full-thickness biopsy. Inadequate biopsies results in diagnostic delays due to need for repeat biopsy and hence longer hospital stay, increased cost of treatment, prolonged parental anxiety and unnecessary

work for the pathologist [1] [10]. The delay in their definitive management represents extra time during which complications like enterocolitis can occur [8]

Specimen obtained using the full-thickness rectal biopsy showed that 48.4 percent (15) had no ganglion implying a diagnosis of Hirschsprung's disease, and 51.6 percent (16) were normal (Table 4.9). For the superficial rectal biopsy 87.1 percent (27) had no ganglia while 12.9 percent (4) were normal (Table 4.10). These results imply that specimens obtained by the superficial-thickness rectal biopsy missed 25 percent (12) of the 16 cases that were found normal using the full-thickness rectal biopsy procedure (Table 4.11). 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 [28].This implies that the proposed method would result in a number of normal patients being wrongly diagnosed with the disease.

The high number of false positives reported for Hirschsprung's disease using the proposed instrument to obtain superficial rectal biopsy in this study compared to the standard full-thickness biopsy may be attributed to obtaining biopsy material that was too superficial and not containing the muscularis mucosa. In addition, since biopsies were obtained at an estimated distance of 1.5cm above the dentate line to avoid the physiological agangliotic zone, and that the number of biopsies were restricted to one biopsy for each method, there was no objective way of ensuring that none of the biopsies were from within the agangliotic zone. Studies have shown that the probability of obtaining adequate sample or identifying ganglion cells increases with more biopsies of

at least two or more above the dentate line at succeeding higher levels [3]. Even though the two biopsy specimens were obtained at the same time from each patient by the researcher, studies have shown that the level of the surgeon performing the biopsy does not impact on the yield. [8]. In another study by C.L. Stewart et al. 2016, it was shown that pediatric surgeons were significantly more likely to perform adequate biopsies compared to gastroenterologists. The same study further states that the adequacy of a rectal biopsy is related to the instruments used and the thickness of the rectal wall. [42]. The proposed instrument in this study did not perform as expected in obtaining adequate superficial biopsies because a high number of normal patients were wrongly diagnosed with the disease implying an increased number of cases would require a re-biopsy.

The correlation results (Table 4.12) shows that there is a significant strong positive correlation between the full-thickness rectal biopsy for adequate diagnosis and the full-thickness biopsy with presence of ganglion presented by 0.63**. This signifies that the specimen obtained using this procedure is likely to demonstrate the presence of ganglia in a normal patient. 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. Comparing the full-thickness biopsy with presence of ganglion and superficial-thickness biopsy adequate for diagnosis there was an insignificant very weak positive relationship of 0.18. Thus a sample obtained from a suspected patient with normal bowel is less likely to demonstrate the presence of ganglia when superficial-thickness procedure is used. The other insignificant weak positive correlation of 0.18 was noticed between a full-thickness biopsy with presence of ganglion and a superficial-thickness biopsy with ganglion. The most important relationships in this case were very weak and insignificant. This also indicates that the superficial-

thickness biopsy using artery forceps was not able to obtain adequate specimen with ganglion in normal patients for the disease to be ruled out.

The proposed test gave a sensitivity of 93.23 percent and a specificity of only 13.33 percent (Table 4.13). The high sensitivity implies the test is able to detect patients with the disease but the low specificity implies that it is not able to detect those without the disease. This therefore means that this test will be diagnosing those with the disease together with those without the disease as being positive for Hirschsprung's disease because of the high sensitivity and poor specificity.

The positive predictive value was at 51.85 percent whereas the negative predictive value was found to be 66.67 percent (Table 4.13). This further reinforces the fact that this proposed procedure would inadvertently result in a lot more patients being missed out.
None of the patients who were biopsied suffered any complications during or after the procedure. The complication rates for full-thickness and rectal suction biopsies have been reported at 6.6 percent and from 0 to 15 percent respectively. 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 the nature of the biopsy is more superficial unlike the full-thickness biopsy. All full-thickness biopsy sites were sutured after obtaining a specimen. The superficial thickness biopsy sites using curved artery forceps were not sutured but packed with Vaseline gauze except in 13 patients who showed oozing of blood who were sutured. This demonstrates that the proposed method may be more cost effective in terms of use of consumables during the procedure.

The cost of taking a biopsy may be reduced somewhat if the procedure can be performed as out-patient procedure. In a study involving the use of jumbo biopsy forceps, it was stated that the procedure could be performed in a cooperative, nonanxious patient just as a suction biopsy by the bedside [10]. This meant eliminating the need for thearter and accompanying staff as well as anesthesia. The forceps in our study however, was not able to provide adequate specimens for diagnosis hence making it a poor choice to consider for use altogether.

Whereas in some centres it has been demonstrated that grasp and cut biopsy of obtaining superficial rectal biopsy is adequate to demonstrate whether one has Hirschsprung's disease or not, this study has failed to show similar results. Furthermore, this study demonstrates that at times improvisations that appear to work come to fall short of expectations. This calls for higher authorities to make available the specific tools for such procedures. It is against this background that the full-thickness biopsy continues to remain the only plausible procedure for adequate diagnosis of Hirschsprung's disease at the University Teaching Hospital and Zambia at large.

CHAPTER SIX: CONCLUSION

The proposed method gave only 4 (12.9%) adequate specimen for the diagnosis of Hirschsprung's disease compared to 19 (61.3%) using the full thickness method.

The proposed method gave a low diagnostic yield of 4 (12.9%) compared to 16 (51.6%) using the traditional full thickness biopsy.

No complications were recorded in the whole duration of the study on any of the patients.

This study, therefore, demonstrates that the superficial-thickness rectal biopsy using artery forceps on 31 patients at University Teaching Hospital has a high sensitivity and poor specificity and low predictive value implying that it stands a poor chance of being adopted as a diagnostic tool for the diagnosis of Hirschsprung's disease.

6.1 Study Limitations

Some limitations had been identified as the study was taking place as follows:

(i) The study was dependent on patients scheduled for full-thickness biopsy, and thus successful enrolment of patient was limited to those patients with chronic constipation or history of failure to pass meconium within 24 hours. In other words, the sampling procedure was convenient and not strictly random.

(ii) A large number of specimens collected for the superficial-thickness biopsy procedure were found to be inadequate due to possibly the collection technique of the proposed instrument, the curved artery forceps.

(iii) Inability to perform multiple biopsies for each method which has been shown to increase the diagnostic yield

(iv) Inability to stain specimen for acetylcholinesterase to increase diagnostic yields

6.2 Recommendations

(i) Continue with the traditional full-thickness biopsy procedure

(ii) Although the curved artery forceps did not perform as well as expected in obtaining adequate biopsies, studies have demonstrated that some forceps perform as well as rectal suction biopsy equipment and hence further studies would be needed to compare the different forceps in future with a larger sample size.

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APPENDICES

Appendix I: Variables

S/N	Categorical Variables	Non-categorical Variables
1	**Sex** - Gender of patient	**ID** - ID of patient
2	**Religion** - Religion of patient	**Weight** - Weight of patient in grams
3	**HIV** - HIV status of patient	**Age -** Age of patient in years
4	**Pmlt24** - Did patient pass meconium within 24 hours?	
5	**lcc** - local causes of constipation present	
6	**fba** - full thickness biopsy for adequate diagnosis	
7	**fbg** - full thickness biopsy with presence of ganglion	
8	**sba** - Superficial thickness biopsy adequate for diagnosis	
9	**sbg** - Superficial thickness biopsy with ganglion	

Appendix II: Data Collection Tool

A. History Taking

Participant Number: Date enrolled:

Name:

Date of birth: Weight:

Age at presentation:

Age at surgery (if performed):

Mode of delivery:

- Vaginal Delivery ☐ Yes ☐ No
- Caesarean section ☐ Yes ☐ No

Maturity and gestational age at delivery:

- Preterm (<37 weeks Gestation) ☐ Yes ☐ No
- Term (≥37 weeks Gestation) ☐ Yes ☐ No

Perinatal morbidity ☐ Yes ☐ No

Medical disease of importance (confirmed and reinforced in general examination):

Associated anomalies (confirmed and reinforced in general examination):

Number of siblings and order among them:

Family history (occurrence of relatives and siblings with similar conditions):

Consanguinity of the parents:

Description of the first presentation (regarding the timing, the complaint and the actions taken):

Details of previous admissions for attacks of

- Enterocolitis:

- Intestinal obstruction :

Course of the disease:

Compliance to laxative treatment and response to conservative measures (especially in cases were surgery was excluded due to a diagnosis of idiopathic constipation):

☐ Compliant ☐ Not Compliant

Postoperative complications and follow-up (in cases were surgery was performed):

B. General Examination

Confirming the presence or absence of associated medical diseases of importance and associated congenital anomalies:

Assessment of

- Nutritional status:
- Weight:

Assessment of readiness for further procedures or surgery: ☐ Fit ☐ Not fit

Exclusion of the presence of enterocolitis prior to contrast enema/biopsy

Fever ☐ Yes ☐ No

Diarrhea ☐ Yes ☐ No

Abdominal Examination

Assessment of the abdominal girth and the pattern, course, distribution and degree of distension if present:

Assessment for signs of intestinal obstruction:

- Visible peristalsis ☐ Yes ☐ No
- Visible congested/distended veins along abdominal wall ☐ Yes ☐ No

Rectal Examination

Assessment of the caliber and status of the anorectum

- Presence of any tightness or spasticity:
- Finger withdrawal test:

Exclusion of the presence of any local causes for constipation; covered anus, anal fissures or perianal suppuration.

☐ Present ☐ Not Present

Exclusion of the presence of enterocolitis prior to contrast enema/biopsy (confirming absence of fluid explosive diarrhea on examination)

☐ Present ☐ Not Present

Blood Results

HIV Status ☐ P ☐ N

C. Procedure done

- Full-thickness rectal biopsy Number of samples ☐
- Grasp biopsy with artery forceps Number of samples ☐
- Adequacy of specimen; submucosa present ☐ Y ☐ N

  Adequate for Diagnosis ☐ Y ☐ N

Results

Method used	Ganglion Present	Ganglion not seen
-Full-thickness Rectal Biopsy	☐	☐
-Grasp Biopsy	☐	☐

Appendix III: Patient Information Sheet

Comparing Rectal Biopsy Using Artery Forceps and Full-thickness Rectal Biopsy in Diagnosing Hirschsprung's Disease at The University Teaching Hospital, Lusaka

Introduction

I, Chizoma Grainer, a Master of Medicine (MMED) student in Pediatric General Surgery in the School of Medicine at the University 0f Zambia, hereby request your participation in the above mentioned study. This study is in partial fulfilment for the award of a Master of Medicine in Pediatric General Surgery. I kindly request you to carefully read this document and ask me anything you don't understand. I want you to understand the purpose of the study and what is expected of you. Kindly remember that participation in this study is absolutely voluntary. If you agree to take part in this study, you will be asked to sign this consent form in the presence of a witness.

The aim of the study

The purpose of the study is to assess the accuracy of making a diagnosis of Hirschsprung's disease from specimen obtained using the grasp biopsy compared to the full thickness biopsy at the University Teaching Hospital. This procedure if found accurate will help surgeons obtain tissue samples much more easily and quickly with the likelihood of becoming an outpatient procedure especially in the older children.

Procedure of the study

If you (Parent/Guardian) agree to participate in this study, we will obtain information about you and the patient with the aid of a questionnaire. Your contact details will be required. The study will not interfere with the preliminary investigations required to be done concerning collection of Blood samples for baseline investigations and establishing the status of the patient. X-rays with contrast enemas will be obtained at the radiology department when deemed necessary in the preliminary investigations to suggest the diagnosis and confirmation of the transition zone. For the definitive diagnosis of the condition, biopsy samples will be obtained using the two methods. Rectal biopsy is a procedure involving the acquisition of tissue sample from the rectum with the patient put under general anaesthesia. The current method of obtaining tissue samples involves obtaining thick tissue samples involving most layers of the rectal wall. The proposed method will obtain only superficial tissue samples that is believed will be adequate enough to make a diagnosis of the condition. The results will be kept confidential, however, they can be availed to you if you so wish.

Possible risks and discomforts

Participation in this study will not expose the patient to any additional risks. However, the definitive diagnosis of this condition requires obtaining tissue from the rectum.

Commonly recorded complications are bleeding and perforation although these are rare. The proposed method will not increase on the risk because the tissue sample will be obtained from the superficial layers of the rectal wall. The patient will be in admission following the operation and should any complication arise, appropriate interventions will be instituted to arrest the situation. The patient will benefit from a uniform and effective assessment, diagnosis and eventual treatment of the condition.

Confidentiality

All the information collected is strictly confidential. Data that will be collected, analysed, and reported on will not include the name of the patient and therefore cannot be traced.

Consent

Participation is absolutely voluntary and can withdraw freely from the study at any time for any reason without any consequences.

Should there be any concerns or need for any clarifications, please contact Dr. Chizoma Grainer or The University of Zambia Research Ethics Committee (UNZAREC) on the following respective addresses;

Dr. Chizoma Grainer

Department of Surgery

Pediatric Surgery

University Teaching Hospital

P.O Box 51292,

Lusaka.

Phone Number: +260974120082, or

The University of Zambia Research Ethics Committee,

School of Medicine

Ridgeway campus

Nationalist Road

Lusaka.

Phone Number: +260-211-256067

E-mail: unzarec@unza.zm

Appendix IV: Consent Form

I, _____ hereby confirm that the nature of this clinical study has been sufficiently explained to me. I, as the guardian/parent of the patient, am aware that personal details will be kept confidential and I understand that I may voluntarily, at any point, withdraw my child's participation without suffering any consequences. I have been given sufficient time to ask questions and seek clarifications, and of my own free will declare my child's participation in this research.

I have received a signed copy of this agreement

_____ _____ _____

Name of Participant (Print) Guardian/Parent of Participant (Signature or thumbprint) Date

_____ _____ _____

Witness (Print Name) Witness (Signature) Date

_____ _____ _____

Researcher (Print Name) Witness (Signature) Date

Appendix V: Child Assent Form

I am Dr Chizoma Grainer from the University Teaching Hospital. I am doing a study to find out if a proposed method of getting a tissue sample from the rectum will be as good as the method we always use. This proposed method will get a sample of tissue that will be more superficial compared to the method we always use. Each method will be used to get a sample of tissue from the rectum so that two samples will be submitted histological diagnosis. You will not feel any pain during this process as you will be put to sleep in theatre.

The results that will be obtained will be communicated to you and your guardians/parents.

If you do not want to take part in this study, you do not have to, and if you feel as though you would like to stop at any point during the study, you are free to do so.

You should discuss with your parent/guardian before you agree to take part. Your parents will be spoken to and will be asked for permission for you to participate, but if you do not want to, you do not have to.

If you have any questions, feel free to ask them, now or later, and I will do my best to answer them. If you think of a question later, you or your parents can contact me on +260-974-120-082, or find me at the University Teaching Hospital in the department of surgery.

Sign this form only if you:

• Have understood what will happen to you during the study

• Have had all your questions answered

• Have talked to your parents/ guardian about the study

• Agree to take part in this study

I _____ (Participants name) in the presence of my parents/guardian and with their consent, do agree voluntarily to participate in this study.

Signature/thumb print: _____ Date _____

Investigators name: _____

Signature/thumb print: _____ Date: _____