A REVIEW OF HIRSCHSPRUNG'S DISEASE IN HOSPITAL UNIVERSITI SAINS MALAYSIA 1999-2004

By

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4. ABBREVIATIONS

ARM	Anorectal Manometry
HUSM	Hospital Universiti Sains Malaysia
ENS	Enteric Nervous System
ERPT	Endorectal pull-through
NC	Neural Crest
NCAM	Neural cell adhesion molecule
MEN	Multiple Endocrine Neoplasia
FTMC	familial medullary thyroid cancer
GDNF	Glial Cell line derived neurotrophic factor
GDNF R- α	GNDF receptor alpha
NTN	Neurturin
PSP	Persephin
ART	Artemin
WS4	Waardenberg Shah Syndrome
EDNRB	Endothelin B receptor
EDN-3	Endothelin 3
ICC	Interstitial Cell of Cajal
NO	Nitric Oxide
VIP	Vasoactive Inhibitory Peptide
HAEC	Hirschsprung's Associated Enterocolitis
GIT	Gastro intestinal tract
CNS	Central nervous System

CE	Contrast Enema
H & E	Haematoxylin and Eosin
TCA	Total Colonic aganglionosis
IAS	Internal Anal Sphincter
TEPT	Transanal Endorectal Pull-through
TAPT	Transanal Pull-through
HD	Hirschsprung's Disease
PFO	Patent Foramen Ovale
BCLP	Bilateral Cleft lip and Palate
G6PD	Glucose-6-phosphate dehydrogenase
RAIR	Rectoanal Inhibitory Reflex
SPSS	Statistical Program for Social Studies

5. List of Flow Charts, tables and figures.

6. Abstrak:

Pengenalan

Penyakit Hirschsprung ialah penyakit masalah perkembangan saraf saluran pemakanan yang menyebabkan pengembangan usus besar yang melampau and menyebabkan masalah usus tersumbat pada masa neonat. Hospital USM adalah satu-satunya unit surgeri pediatrik di utara pantai timur M\laysia sehingga hujung tahun 2005. Semua rujukan kes berhubung dengan penyakit 'Hirschsprung' telah dirawat di sini. Secara amnya perawatan bermula dengan pencucian rektum sehingga diagnosa yang pasti telah didapati melalui biopsi rektum. Selepas itu pembedahan untuk membuat kolostomi akan dilakukan. Pada umur bayi lebih kurang setahun atau berat badan mencapai 10 kilogram, pembedahan definitif akan dijalankan. Sebelum tahun 2004 pembedahan yang selalunya dijalankan di Hospital USM ialah prosedur Duhamel. Selepas pembedahan ini pesakit akan menjalani satu lagi pembedahan untuk menutup kolostomi mereka.

Objektif:

Kajian ini adalah bertujuan untuk mendapatkan data mengenai penyakit ini dalam konteks demografik, simptom awalan dan umur pesakit yang menunjukkan simptom penyakit 'Hirschsprung' dan waktu diagnosa. Data mengenai cara diagnosa, cara perawatan, kesan jangka panjang dan masalh masalah mendiagnosis pesakit pesakit juga dikaji.

Metodologi :

Kajian ini adalah retrospektif yang merangkumi semua pesakit yang didiagnosakan mempunyai penyakit ini dan menjalani pembedahan definitif mereka di hospital ini. Tarikh kajian ini adalah dari Februari 1999 sehingga Februari 2004; merangkumi 5

tahun kajian. Pesakit yang tidak menerima nasihat pembedahan definitif tidak dimasukkan ke dalam kajian ini.

Keputusan:

Terdapat 94 pesakit yang dapat dikaji untuk kajian ini. Nisbah lelaki kepada perempuan ialah 4:1. Umur pesakit menunjukkan simptom simptom penyakit ini adalah 2 hari sehingga 13 tahun. Tujuh puluh tiga peratus (73.4%) pesakit datang ke hospital dalam umur kurang dari 3 bulan. Simptom simptom adalah seperti berikut; kembung perut (87.2%), muntah (61.7%), tidak mahu menyusu (55.3%), tidak membuang mekonium sejak lahir (47.9%) dan sembelit (46.8%). Tujuh belas peratus (17%) daripada pesakit mempunyai kecacatan lain dan 3.2% mempunyai adik beradik yang mempunyai penyakit yang sama. Enam puluh tiga peratus (63.4%) didiagnosakan menggunakan biopsi rektum secara sedutan. Sembilan puluh lima peratus (95.7%) daripada pesakit menjalani pembedahan Duhamel untuk rawatan definitif, 3 'transanal pull-through' dan 1 prosedur Soave. Terdapat satu kematian dan ia disebabkan oleh jangkitan enterokolitis. Pesakit ini juga menghidapi sindrom Down dan penyakit kongenital jantung berlubang.

Dalam rawatan susulan 74% tidak mempunyai masalah besar seperti sembelit, enterokolitis, hilang kawalan untuk membuang air besar dan keperluan untuk pembedahan tambahan. Terdapat 10.6% daripada pesakit yang memerlukan pembedahan tambahan. Lima orang pesakit telah menjalani pembedahan definitif semula.

Kesimpulan:

Data demografik pesakit adalah hampir serupa dengan yang telah dikaji di tempat tempat lain. Simptom awalan yang membawa kepada diagnosa juga hampir sama

dengan kajian kajian lain. Pembedahan yang dijalankan di Hospital USM adalah majoritinya prosedur Duhamel pada masa kajian dijalankan dan sedang menuju ke arah satu fasa pembedahan sahaja pada masa kini. Pembedahan yang dilakukan adalah selamat dan tiada komplikasi yang membahayakan pesakit.

7. Abstract:

Introduction

Hirschsprung's disease is a developmental disorder of the enteric nervous system causing congenital megacolon and the commonest cause of intestinal obstruction in the neonatal period. Being the only Pediatric surgical unit until the end of 2005 Hospital USM undertook all the references for Hirschsprung's disease in the upper east coast region of Malaysia. The treatment of the disease started when a patient is suspected of having the disease clinically. The patient would receive rectal irrigation until the diagnosis is confirmed by rectal biopsy. When the diagnosis is confirmed the child would have a colostomy created. When the child reached about one year old or weight of 10 kilograms, the definitive procedure would be done. Until 2004, the procedure of choice in the hospital was Duhamel procedure. After the definitive procedure, the colostomy would be closed.

Objective:

The objective of the study is to review the children presenting with Hirschsprung's disease to the hospital and described the demographics, mode and age of presentations, diagnosis, operative treatment, complications and outcome of the patients.

Methodology:

The study was retrospective review study of the patients who were diagnosed and had their definitive pull-through procedure in the hospital during a period of 5 years (from February 1999 – February 2004). Patients who defaulted prior to the definitive procedure were excluded.

Results:

There were 94 patients whose data was available for the review. There were 4: 1 male to female ratio with age of presentation ranging from 1 day old and 13 years old with 73.4% presented within the age of 3 months. The most common of clinical presentation was abdominal distension (87.2%) followed by vomiting (61.7%), poor feeding (55.3%), delayed passage of meconium (47.9%) and chronic constipation (46.8%). Seventeen percent (17%) of patients had other associated anomalies and 3.2% had a family history. Sixty three percent (63.4%) were diagnosed with rectal suction biopsy. Ninety five percent (95.7%) of patients had Duhamel type of pull-through procedure with 3 had transanal pull-through and only one who had Soave. There was a mortality recorded for the study and it was attributed to enterocolitis. The patient was also having Down syndrome and congenital heart disease.

In the follow up period an average of 74% were free of complications of constipation, enterocolitis, incontinent, redo operation. There was 10.6% reoperation rate. Five patients needed a complete redo of their definitive procedures.

Conclusion

The demographics finding of the study is quite similar to the patterns in the other parts of the world. The presenting symptoms were also quite similar to other studies. The definitive procedure of choice during the study time was Duhamel operation. The operative outcome was safe and acceptable.

1. INTRODUCTION

Hirschsprung's disease is a developmental disorder of the enteric nervous system (ENS) characterized by absence of ganglion cells in the myenteric and submucosal plexuses along a variable portion of the distal intestine. It is the most common cause of bowel obstruction in the neonatal period.[1]

In the 1691 Frederick Ruysch described a 5-year old child who might have Hirschsprung's disease who died of intestinal obstruction and constipation. In 1800 Domenico Battini's account of a child with congenital megacolon was published after his death[1]. In 1887, Hirschsprung, in his original report, described 2 patients, aged 9 and 11 months, with megacolon. Both died, and at postmortem examination he described severe inflammation and ulcer formation in the colon[2].

Tittel in 1901 was the one who first noted the absence of ganglion cells in the distal colon of a 15-month old child with Hirschsprung's disease[1]. Then it was established that abnormalities of innervation within the colon and the absence of ganglion cells is pathognomonic of Hirschsprung's disease.

Ehrenpreis noted in 1946 that the proximal colon was dilated and hypertrophied due to distal obstruction[1]. Whitehouse and Kernohan presented summary of the literature and a series of their own patients that documented aganglionosis within the distal colon or rectum was the cause of the functional obstruction[1]. In 1949, Swenson et al published the discovery of the cause of the disease was based on a series of clinical observations indicating that there was a defective segment of distal colon producing a partial bowel obstruction. Peristaltic tracings of the dilated proximal colon recorded progressive

contractions[3]. However, this peristaltic wave did not enter the more distal narrow segment. This was suggestive evidence of a physiologic defect in that distal segment. Therefore, an operative technique was devised. In 1949, Swenson and colleagues published an article recommending rectosigmoidectomy with preservation of the sphincters as the optimal treatment of this disease[3]. The procedure was called Swenson pull-through. However, this initial procedure caused a lot of enterocolitis episodes which led to modifications of the procedure.

In 1956 Bernard Duhamel proposed a retro-rectal and trans-anal pull-through operation for the treatment of Hirschsprung's disease[4]. The procedure, originally intended to be palliative, rapidly became a radical method. As experience was gained, certain problems appeared which induced several authors to design improvements and modifications of the details of the original technique[4].

The Soave procedure (or endorectal pullthrough) was first performed in the 1960 by Franco Soave.[3] This operation was designed to avoid injury to pelvic vessels and nerves and protect the internal sphincter, all of which are theoretically at risk during the Swenson and Duhamel procedures. The operation consists of a mucosal proctectomy with preservation of the rectal muscular cuff, and the normally innervated colon is pulled through the muscular cuff and anastomosed just above the dentate line. In the original description the pulled-through bowel was left hanging out for several weeks and relied on scarification of the 2 segments of intestine to support the anastomosis. Boley's modification of the Soave procedure in 1964 , in which the anastomosis is performed primarily, is employed by most surgeons today.[3] Other approaches that are used more frequently outside of North America include the

Rehbein procedure and the use of long myectomy without resection. The Rehbien operation involves a somewhat higher anastomosis than the previously mentioned operations, although long-term follow-up suggests very good results in experienced hands[4].

Recently, the multi-stage approaches have been challenged. The use of a primary endorectal pull-through (ERPT) in the management of neonates with Hirschsprung's disease represents a significant change from the classic approach to its treatment. The first successful report of a primary pull-through for Hirschsprung's disease came from So et al in 1980. [5] Subsequently, due to the simplified nature of this approach and the potential for cost savings, several groups have reported on the use of this procedure and its safety, therefore improving morbidity and mortality rates in infants with Hirschsprung's disease.

This study documented the number of patients treated in Hospital USM for the period of 5 years from February 1999 to February 2004. Until late 2005, Hospital USM was the only Pediatric Surgical Unit in the East Coast of Malaysia.

2. LITERATURE REVIEW

2.1 Epidemiology

Hirschsprung's disease occurs in about 1 in 5000 live births (1/5257 US, 1/3070 Oman, 1/7165 Australia and 1:4697 in Japan).[3, 6-8] The male to female sex ratio is 4:1.

The ratio however, dropped to 1:1 in total colonic aganglionosis. There is no association between race, geographical area and incidence of aganglionosis. Approximately 10% of children with Hirschsprung's have positive family history, especially those with longer segment disease.[9]

2.2.Pathology

Grossly the features of Hirschsprung's disease depend on the time of it being diagnosed with transition zone features being more obvious. Classically the appearance of the bowel was contracted ganglionic segment, funnel shaped transition zone and dilated proximal normal colon.

The absence of ganglion cells in a variable length of distal bowel commencing from the rectum upwards thus results in a normal caliber rectum with dilatation of the normally innervated proximal colon. The absence of the ganglions is thus the essential cause of Hirschsprung's disease. As the ganglia are part of the enteric nervous system that coordinate the motility of the bowel, their absence made the bowel become dysmotile, aperistaltic and causes the symptoms classical to the disease.

2.2.1. Etiology

The most widely accepted etio-pathogenic hypothesis is based on a defect of craniocaudal migration of neuroblasts originating from the neural crest (NC), that under normal circumstances reach the small intestine in the 7th week of gestation and the rectum in the 12th week.[11] The earlier the migration arrest, the longer the distal aganglionic intestinal portion.

The neural crest is one of the earliest organs to form within the developing embryo. The cells contribute to a vast amount of structures throughout the body. They follow migratory pathways that are dependent on their axial level of origin. Once they reach their final destination, neuroblasts differentiate into numerous cell types which include adrenal medulla, neurons and glia of the sympathetic and parasympathetic nervous system, melanocytes and neuroendocrine cells[10].

In 1974 Bolande suggested neurocristopathies for the conditions arising from neural crest derived tissues, and divided them into simple and complex. Hirschsprung's disease was classified as a simple neuroscristopathy because it is characterized by a single pathological process, of unifocal and localized condition[11].

The ganglion cells of the ENS (enteric nervous system) thus originate from the neural crest. These cells migrate first into the Auerbach's (myenteric) plexus and then into Meissner's (submucosal) plexus in a cranio-caudal fashion as mentioned before.

The vagal neural crest generally is considered to be the source of both neurons and supportive cells of the ENS along the digestive tract. The vagal neural crest also

originates cardiac ganglia and is involved in the development of cardiac outflow tract, thymic stromal cells and the parathyroids[11].

Using ablation techniques Peters-Vander Sanden et al, studied regional differences within the vagal neural crest with regard to the formation of the ENS.[11] They were able to show that ablation of the total vagal neural crest resulted in total aganglionosis from anus through midgut, the foregut (esophagus, stomach and duodenum) being normally ganglionic. Ablation of the vagal crest adjacent to somites 3 to 5 resulted in aganglionosis of the hindgut only. Ablations of the vagal neural crest not including this segment had no effect on the formation of the ENS[11].

In any case to be able to contribute to ENS development, NC cells have to leave the neural tube, migrate to the gut, enter it at some point and start their cranio-caudal migration along the hind gut. These events are regulated by molecular and cellular mechanisms that are not completely understood[12].

The other theory is that neural crest cells arrived at their destination but then failed to survive, proliferate or differentiate due to abnormalities within their microenvironment[13]. Fibronectin and laminin are glycoproteins that facilitate neural cell migration and development. Gaillard et al have shown that there is abnormal distribution of these proteins in the bowel of patients with Hirschsprung's disease which may prevent the migrations of ganglion cells into the extracellular matrix. [11] Langer et al showed abnormal cell to cell interactions between aganglionic smooth muscle and ganglion cells. [14]

Other studies have shown differential expression of the neural cell adhesion molecule (NCAM) in aganglionic compared with normal bowel. [11] The absence of neurotrophic factors such as neutrophen was also noted in patients with Hirschsprung's disease.[15] These support the concept that the microenvironment may also play a role in producing the distal aganglionosis that is characteristic of Hirschsprung's disease.

2.2.2. The major gene for Hirschsprung's disease

Approximately 10% of children with Hirschsprung's disease have positive family history, especially those with longer segment disease. Males are 4 times more likely to be affected than females [16]. Siblings of female index patients have a 360 fold increased risk and siblings of male patients have a 130 fold risk of developing Hirschsprung's disease[1]. Children with Down syndrome and other genetic, abnormalities also have a higher incidence of Hirschsprung's disease and the incidence of associated congenital anomalies is approximately 20%[1].

The first step toward understanding of the molecular basis of Hirschsprung's disease as well as the nature of its genetic transmission was the observation of a young female patient with total colonic aganglionosis carrying a de novo interstitial deletion of chromosome 10 (46xx, del10q11.21-q21.2).[11] Human-hamster somatic cell hybrids retaining the deleted (Hy185-O) and the non-deleted (Hy179-Q) chromosome 10 were produced from lymphocytes of the total colonic aganglionosis patient using an immunomagnetic positive selection method . The availability of these 2 somatic cell hybrids allowed the mapping of a series of chromosome 10-specific polymorphic markers, either inside or outside the deletion[11].

Two studies, 1 using a cohort of 15 families with Hirschsprung's disease and the other done by analyzing Hirschsprung's disease pedigree, confirmed that the genetic abnormality was in the proximal portion of chromosome 10[11].

The refinement of the genetic and physical maps of the proximal portion of the long arm of chromosome 10 found genes for multiple endocrine neoplasia type 2A and 2B (MEN 2A, MEN2B) as well as the gene responsible for familial medullary thyroid cancer (FMTC). Two additional interstitial deletions of chromosome 10q associated with Hirschsprung's disease were observed. The characterization of the smallest region of overlap (SRO) among the 3 deleted chromosomes 10 allowed narrowing the candidate Hirschsprung's disease region to an interval of less than 250kb [11]. The RET proto-oncegene was the only cloned gene known to be located in this interval [1].

RET proto-oncegene encodes a transmembrane receptor tyrosine kinase on 10q11.2 and encodes 20 exons. Receptor tyrosine kinases are essential for normal growth and differentiation of cell lines. RET knocked out mice show few neural crest cells, which do not migrate beyond the esophagus, defects in sympathetic innervations, as well as the absence of renal development[11]. Mutations in the RET proto-oncogene have been found in 17-38% of children with short segment Hirschsprungs disease, and 70-80% of those with long segment involvement. Missence and nonsense mutations and a few base-pair deletions or insertions of the RET proto-oncegene have been identified since then in Hirschsprungs disease patients by different groups.[11]

Other genetic alterations may also be the cause Hirschsprungs disease, as only 50% of familial and 20% of sporadic cases showed RET proto-oncegene mutations.

RET polymorphism may also predispose to the isolated or sporadic from of Hirschsprungs disease. Strong association between specific alleles of the RET proto-oncegene and Hirschsprung's disease has been noted, specifically c135g/A and c2307G that may potentially cause Hirschsprung's disease in the recessive or in dose dependent manner.[18] Patients with MEN 2B often developed ganglioneuromatosis of the gastrointestinal tract, which can be associated with distension, megacolon and loss of tone despite histological normal ganglion cells [19].

A possible explanation for coexistence of MEN 2 and Hirschsprung's disease could be activation of apoptosis in the enteric ganglion cells in response to an inappropriate mitogenic signal transduced by the gain of function mutation of the RET proto-oncegene [1].

Glial cell line derived neurotrophic factor (GDNF) is the first RET receptor ligand identified GDNF is proposed to act as a ligand for a multisubunit receptor in which the glycosylphosphatidylinositol-linked protein (GDNF R-α) provides the ligand binding, and RET provides the signalling component. Functional assays have shown that, in the absence of either GDNF or GDNFR -α, RET signaling is reduced or absent [11]. Because RET mutation did not account for all the Hirschsprungs disease cases studied, GDNF appeared to be an ideal candidate for mutation analysis studies. Martucciello et al in the Gaslini Children's Hospital confirmed the infrequency of GDNF mutations in association with Hirschsprungs disease. That is, 4.6% of all Hirschsprung's disease cases studied agreed with mutation rates reported by other investigators which ranged from 0.9% to 5.5%.[11] GDNF immunoreactivity was localized in the ganglia of

myenteric and submucous plexuses. In the normal colon and in the ganglionic segment of Hirschsprungs disease, a strong granular red staining was obtained in the satellite elements and the cellular membranes of the ganglion cells GDNF immunoreactivity was absent in the aganglionic segment of Hirschsprung's disease[11].

GDNF expression deficit in the distal aganglionic segment could be an important cofactor in Hirschsprungs disease pathogenesis. Absence of GDNF in the distal hindgut could determine a missed activation and phosphorylation of the RET receptor in the absence of RET proto-oncogence mutations causing enteric neuroblast migration arrest and Hirschsprung's disease[11].

In addition to GDNF, RET has at least 3 other ligands that are members of the GDNF family, Neurturin (NTN), persephin (PSP) and artemin (ART). Neurturin has also been found to be important for development in a subset of parasympathetic and enteric neurons. [20] Mice that lack NTN have defect in the cholinergic innervations of the colon[11].

Waardenburg-Shah (WS4) syndrome combined features of Waardenburg and long segment Hirschsprung's Disease. There are 3 known gene mutations that cause WS4 including the Endothelin-B receptor (EDNRB), the gene for the ligand endothelin-3 (EDN-3) and sox10[1]. These genes are involved in the signalling and control of the normal development of neural crest stem cells, which differentiate into both melanocytes and enteric ganglia. Sox10 is a member of the Sox gene family related to HMG box region of the testis determining gene SRY. Analysis of the mutation appears to show a correlation between the specific location of the mutation in the sox10

sequence and the severity of intestinal aganglionosis. Mutations closer to the HMG portion of the gene lead to short-segment Hirschsprung's disease whereas mutations that affect the C-terminal transactivation domain lead to long segment or total colonic Hirschsprung's disease [22].

ZFHX1b gene is located in the deleted segment of chromosome 2q22 and encodes for the transcription factor SMAD interacting protein 1 (SIP1). SIP1 is a signal transducer involved in the transforming growth factor β (TGFβ) signaling pathway. Interruption of SMAD signalling pathway caused a syndrome that includes mental retardation, microcephaly, distinct facial features and Hirschsprung's disease. This showed SIP1 importance in normal neural crest cell development[11].

PHOX2B is also implicated in Hirschsprung's disease whereby it may play a regulatory role in RET expression and account for the lack of enteric nervous system development in Hirschsprung's disease[23].

2.3. Pathophysiology of Hirschprung's Disease.

The normal motility in the gastro intestinal tract depends on the enteric nervous system, the smooth muscle layers and the interstitial cells of Cajal (ICCs). Neural crest cells populate submucosal and myenteric plexuses. The neurons within these plexuses comprise of the enteric nervous system which control the gut mobility, secretion, absorption and blood flow. These neurones are responsible for coordination of the intestinal smooth muscle. The absence of ganglia in the subumucosal and myenteric

plexuses results in the abnormal bowel motility that is pathognomonic of Hirschsprung's disease.

The smooth muscle is in a state of relaxation under normal physiologic condition. Intrinsic nervous system, to which the ganglion cells belong, is unique to the intestine. These nerves are inhibitory and utilize peptidergic neurotransmitters such as vasoactive inhibitory peptide (VIP) and substance P.

They act on smooth muscle through the release of nitric oxide which is synthesized by the activation of neuronal NO (nitric oxide) synthases. The absence of ganglion cells is associated with a twofold increase in the innervation of the aganglionic intestine by extrinsic adrenergic and cholinergic fibers.

Extrinsic cholinergic fibers mediate signal for muscular contraction using acetylcholine as the main neurotransmitter. Cholinergic innervation is markedly increased within the aganglionic segment, and since it is predominantly excitatory the aganglionic bowel is chronically contracted. This functional obstruction leads to the clinical presentation characteristic of Hirschsprung's disease[11].

The interstitial cells of Cajal (ICCs) are pacemaker cells found within the smooth muscle of the intestine, that generate physiological slow waves in the gastrointestinal tract and have an important role in the control of gut motility. These cells mediate between the enteric nerves and smooth muscle cells of the gut. Deficiency of ICC has been found within aganglionic bowel.

2.4. Clinical Presentation

About 50% to 90% of children present during the neonatal period. The common presenting features in the neonatal period were abdominal distension, feeding intolerance with bilious aspirates or bilious vomiting and delayed passage of meconium.

Delayed passage of meconium is defined as failure to pass meconium in the first 48 hours of life. Normally, 95% of normal term infants pass meconium in the first 24 hours of life and less than 10% of children with Hirschsprung's disease passed meconium during the time.

Due to functional distal bowel obstruction air and intestinal secretion collects in the intestinal lumen causing abdominal distension and vomiting (or bilious nasogastric tube aspirate). Classically there will be a gush of feces and flatus upon digital rectal examination. This is due to high pressure in the rectum which was unable to contract against anal sphincter.

After the neonatal period the children would present with chronic constipation, especially during the time of weaning for breastfed babies. Most of those presenting out of the neonatal period are those with short segment diseases, however even those with total colonic involvement may present after the neonatal period.

Chronic constipation otherwise, in older childhood with accompanying soiling is a common and persistent problem in childhood and account for about 3% visits to pediatric out-patient clinic and 25% of pediatric gastroenterology clinics.[24]

Hirschsprung's disease is a rare cause of constipation but need to be considered and ruled out as a cause for children of any age with severe constipation.[24] Clinical features that indicated invasive investigations of chronic constipation to exclude Hirschsprung's disease is by recognition from the history of failure to pass meconium in the first 48 hours of life, low intermittent intestinal obstruction of unknown cause, severe constipation dependant on enemas, chronic abdominal distension and failure to thrive. However, many parents do not remember the meconium history.

2.5. Enterocolitis

Another clinical presentation that is pertinent to Hirschsprungs disease is enterocolitis. The incidence of enterocolitis before the diagnosis of Hirschsprung's disease is established as between 15 and 50%[25]. The original description of the entity is credited to Bill and Chapman in 1962.[3]

This condition is characterized by fever, abdominal distension, diarrhea and occasionally vomiting. The condition can be severe and lead to severe dehydration, sepsis and death. There is inflammation of the colonic mucosa which can lead to full-thickness necrosis and eventually perforate causing fecal peritonitis. Stasis and bacterial overgrowth caused by functional obstruction of the aganglionic bowel has been thought to be etiopathogenic to the condition.

The pathogenic organisms include *Escherichia Coli,* Methicillin Resistant *Staphylococcus Aureus, Klebsiella pneumonia, Enterococus fecalis* and *Clostridium dificile* and other common pathogens [16]. Alterations in intestinal mucin production and alterations in the mucosal production of immunoglobulins causing loss of intestinal

barrier function which allow bacterial translocation are also implicated as etiology of enterocolitis associated with Hirschsprung's disease . [26]

The timing of (Hirschsprung's associated enterocolitis) HAEC and the clinical course of Hirschsprung's disease show that the two times an infant has the greatest risk for HAEC development are before the diagnosis of Hirschsprung's disease has been made and after the definitive surgery.

Many cases of diarrhea or abdominal distension may be misdiagnosed as gastroenteritis or the obstructing sphincter syndrome.

Table 1: The clinical Grading System for HAEC[27]

Grade	Clinical Symptoms
I	Mild explosive diarrhea, mild or moderate abdominal distension; no systemic manifestations.
II	Moderate explosive diarrhea, moderate to severe abdominal distension and mild systemic symptoms.
III	Severe explosive diarrhea, marked abdominal distension and shock or impending shock.

An occasional case of HAEC may present as perforation of the bowel proximal to the aganglionic segment. After surgical reconstruction the incidence of enterocolitis is between 2% and 33% with a mortality rate varying from 0% to 33%.[25]

There has been a clear decline in the incidence of HAEC over the past 40 years due to improved and more prompt diagnosis. The higher risk for HAEC is longer segment aganglionosis and trisomy 21 (Down's).

2.6. Associated congenital anomalies.

Associated congenital anomalies occur in at least 5-32% of patients and certain syndromic phenotypes have been linked to distinct genetic sites, indicating underlying genetic associations of the disease and probable gene-gene interaction in its pathogenesis. Clear-cut associations with Hirschsprung's disease include Down's syndrome, dominant sensorineural deafness, Waardenburg syndrome, neurofibromatosis, neuroblastoma, phaeochromocytoma, the MEN type 2B syndrome and other abnormalities. Individual anomalies vary from 2.97% to 8%, the most frequent being the gastrointestinal tract (GIT) (8.05%), the central nervous system (CNS) and sensorineural anomalies (6.79%)and the genito-urinary tract (6.05%). Other associated systems include the musculoskeletal (5.12%), cardiovascular systems (4.99%), craniofacial and eye abnormalities (3%) and less frequently the skin and integumentary system (ectodermal dysplasia) and syndromes related to cholesterol and fat metabolism[28].

In addition to associations with neuroblastoma and tumours related to MEN2B, Hirschsprung's disease may also be associated with tumors of neural origin such as ganglioneuroma, ganglioneuroblastoma, retinoblastoma and tumors associated with neurofibromatosis and other autonomic nervous system disturbances[28].

2.7. Diagnosis

Because of the recent advances in perinatal medicine, almost all patients with symptoms akin to Hirschsprung's disease are referred to pediatric surgical facility in the neonatal period. The investigations are described below.

2.7.1. Contrast Enema

Neonatal plain radiographs cannot differentiate colon from small bowel. In a study comparing all the diagnostic methods de Lorijin et al showed that the sensitivity and specificity of contrast enema (CE) was 76% and 97% respectively[29]. The presence of a caliber change, with a dilated normal colon to a narrowed aganglionic bowel may be demonstrated with contrast enema with demarcation of transition zone (Figure 1)[1].

There was no significant difference between contrast enema appearance in neonates compared to infants more than a month old infants (100% vs 90% P=0.27) or in children > 1 year old compared with younger children (94% vs 89% respectively P=0.21)[29].

Since only approximately 75% of neonates with Hirschsprung's disease will demonstrate a transition zone, absence of a transition zone (a caliber change) does not exclude the diagnosis.[30] CE is done by administering barium or water based contrast rectally.

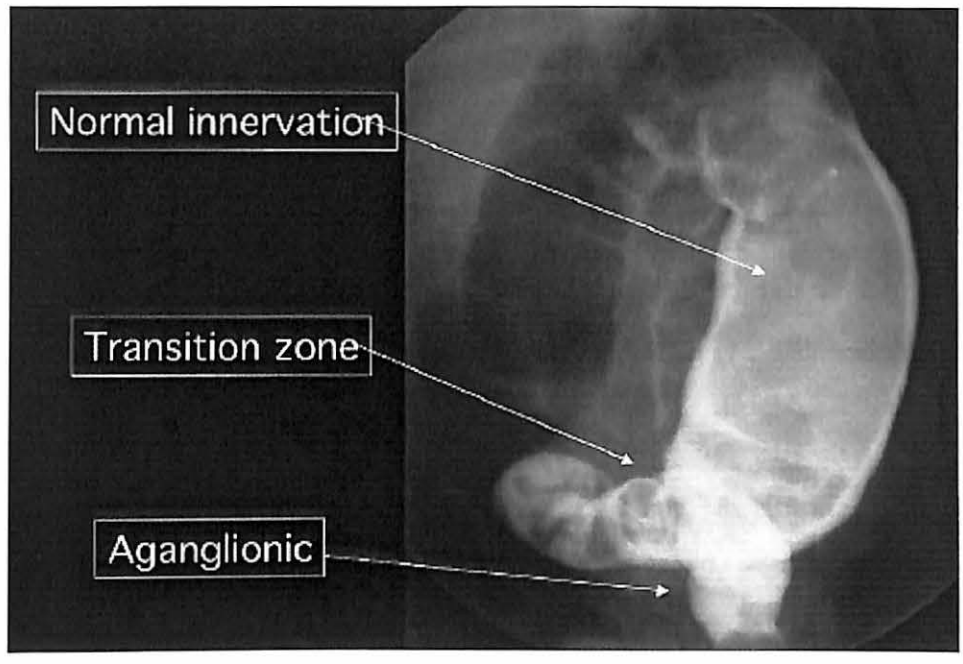

Figure 1 : The pathognomonic findings of Hirschsprung's disease on contrast enema is a transition zone between normal and aganglionic bowel.

2.7.2. Anorectal Manometry

Anorectal manometry assesses the rectoanal inhibitory reflex which is absent in children with Hirschsprung's disease. Rectoanal inhibitory reflex is absent in the majority of children with Hirschsprung's disease thus the use of anorectal manometry is helpful in diagnosing Hirschsprung's disease. However, it is unreliable in newborns less than 39 weeks of gestation or in those who weigh less than 2700g [29]. The test was feasible to be done in older children. Lorijin et al showed a sensitivity rate of 83% and specificity rate of 93%[29]. However technical factors such as air leak in the circuit and insufficient inflation of the balloon may cause false positive test results. False negative results may be due to the relaxation of external anal sphincter rather than the internal anal sphincter.

2.7.3. Rectal Biopsy

Definitive diagnosis is based on histological evaluation of a rectal biopsy, showing the presence or absence of ganglion cells and the finding of hypertrophied nerve bundles. Swenson advocated full-thickness rectal biopsy first in 1955 to establish diagnosis[29]. Helen Noblett in 1969, introduced a rectal suction biopsy tube which was developed specifically for taking rectal biopsies suitable for the diagnosis of Hirschsprung's disease.[31]

Rectal suction biopsy thence became the most common technique used to sample mucosa and the underlying submucosa because of it being non invasive and simple and could be done in the ward or in the out patient department. The biopsy is taken at 0.5 to 1.0 cm above the dentate line. The samples were frozen immediately by liquid nitrogen and stored at – 80^{o} C. Haematoxylin and Eosin (H & E) staining of the rectal biopsy showed absence of ganglion and hypertrophic nerve bundles (Figure 2 and 3).

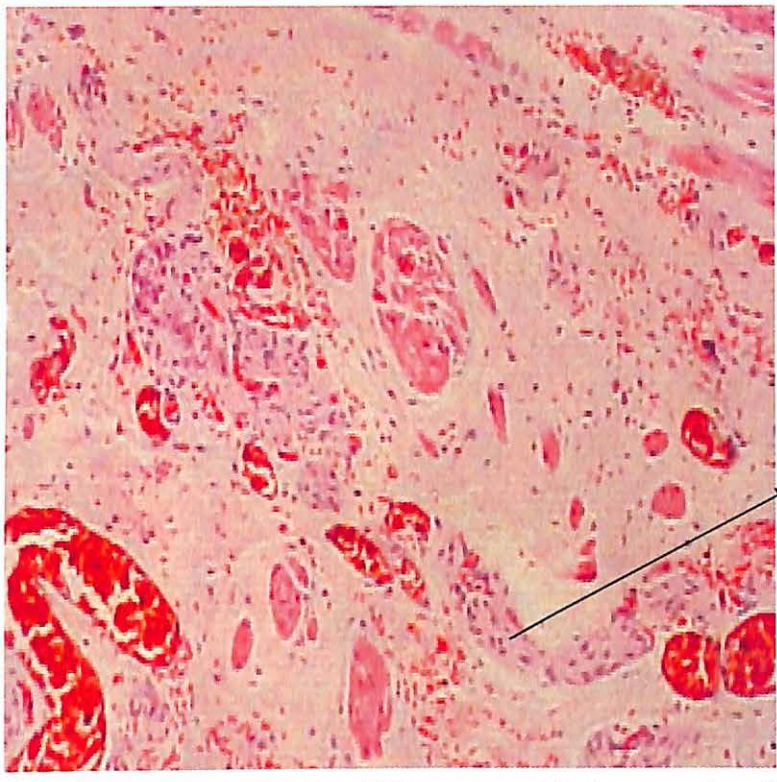

Figure 2: Appearance of Hypertrophied nerve bundles on H & E (Courtesy of Dr Shahriman HUSM Pathology Department)

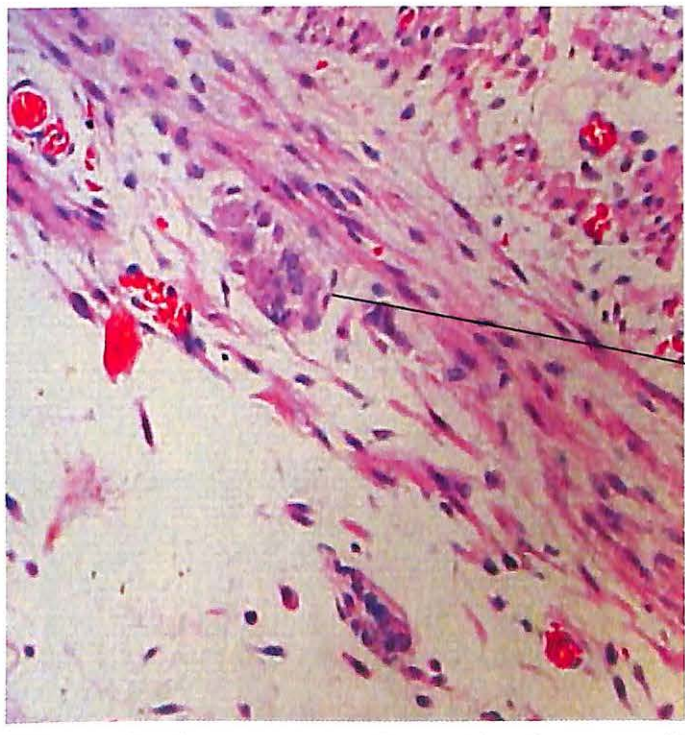

Figure 3 : Appearance of ganglion in normally innervated bowel on H&E (Courtesy of Dr Shahriman HUSM Pathology Department)

Evaluation of suction biopsies may be enhanced by staining for actylcholinestrase, which has a characteristic staining pattern in the submucosa and mucosa. Acetylcholinesterase staining was done according to the modified Karnofsky and Roots method. Martucciello et al accepted rectal suction biopsy as the current gold standard in the diagnosis of Hirschsprung's disease [32].

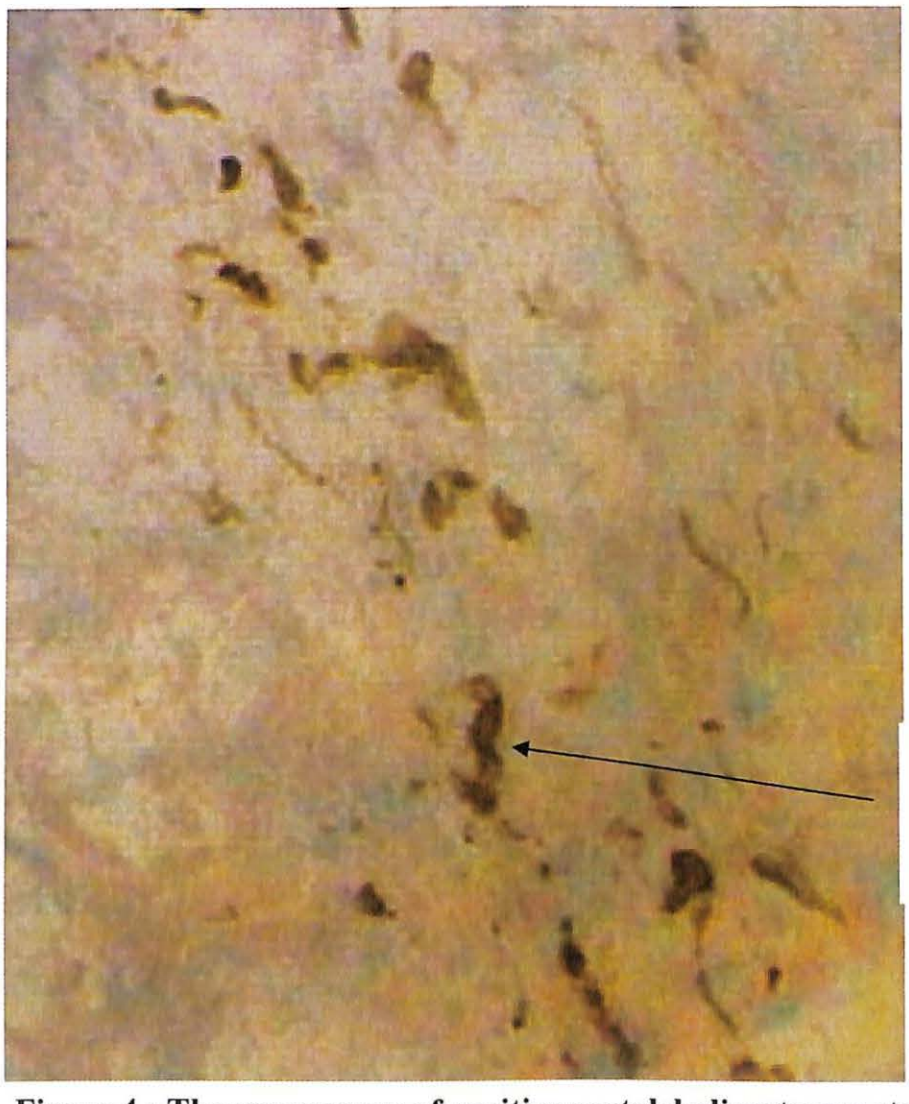

Figure 4 : The appearance of positive acetylcholinesterase staining. (Courtesy of Dr Shahriman HUSM Pathology Department)

Actylcholinestrase staining of rectal mucosal biopsy in the neonatal period has become essential for a definite diagnosis of Hirschsprung's disease (Figure 4). Studies have

shown that it is an effective examination with 100% specificity but with slightly low sensitivity (91% - 93%). [29,33] Possible false negative results include variability in the biopsy site, too superficially taken biopsy material that lacks muscularis mucosa, immaturity of the enzyme system, technical variations in performance of the stain and the experience of individual pathologists.[34] Martucciello et al agreed that European and Asian investigators routinely use acetylcholinestrase staining to diagnose Hirschsprung's disease, whereas the American contingent think that H & E is more user friendly, cheaper and more reliable to diagnose Hirschsprung's disease[32]. Thus, even though actylcholinestrase staining is an extremely useful for diagnosis only very specialized centers can rely on this staining technique for diagnosis[32].

2.8. The extent of aganglionosis

Rectosigmoid aganglionosis occurs in over 75% of all patients with Hirschsprung's disease. 10-15% of cases have long segment disease which extends into the colon proximal to the sigmoid and up to right colon. Total colonic aganglionosis is where the aganglionosis extends to the whole of the colon and 30 cm of the terminal ileum and accounts for 3 – 13% of infants with Hirschsprung's disease. In aganglionosis that involves small intestine, the aganglionosis extended orally more that 30cm of the terminal ileum. When evaluating Hirschsprung's disease from an etiologic standpoint, the long-segment-type cases differ from the short-segment-type cases in regard to the sex ratio and the incidence of familial occurrence.

Ultrashort Hirschsprung's disease (UHD) was enzyme-histochemically characterised about 35 years ago. Its existence is still ignored. Reliable diagnosis requires contrast

enema to exclude Hirschsprung's disease. It showed no reflux of contrast observed during pressing and crying. The final proof of UHD is enzyme-histochemical biopsy examination of distal rectal mucosa. The biopsies must be taken from the dentate line and 1,2,4 and 6 cm from the dentate line. Nets of nerve fibres with acetylcholinesterase activity can only be found in the muscularis mucosae and musculus corrugator cutis ani (MCCA). Its incidence is about 13.4%.[35]

2.8.1. Total colonic aganglionosis

Total colonic aganglionosis (TCA) accounts for approximately 3% to 12% of infants with Hirschsprung's disease and is associated with increased morbidity and mortality[29]. Total colonic aganglionosis can be very difficult to diagnose. Radiographic studies may show dilated loops of intestine, and an enema contrast study may show a question-mark–shaped colon, which results from the rounded edges of the splenic and hepatic flexures of an unused colon (Figure 5) [1].

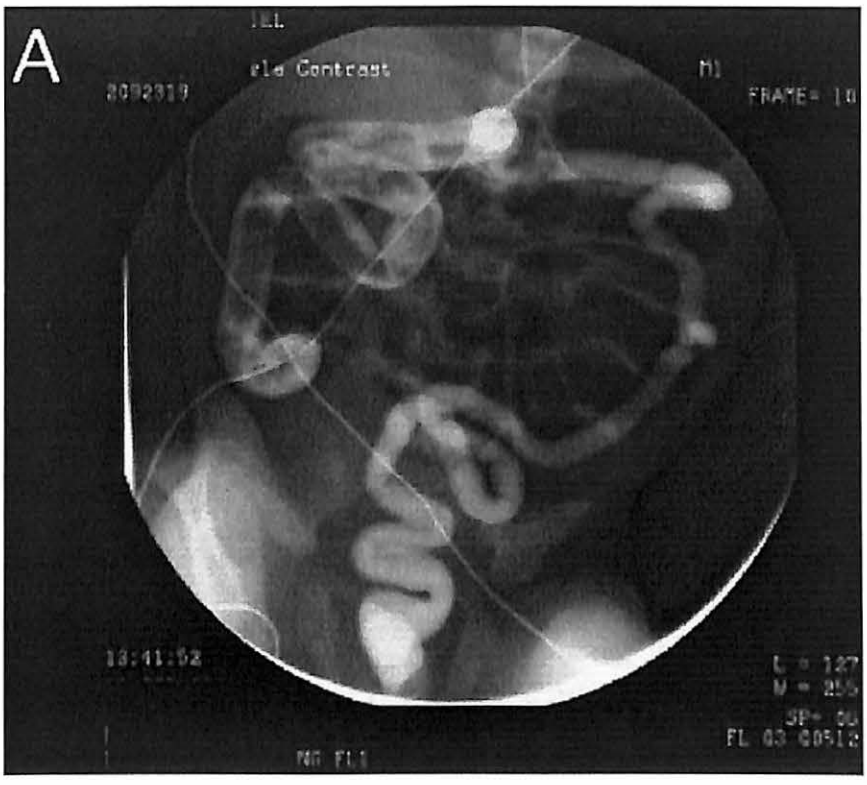

Figure 5 : The appearance of Barium enema of total colonic aganglionosis showing presence of microcolon appearance till the caecum[1].

The diagnosis is generally made at the time of laparotomy for suspected intestinal obstruction or perforation or while a leveling colostomy for Hirschsprung's disease is being done. A frozen section of an aganglionic appendix is almost always diagnostic. Total colonic aganglionosis has higher association with family history ranging from 12.4 to 33%.[11] The male to- female ratio is also less at 1.3:1.[11] Bickler et al found the proximal extent of aganglionosis was the terminal ileum in 76%, mid ileum in 19%, and the jejunum in 5%.[36]

Treatment begins with the creation of a properly placed enterostomy. Nutrition is usually initiated parenterally and slowly converted to the enteral route. Failure to thrive