A REVIEW ON RECENT PROGRESS IN CONGENITAL AGANGLIONIC MEGACOLON: DIAGNOSIS, TREATMENT, AND FUTURE DIRECTIONS

Mrs.S.L.Priyanka, tharlampudi Bharathi, shaik Sufiya, talamala Manasa, shaik Sadiya, tellamekala Geetha sravnthi, Dr nvv jagan mohanreddy, Dr D Narendra

Gmail: spriyanka.pharma@gmail.com

ABSTRACT:

Congenital Aganglionic Megacolon, commonly known as Hirschsprung disease, it is a developmental disorder of the gastrointestinal tract characterized by the absence of enteric ganglion cells in the distal bowel. This absence leads to impaired intestinal motility and results in functional intestinal obstruction, primarily affected neonates and infants. The condition arises due to the failure of neural crest cell migration during embryonic development. It has an estimated incidence of approximately 1 in 5000 live births and shows a higher prevalence in males. Recent advances in molecular genetics have identified several susceptibility genes, including RET, EDNRB, EDN3, SOX10, and PHOX2B, highlighting the complex genetic and multifactorial basis of the disorder. Diagnosis relies on a combination of clinical evaluation, contrast enema studies, anorectal manometry, and confirmatory rectal biopsy demonstrating aganglionosis and hypertrophic nerve fibers. Histopathological examination remains the gold standard for defienity his review summarizes the current understanding of the epidemiology, embryology, genetics, pathophysiology, clinical presentation, diagnostic approaches, treatment modalities, and long-term outcomes of Hirschsprung disease. Emphasis is placed on emerging diagnostic biomarkers, advances in minimally invasive surgery, and future directions in regenerative and stem cell-based therapies. A comprehensive understanding of these developments is essential for improving early diagnosis, optimizing patient management, and enhancing quality of life for affected individuals. Genetic factors play a significant role in its pathogenesis, with mutations in the RET proto-oncogene being most commonly implicated. Clinically, patients present with symptoms such as delayed passage of meconium, abdominal distension, and chronic constipation. Diagnosis is confirmed through biopsy and imaging studies.

KEY WORDS :

crest cells Neural, Intestinal obstruction, Chronic constipation, Hirschsprung-associated enterocolitis (HAEC), Rectal biopsy, Anorectal manometry, Pull-through surgery, RET gene, Pediatric gastrointestinal disorders, Colonic dysmotility, Enteric neuropathy.

1.INTRODUCTION:

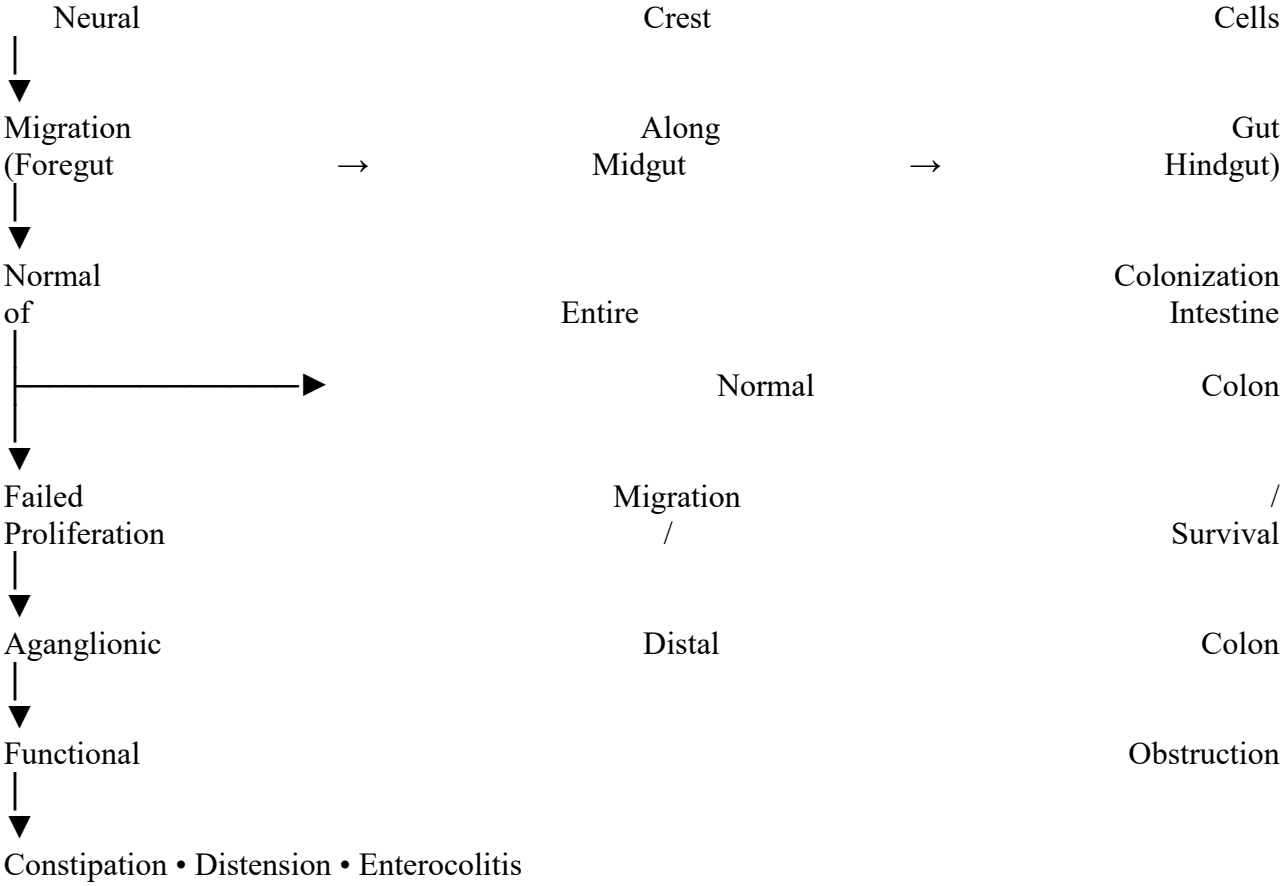
Hirschsprung's disease (HD) was initially reported by the Danish pediatrician Harald Hirschsprung in 2 infant patients in 1886. He described it as colonic hypertrophy accompanied by symptoms such as severe constipation without any mechanical obstructive justification, which is why it is attributed the origin of the disease itself is colon hypertrophy. later it was shown that at the rectal level and ascending colon the plexus of Auerbach and Meissner lacked ganglion cells. Likewise, alterations in innervation were described at the level of the circular muscular and mucosal layer, which leads to greater difficulty in intestinal relaxation and, therefore, normal intestinal evacuation.[1]

Once considered deadly, surgical treatment has reduced the disease mortality to 3% in developed countries.[2] HD most often affects the newborn. Its prevalence varies from 1 to 1.63 per 10,000 births. It is responsible for non-specific symptomatology, including chronic constipation and intestinal obstruction [3]
Hirschsprung disease occurs in 1/5000 live births and has an overall 4:1 male predominance Sex ratio inversion in total or extensive colonic form is observed in the literature.[4] HD is a congenital disorder, presenting mainly in the neonatal period. The diagnosis is made in 65% of the cases before the age of 1 month and in 95% of the cases before the age of one year. It is rarely diagnosed during adulthood, although the eldest age at presentation reported in Butler Tjaden NE, Trainor PA. The developmental Etiology and pathogenesis of Hirschsprung disease. Transfer the literature is seventy-four years.[5]

Fecal incontinence is just one of the few complications to resulting from HD. Management by an interdisciplinary team is of great importance to provide an adequate quality of life in adulthood for these patients, so follow-up by surgery, pediatrics and psychology should not be missing in the follow-up of these patients.[6]
Patients with chronic constipation, nutritional and growth disorders are commonly observed. Upon rectal examination, these patients present hypertonia of the anal sphincter, and the rectal ampulla is empty. It is frequently found as a case of enterocolitis that has symptoms of fever, abdominal distension, this because of the dilation of the loops which generates an increase in intraluminal pressure which ends up reducing blood flow, creating an environment for bacterial stagnation and growth. [7]

1:1Neural crest cells:

Neural crest cells are multipotent, migratory stem cells unique to vertebrate embryos. Often dubbed the “fourth germ layer”, they originate during neurulation at the border of the neural plate and epidermis. Following an epithelial-to-mesenchymal transition, they disperse throughout the body to form diverse tissues, including facial bones, peripheral nerves, and pigment cells.
Neural crest cells are unique because, after their formation, they undergo extensive migration to different parts of the body where they contribute to the development of the peripheral nervous system, craniofacial structures, melanocytes, endocrine tissues, and components of the cardiovascular system.



1:2 Intestinal obstruction:

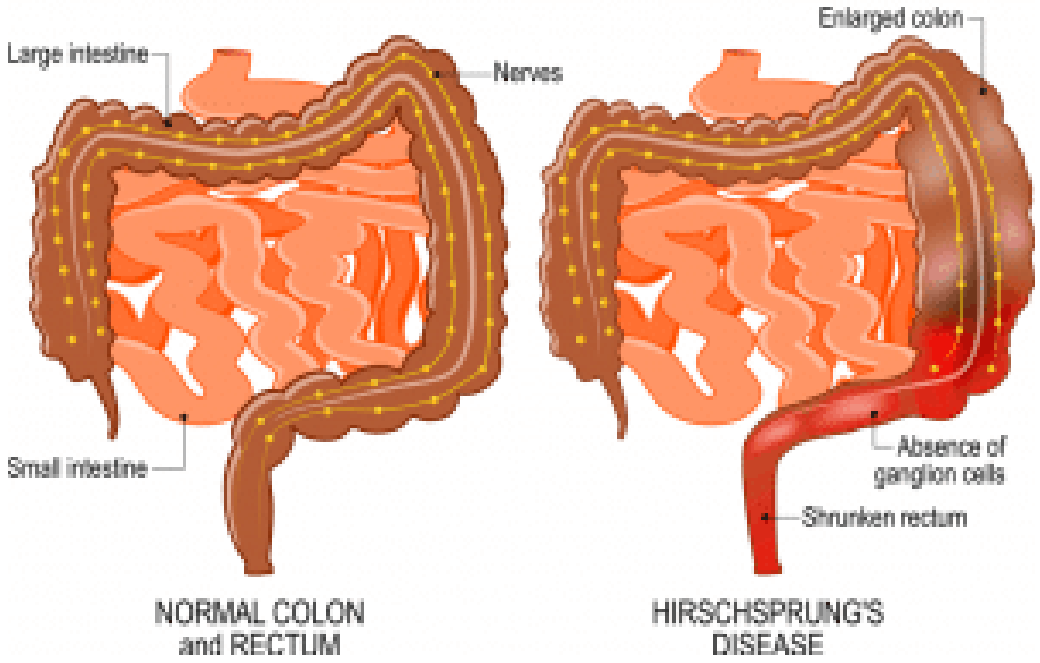
Intestinal obstruction is a common surgical emergency characterized by the interruption of the normal flow of intestinal contents through the gastrointestinal tract. It may occur due to a mechanical blockage or functional impairment of intestinal motility. The condition is associated with significant morbidity and mortality if not diagnosed and managed promptly. Small bowel obstruction accounts for the majority of cases, while large bowel obstruction is less common but often associated with malignancy. Early recognition and appropriate intervention are essential to prevent complications such as bowel ischemia, necrosis, perforation, sepsis, and death

Intestinal obstruction in Hirschsprung disease is a condition where there is blockage of stool and gas passage due to aganglionosis of the distal bowel, leading to failure of peristalsis and functional bowel obstruction.

1:3 Hirschsprung-associated enterocolitis [HAE]:

Hirschsprung-associated enterocolitis (HAEC) is a potentially life-threatening inflammation of the intestine that affects individuals with Hirschsprung disease (HD). It is the leading of cause morbidity and mortality in HD patients and can occur either before surgical repair or in the postoperative period. It is the leading cause of hospitalization and death in these patients, often presenting with explosive diarrhea, fever, vomiting, severe abdominal distention, and lethargy. HAE is a rare genetic disease characterized by severe, unpredictable, and potentially life-threatening swelling attacks. These episodes affect the face, limbs, airways, and digestive tract. It is caused by a deficiency or malfunction of the C1 inhibitor protein, leading to an overproduction of bradykinin. HSCR is unknown. However, theories include premature differentiation of neural crest cells leading to lack of further migration and local ganglion cell destruction . Long-segment disease, that is aganglionosis proximal to the splenic flexure, may confer increased risk of HAEC due to dysmotility of the residual segment leading to stasis of luminal contents. In 1995, showed significantly higher rates of HAEC in patients with long-segment disease compared to those with short-aganglionic segment disease, 49% vs. 31%, respectively.[8]

HAEC in the pre-operative period warrant consideration of diversion when determining operative strategy. It has been suggested that stable children with delayed presentation of HD and HAEC may benefit from prolonged decompression with rectal irrigations and delayed pull-through to allow the bowel to resume a more normal caliber. A 2011 survey of pediatric surgeons in the U.K. and Ireland found that 19 of 34 would change to a staged operation in the setting of HAEC and 7 of 34 would do so in the setting of Down's syndrome. It is important to note that diversion will virtually always improve a patient's condition, but may not resolve HAEC in all cases.[9]



Hirschsprung-associated enterocolitis (HAEC) was first recognized in the late 19 century by Härald Hirschsprung who included it in his hallmark description of congenital megacolon. HAEC is a condition of intestinal inflammation characterized clinically by fever, abdominal distention, diarrhea and sepsis One of the keys to prevention of HAEC is early diagnosis of HD in the perinatal period. Hirschsprung disease occurs in approximately 1 in 5000 live births(and should be considered in infants who fail to pass meconium in the first 24 hours of life. Failure to recognize HD in the early perinatal period places children at greater risk of HAEC with HAEC complicating 18% to as many as 50% of these children in the pre-operative period. One study showed that the incidence of HAEC was 24% for infants diagnosed with HD after the first week of life compared to 11% if diagnosed within the first week. Further supporting this finding, another study found a greater delay in passage of meconium (53 hrs. vs. 44 hrs.) and a much more significant delay in HD diagnosis (16.6 vs. 4.6 days) in children. [.10]

HAEC regardless of therapy. (11) Reding and colleagues reported twelve cases of pre-operative HAEC, four of which redeveloped HAEC in the post-operative period. A retrospective study of 168 patients with HD found that 57 patients developed 119 episodes of HAEC. Twenty-one children developed HAEC pre-operatively with eight recurring in the post-operative period. The remaining 36 children developed only post-surgical HAEC. Other authors have suggested that HAEC may be associated with female gender(and while increased incidence of HAEC has been reported in girls (42% vs. 30%, p = 0.16), the observation did not reach significance. long-segment disease, that is aganglionosis proximal to the splenic flexure, may confer increased risk of HAEC due to dysmotility of the residual segment leading to stasis of luminal contents. In 1995, Elhalaby showed significantly higher rates of HAEC in patients with long-segment disease compared to those with short-aganglionic segment disease, 49% vs. 31%, respectively.[12] Similar data from Reding et al. report a 56% incidence of HAEC in the pre-operative period among children with long-segment disease compared to a 16% incidence among children with short-segment disease ($p<0.01$).

More recent data from Lacher et al. showed a lower overall incidence of HAEC, but demonstrated continued increase in prevalence of HAEC in the long-segment cohort (17.6% vs. 11.4%) in both the pre- or post-operative periods. Finally, while not including a scoring system, the American Pediatric Surgery Association (APSA) released its guidelines in 2017.

Conversely, establishing a diagnosis of HAEC can be challenging prior to the diagnosis of HD being made. Hirschsprung-associated enterocolitis can be the presenting symptom in some HD patients, and may not be

immediately recognized due to the relative rarity of HD[13] These factors may potentially lead to a delay in diagnosis. Hence, evaluating physicians and surgeons should keep HAEC in mind as a potential diagnosis, especially when assessing a newborn with possible necrotizing enterocolitis or distal bowel obstruction with loose stools.

Segment affected by HD	characteristic
Short segment disease	Absence of ganglion cells at the rectal level and even the lower part of the sigmoid colon.
Long segment disease	Absence of ganglion cells at the rectal level and in greater quantities at the colonic level compared to short segment disease, but at least nerve cells are found in one segment of the colon.
Total colonic disease	Absence of ganglion cells in the tip of the small intestine.

In Hirschsprung disease, there is a disruption of the migration process and differentiation of neural crest cells at the level of the enteric nervous system, which is under the control of the RET gene and its ligands. This disturbance causes a total absence of GC in the nerve plexuses. It leads to the overactivity of the intestine with the persistent release of the acetylcholine. Subsequently, there is a continuous contraction of the narrowed (affected) colonic segment and progressive secondary dilatation of the healthy proximal colon.[14] HD belongs to a group of diseases called dysganglionosis, which includes different anomalies of genetic origin. It is known that the main etiology is due to a failure that occurs between the fifth and sixth week of gestation in which the ganglion cells must migrate from the neural crest, and it is even believed that there could be a defect in the extracellular matrix of the intestinal wall. which would prevent adequate colonization by cells from the neural crest. Finally, it was shown that accompanied by this pathology, patients generally present an altered mucosal barrier in the colon, making them prone to other disease .[15]

2.HISTOPATHOLOGY:

The diagnosis of HD is based on a combination of clinical features, radiological findings, and histopathological evaluation of the biopsied sample. Indeed, the histopathologic examination of the rectal biopsy confirms the diagnosis of the disease by highlighting the association of the absence of GC in the submucosal and myenteric plexus with hypertrophy of nerve fibers in the aganglionic segment. GC are polygonal cells with abundant fibrillar eosinophilic cytoplasm, eccentric nucleus, and a large nucleolus. To reduce the rate of inconclusive results related to inadequate biopsies, the International Gastroenterology Committee of 2009 defined the criteria for performing preoperative biopsies necessary to ensure a good interpretation. At least two biopsies should be required, with a minimum diameter of 3mm. The biopsy should have as much mucosa as the submucosa. It should be well-oriented and included in the correct axis to avoid tissue loss between the different cutting levels.

Preoperative biopsies should be performed at least 2 cm above the dentine line. This area is physiologically devoid of GC and shows physiological hyperplasia of the nerve fibers. Conventional histopathology with hematoxylin-eosin (H and E) stain is commonly used in the diagnosis of HD.[16]

Acetylcholinesterase (AChE) staining is an ancillary method to identify the increased activity of parasympathetic nerve fibers in the lamina propria and the muscularis mucosa and, thus, help in making the diagnosis, especially in difficult cases. Normally innervated intestine does not stain with AChE. However, AChE staining is a laborious technique, is time-consuming, and needs experienced technicians and pathologists. mmunohistochemistry (IHC) using the calretinin antibody has emerged as a useful technique to confirm or rule out the diagnosis of HD. Calretinin is a vitamin D-dependent protein that binds and buffers calcium in nerve fibers. Its absence causes the accumulation of intra-cytoplasmic calcium with consequent hyperexcitability and cell degeneration. It is expressed physiologically in several cells of human tissue, particularly the central and peripheral nervous systems.[17]
In 2004, Barschak et al. associated the loss of calretinin expression with the absence of GC, a characteristic of HD. Immunolabeling with anti-calretinin antibody results in GC positivity and interstitial nerve fibers of the lamina propria, muscularis mucosa, and submucosa. In the latest recommendations published in 2009, the International Gastroenterology Committee introduced the loss of calretinin expression as one of the diagnostic criteria for HD, Frozen section examination is essential in the management of HD. It can be achieved in two circumstances: to establish the diagnosis of HD and to identify the ganglionic zone during endo-anal resection. This diagnosis is made on biopsies, including only the muscularis of all the colonic walls. It is increasingly recommended to perform circumferential biopsies to make a definitive diagnosis.[18]

2.1 RETproto-oncogene:
RET is a gene that codes for proteins that assist cells of the neural crest in their movement through the digestive tract during the development of the embryo. Those neural crest cells eventually form bundles of nerve cells called ganglions. *EDNRB* codes for proteins that connect these nerve cells to the digestive tract. Thus, mutations in these two genes could directly lead to the absence of certain nerve fibers in the colon. Research suggests that several genes are associated with Hirschsprung's disease.[19]
Also, new research suggests that mutations in genomic sequences involved in regulating *EDNRB* have a bigger impact on Hirschsprung's disease than previously thought.

RET can mutate in many ways and is associated with Down syndrome. Since Down syndrome is comorbid in 2% of Hirschsprung's cases, a likelihood exists that *RET* is involved heavily in both Hirschsprung's disease and Down syndrome. *RET* is a associated with medullary thyroid cancer and neuroblastoma, which is a type of cancer common in children. Both of these disorders are more common in Hirschsprung's patients than in the general population. One function that *RE*T controls is the travel of the neural crest cells through the intestines in the developing fetus. The earlier the *RET* mutation occurs in Hirschsprung's disease, the more severe the disorder becomes.

2.2 OTHER GENES :
Common and rare DNA variations in the neuregulin 1 (*NRG1*) and NRG3 (*NRG3*) were first shown to be associated with the disease in Chinse patients through a Genome Wide Association Study by the Hong Kong team in 2009 and 2012, respectively Subsequent studies in both Asian and Caucasian patients confirmed the initial findings by the University of Hong Kong. Both rare and common variants in these two genes have been identified in additional Chinese,[20] Thai, Korean, Indonesian, and Spanish patients. These two genes are known to play a role in the formation of the enteric nervous system; thus, they are likely to be involved in the pathology of Hirschsprung's disease, at least in some cases.

Another gene associated with this condition is NADPH oxidase, EF-hand calcium binding domain 5 (NOX5).[21] This gene is located on the long arm of chromosomes .

2.3 prognosis :

Prognosis: The prognosis of Hirschsprung disease is generally excellent with early diagnosis and definitive surgical treatment. More than 90% of patients achieve normal growth and satisfactory bowel function. Short-segment disease has the best outcome. Long-term complications include constipation, fecal incontinence, and recurrent enterocolitis. Mortality is low with modern management, and most patients lead a normal adult life with good quality of life. Long term prognosis is generally positive, with many patients acquiring faecal continence. Outcome is usually dependent on the extent of aganglionosis with longer sections correlating with increasing complications. The overall prognosis is excellent when diagnosed early and treated appropriately. More than 90% of children survive and achieve good quality of life after definitive surgery.Most children attain normal growth, development, and social functioning. Lifelong follow-up may be required in some patients because bowel function problems can persist.

The quality of life in patients with HD depends on the degree of fecal continence. There is still a dearth of literature highlighting the quality of life in long-standing HD. In one of the unique, comprehensive surveys by a nationwide French study, approximately 3000 patients of the adult population with Hirschprung disease were evaluated utilizing a modified questionnaire. The original questionnaire was described by a Dutch team in 2001 and was pointing not only to HD but also to the wide spectrum of anorectal malformations.[22]

3.Pathophysiology:

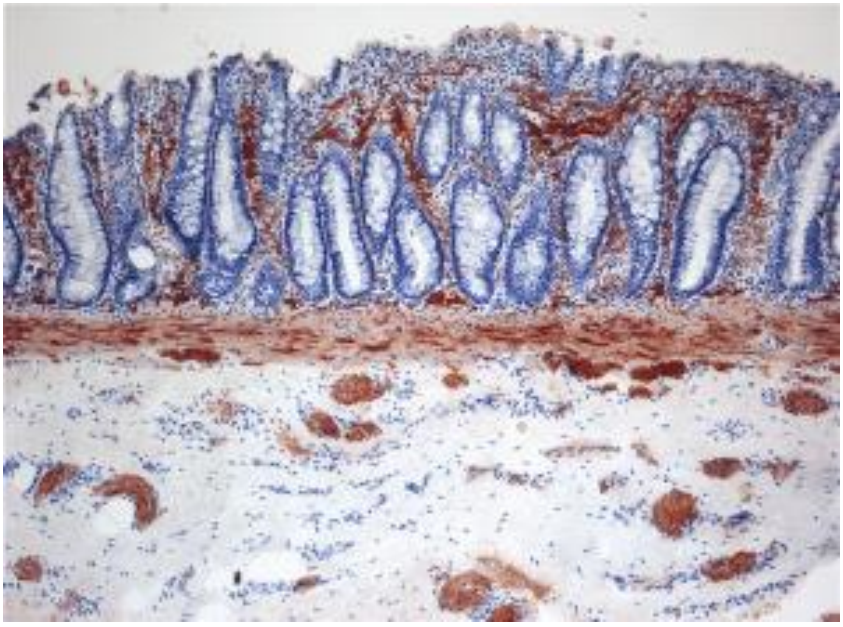
Disturbed rostro caudal migration of the neural crest cells (NCC) along a variable length of the intestine is responsible for HD. GC first migrates to a myenteric plexus and then to the submucosal plexus. Animal models have also highlighted an arrest or delay in the migration of NCCs as the factor behind the pathogenesis of HD.[23] HD affects the rectosigmoid colon in approximately 80 percent of patients. This is short-segment disease. The aganglionosis extends proximally to the sigmoid colon In 15 to 20 %of patients. This is long-segment disease. The entire colon is affected in only 5%. This is known as total colonic aganglionosis.

The diagnosis of HD is based on a combination of clinical features, radiological findings, and histopathological evaluation of the biopsied sample. Indeed, the histopathologic examination of the rectal biopsy confirms the diagnosis of the disease by highlighting the association of the absence of GC in the submucosal and myenteric. The innervation of the intestine occurs through plexi that are distributed largely in the submucosal (Meissner) and myenteric (Auerbach) layers. During embryonic development, enteric ganglion cells migrate from the neural crest in a cranio-caudal fashion. By the end of the first trimester, these cells have reached the colon. A disruption in this migration process leads to varying degrees of aganglionosis in the affected bowel. In 75% of cases, this gives rise to classical recto-sigmoid diseaseThe clinical presentation of HD is most frequently in the term, newborn infant presenting with abdominal distension, bilious vomiting, delayed passage of meconium, and feed intolerance. In 99% of term infants without HD, the first stool is passed within 24 hours. However, most children who pass stool after 24 hours do not have HD. Delayed passage of meconium is also seen in the premature infant as an indication of functional dysmotility.

- short segment disease: ~75% *
  - rectal and distal sigmoid colonic involvement only
- long segment: ~15%
  - typically extends to splenic flexure / transverse colon
- total colonic aganglionosis: ~8% (range 2-13%)
  - also known as Zuelzer-Wilson syndrome
  - occasional extension of aganglionosis into the small bowel

- ultrashort segment disease
  - 3-4 cm of internal anal sphincter only
  - controversial entity

Hirschsprung Disease is a congenital disorder of the enteric nervous system in which there is failure of migration of neural crest cells into the distal bowel during fetal development. The main pathological feature is absence of ganglion cells (aganglionosis) in both the submucosal (Meissner) plexus and myenteric (Auerbach) plexus, leading to functional intestinal obstruction. The pathology of Hirschsprung disease is characterized by congenital absence of ganglion cells in the distal bowel, leading to a spastic narrow segment, proximal dilation, and functional intestinal obstruction. Histologically, the disease shows aganglionosis, hypertrophied nerve fibers, and increased acetylcholinesterase activity, while modern immunohistochemistry (especially calretinin staining) has greatly improved diagnostic accuracy. Of the Hirschsprung disease.



Histopathology of Hirschsprung's Disease also known as aganglionosis.

The enteric nervous system (ENS) develops from neural crest cells that migrate rostrocaudally along the gastrointestinal tract between the 4th and 12th weeks of gestation. In Hirschsprung disease, this migration, proliferation, differentiation, or survival of neural crest-derived cells is disrupted.

Failure of complete colonization of the bowel by these precursor cells results in an aganglionic segment, usually involving the rectum and extending proximally for varying distances. The most accepted theory of the cause of Hirschsprung is a defect in the craniocaudal migration of neuroblasts originating from the neural crest that occurs during the first 12 weeks of gestation. Defects in the differentiation of neuroblasts into ganglion cells and accelerated ganglion cell destruction within the intestine may also contribute to the disorder [24] During normal prenatal development, cells from the neural crest migrate into the large intestine (colon) to form the networks of nerves called the myenteric plexus (Auerbach plexus) (between the smooth muscle layers of the gastrointestinal tract wall) and the submucosal plexus (Meissner plexus) (within the submucosa of the gastrointestinal tract wall). In Hirschsprung disease, the migration is not complete and part of the colon lacks these nerve bodies that regulate the activity of the colon. The affected segment of the colon cannot relax and pass stool through the colon, creating an obstruction.[25]

4. EPIDERMALOGY:

According to a 1984 study conducted in Maryland, Hirschsprung's disease appears in 18.6 per 100,000 live births .[26]In Japan, it occurs at a similar rate of about one in 5,000 births (20 per 100,000).[1] It is more common in male than female (4.32:1) and in white rather than nonwhite. Nine percent of the Hirschsprung cases were also diagnosed as having Down syndrome. Most cases are diagnosed before the patient is 10 years of age.

HD occurs in 1/5000 live births and has an overall 4:1 male predominance. Sex ratio inversion in total or extensive colonic form is observed in the literature. HD is a congenital disorder, presenting mainly in the neonatal period. The diagnosis is made in 65% of the cases before the age of 1 month and in 95% of the cases before the age of one year. It is rarely diagnosed during adulthood, although the eldest age at presentation reported in the literature is seventy-four years.[27] Hirschsprung disease affects approximately 1:5000-8000 live births. In short-segment disease, there is a significant predilection for males (M:F = ~3.5:1), which reduces with the increasing length of involvement. Interestingly, it is almost never seen in premature infants.

Hirschsprung Disease is a congenital developmental disorder of the enteric nervous system characterized by the absence of ganglion cells in the distal bowel. The disease results from abnormal migration, proliferation, differentiation, or survival of neural crest cells during fetal development. It is one of the most common causes of neonatal intestinal obstruction.

Understanding the epidemiology of Hirschsprung disease is important for identifying risk factors, planning healthcare services, and improving early diagnosis and treatment outcomes.

- Incidence: approximately 1 in 5,000 live births.
- More common in males than females.
- Male-to-female ratio is about 4:1.
- Around 80% of cases are diagnosed during infancy.
- Frequently associated with genetic disorders such as Down Syndrome.
- Family history is present in approximately 20% of patients.

5.Modern Technologies In Diagnosis And Management Of Hirschsprung Disease:

Hirschsprung Disease is a congenital disorder characterized by the absence of ganglion cells in a segment of the intestine. These ganglion cells are part of the enteric nervous system and are responsible for coordinating bowel movements. Without them, the affected bowel segment remains contracted, causing intestinal obstruction, severe constipation, abdominal distension, and difficulty passing stool. The disease is usually diagnosed in infancy, although some mild cases present later in childhood.Recent advances in medical technology have significantly improved the diagnosis, surgical treatment, and long-term management of Hirschsprung disease. Modern technologies focus on earlier detection, minimally invasive interventions, improved pathological diagnosis, genetic analysis, and regenerative medicine approaches.

High-Resolution Anorectal Manometry (HRAM)

High-resolution anorectal manometry is an advanced diagnostic technique used to assess the function of the rectum and anal sphincters

1. Rectal Suction Biopsy Instrument:

Name : Rectal suction biopsy device
Use : Gold standard diagnostic tool
Explanation : Used to collect a small tissue sample from rectum. Helps detect absence of ganglion cells. Confirms diagnosis histologically.

2. Contrast Enema (Barium Enema Setup):

Name: contrast enema apparatus

Use: Imaging of colon

Explanation: Barium dye is introduced into colon X-ray shows narrow distal segment + dilated proximal colon. Helps identify transition zone.

3. **Anorectal Manometry System**:

Name : Anorectal manometry device

Use : functional test

Explanation : Measure pressure in rectum and anal sphincter. In this disease absence of rectoanal inhibitory reflex (RAIR)

4. Histopathology Microscope:

Name : Compound light microscope

Use : Tissue examination

Explanation : used after biopsy confirms absence of ganglion cells may show hypertrophied nerve fibers

5. (IHC) Equipment:

Name : IHC staining system

Use : Advanced confirmation

Explanation : uses markers like calretinin, acetylcholinesterase. Helps confirm aganglionosis when unclear

6.Abdominal X-Ray Machine:

Name : x-ray imaging system

Use : initial screening

Explanation : shows distended bowel loops. suggests intestinal obstruction

7**. Genetic Testing Tools:**

Name : PCR/DNA sequencing equipment

Use : Genetic Analysis

Explanation : Detects Mutations like RET gene. used in research or familial cases

6.Signs and Symptoms :

Typically, Hirschsprung disease is diagnosed shortly after birth, although it may develop well into adulthood. because of the presence of megacolon, or because the baby fails to pass the first stool (meconium)[28] within 48 hours of delivery. Normally, 90% of babies pass their first meconium within 24 hours, Some other symptoms in newborns include a Typically swollen belly, vomiting (green or brown vomit), and flatulence. In old children, symptoms include chronic constipation, swollen belly, fatigue, and failure to thrive. HD is characterized by sudden onset of fever, abdominal distension, vomiting, passage of bloody stools or release of extensive gas after rectal examination. Some other signs and symptoms in newborns include a swollen belly, vomiting (green or brown vomit), and flatulence. In older children, some other signs and symptoms include chronic constipation, flatulence, swollen belly, fatigue, and failure to thrive.[29] Other symptoms include symptoms of bowel perforation such as vomiting, constipation, poor feeding, lethargy, and diarrhea. Symptoms of bowel obstruction would include vomiting of bile and abdominal distension. Those who do not pass stools after 36 to 48 hours

after birth should raise suspicion of Hirschsprung disease Some cases are diagnosed later, into childhood, but usually before age 10. The child may experience fecal retention, constipation, or abdominal distention.

Associated Syndrome :

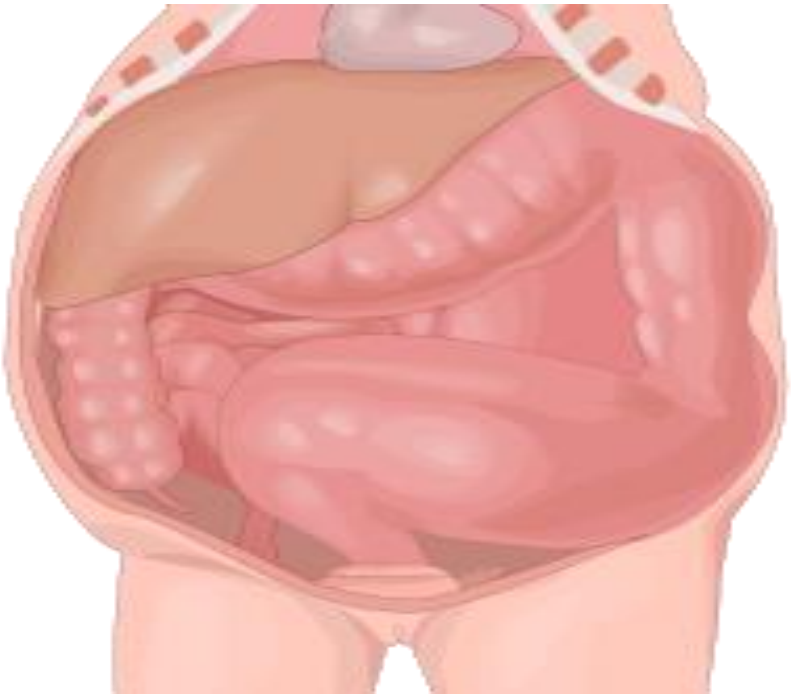
Hirschsprung's disease can also present as part of multi system disorders, such as

- Trisomy 20 Down Syndrome] [30]
- Bardet–Biedl syndrome
- Cartilage–hair hypoplasia[31]
- Congenital central hypoventilation syndrome[32]
- MEN2[33]
- Mowat–Wilson syndrome[34]
- Smith–Lemli–Opitz syndrome[35]

HSCR was also found to be associated with several congenital syndromes. Down syndrome was the most common, affecting 3.8% of the cohort ($n = 59$), followed by Haddad syndrome, which was present in 3.15% ($n = 50$). Other rare associations included multiple endocrine neoplasia and Waardenburg–Shah syndrome, emphasising the importance of a comprehensive genetic and clinical work-up in patients with HSCR.

The disorder may occur by itself or in association with other genetic disorders such as Down syndrome. About half of isolated cases are linked to a specific genetic mutation and about 20% occur within families.[36] Some of these occur in an autosomal dominant manner. The cause of the remaining cases is other unclear. Ifotherwise normal parents have one child with the condition, the next child has a 4% risk of being affected.

Megacolon caused by Hirschsprung disease



TRISOMY:

Trisomy of Hsa21 is associated with reduced brain volume, particularly in the hippocampus and cerebellum.[37] Hypotonia is a hallmark feature of infants with Down syndrome, occurring in nearly all cases. The condition is defined as decreased resistance to passive muscle stretch and is responsible for delayed motor development in these patient. Because of hypotonia, patients exhibit joint laxity, which decreases gait stability and increases the energy required for physical exertion. Individuals with Down syndrome are prone to reduced bone mass and an increased risk of fractures due to reduced physical activity, while ligamentous laxity predisposes these patients to atlantoaxial subluxation.[38] Approximately 5% to 13% of children with Down syndrome experience seizures. Among these, 40% have onset before their first year of life, with infantile spasms being the most common seizure type in this age group. Children with Down syndrome and infantile spasms tend to respond more favorably to antiepileptic therapy than other children with the same condition. Early recognition and treatment are therefore critical, as timely intervention can improve developmental outcomes.[39]

Lennox-Gastaut syndrome is also more prevalent in children with Down syndrome. When it occurs, it typically has a late onset and is associated with reflex seizures, along with an increased rate of electroencephalography (EEG) abnormalities.

Approximately 40% of individuals with Down syndrome develop tonic-clonic or myoclonic seizures within their first 3 decades.[40] Dementia occurs more commonly in patients aged 45 or older and approximately 84% of patients are prone to developing seizures.[41] Seizures appear to be associated with the rapid decline in cognitive function.

Down syndrome Down syndrome was first described in 1866 by the English physician John Langdon Down, who recognized its distinct constellation of physical and developmental features. The genetic basis of this condition was established in 1959 at the Paris laboratory of pediatrician Raymond Turpin, where trisomy 21 was identified by his students, Marthe Gautier and Jérôme Lejeune. The attribution of this discovery remains debated, particularly concerning the respective contributions of Gautier and Lejeune.[42] In people with Down syndrome mosaicism, the prognosis for intelligence and risk of medical complications probably depends on the proportion of abnormal eg, trisomy 21 cells in each different tissue, including the brain. However, in practice, this risk cannot be predicted because it is not feasible to determine the karyotype in every single cell in the body. Some people with Down syndrome mosaicism have very subtle clinical signs and may have normal intelligence; however, even people with Down syndrome without mosaicism can have variable clinical findings. This is also true for affected people who are not mosaic chapters of the clinical parameters.

Bardet–Biedl syndrome:

Bardet-Biedl syndrome is a disorder that affects many parts of the body. The signs and symptoms of this condition vary among affected individuals, even among members of the same familyVision loss is one of the major features of Bardet-Biedl syndrome. Loss of vision occurs as the light-sensing tissue at the back of the eye gradually deteriorates. Problems with night vision become apparent by mid-childhood, followed by blind spots that develop in the side (peripheral) vision. Over time, these blind spots enlarge and merge to producertunnel vision. Most people with Bardet-Biedl syndrome also develop blurred central vision (poor visual acuity) and become legally blind by adolescence or early adulthood

7.For Disease Etiology And Respective Drug Explanation:

In Hirschsprung disease, there is a disruption of the migration process and differentiation of neural system, which is under the control of the RET gene and its ligands. This disturbance causes a total absence of GC in the nerve plexuses. It leads to the overactivity of the intestine with the persistent release of acetylcholine. Subsequently, there is a continuous contraction of the narrowed (affected) colonic segment and progressive secondary dilatation of healthy proximal colon [43]

HD transmission is complex, involving multigenic inheritance. Its penetrance is weak, variable, and sex-dependent. The main gene involved is the proto-oncogene RET found in about 35% of sporadic cases and 49% of familial cases. RET mutations may occur in any of the 21 exons of the gene. More than 100 different mutations have been identified. These include nonsense, missense, deletions, and insertions.[13] The other genes involved in the etiopathogenesis of HD are implicated in only 5 to 10% of cases. It includes the ligands of the RET receptor: glial cell-derived neurotrophic factor (GDNF), endothelin-3, endothelin receptor B (EDNRB), SOX10 transcription factor, and the PHOX2B gene.[44]

8.Treatment of HD:

The Treatment of Hirschsprung Disease is Broadly Divided into three Categories :

1. Initial/ Conservative Management
2.Definitive Treatment
3. Post -operative care and complications

The diagnosis of Hirschsprung disease (HD) almost exclusively demands surgical intervention. Pediatric healthcare providers should possess a comprehensive understanding of the most popular surgical procedures to assist in the bridging referral phase between the surgeon and the patient's family. Rectal irrigation before the surgery and in the management of HAEC is highly recommended. It might have a couple of crucial advantages, including colon size decompression and preventing the most devastating complication, enterocolitis. Surgical planning is profoundly affected by the presence of comorbidities, while short-segment HD without any comorbidities can be subjected to a single-stage pull-through procedure.[45] [46]In contrast, in the presence of HAEC or a remarkably dilated colon, a staged reconstruction, starting with a temporary decompressive colostomy, should be preferred.

The recommended timing for a definite pull-through procedure varies from four to six months following colostomy placement. A variety of pull-through surgeries have been identified. The traditional Swenson's technique involves proctectomy, pulling the healthy ganglionated colon through, and anastomosing it to the anus. Novel surgical procedures (e.g., Duhamel's and Soave's procedures) have the advantage of preserving the intricate innervation of the rectum and bladder.[47] The early postoperative period following Soave's procedure is critical. Frequent regular sessions of mechanical anastomotic dilations, which might be done at home, are highly recommended. All of these procedures have high success rates, and morbidity is minimal .

An alternative approach is to perform a single-stage transanal Soave's procedure early in the neonatal period, which might obviate the need for an abdominal incision and colostomy.[48] However, the complication rates are quite similar to the more invasive procedures.

Colostomy:

he first stage of treatment used to be a reversible colostomy. In this approach, the healthy end of the large intestine is cut and attached to an opening created on the front of the abdomen. The contents of the bowel are discharged through the hole in the abdomen and into a bag. Later, when the patient's weight, age, and condition are right, the "new" functional end of the bowel is connected with the anus. The first surgical treatment involving surgical resection followed by reanastomosis without a colostomy occurred as early as 1933 by Doctor Baird in Birmingham on a one-year-old boy

OTHER PROCEDURES

The Swedish-American surgeon Orvar Swenson (1909–2012), who discovered the cause of Hirschsprung's, first performed its surgical treatment, the pull-through surgery, in 1948.[36] The pull-through procedure repairs the colon by connecting the functioning portion of the bowel to the anus. The pull-through procedure is the typical

method for treating Hirschsprung's in younger patients. Swenson devised the original procedure, and the pull-through surgery has been modified many times.[49]

Currently, several different surgical approaches are used, which include the Swenson, Soave, Duhamel, and Boley procedures. The Swenson procedure leaves a small portion of the diseased bowel. The Soave procedure, named after the Italian pediatric surgeon, Franco Soave [it] (1917–1984), leaves the outer wall of the colon unaltered. The Boley procedure, pioneered by the American surgeon, Scott Boley (born 1941), is a small modification of the Soave procedure, so the term "Soave-Boley" procedure is sometimes used[.50] The Duhamel procedure, named for the French pediatric surgeon Bernard Duhamel (1917–1996), uses a surgical stapler to connect the good and bad bowel.[50]

For the 15% of children who do not obtain full bowel control, other treatments are available. Constipation may be remedied by laxatives or a high-fiber diet. In those patients, serious dehydration can play a major factor in their lifestyles. A lack of bowel control may be addressed by an ileostomy – similar to a colostomy, but uses the end of the small intestine rather than the colon. The Malone antegrade colonic enema (ACE) is also an option.[51] In a Malone ACE, a tube goes through the abdominal wall to the appendix, or if available, to the colon. The bowel is then flushed daily. Children as young as 6 years of age may administer this daily flush on their own.

If the affected portion of the lower intestine is restricted to the lower portion of the rectum, other surgical procedures may be performed, such as a posterior rectal myectomy. The prognosis is good in 70% of cases. Chronic postoperative constipation is present in 7 to 8% of the operated cases. Postoperative enterocolitis, a severe manifestation, is present in the 10–20% of operated patient.

9. **DIFFERENTIAL DIAGNOSIS :**

- **Meconium Plug Syndrome**: Leads to failure to pass meconium but symptoms should resolve after passage of plug. Can be differentiated by barium enema or water-soluble contrast enema (water soluble preferred as less risk of perforation)
- **Meconium Ileus**: Distal small bowel is impacted by meconium leading to abdominal distension and failure to pass meconium.Can be differentiated by radiograph, barium enema or water-soluble contrast enema.
- **Intestinal atresia**: Congenital malformation of any part of the intestine resulting in complete obstruction. The presentation will depend on the site of the blockage but will usually involve abdominal distension and failure to pass meconium.
- **Intestinal malrotation:** This is caused by a congenital anomaly in the rotation of the midgut during embryological development. This can result
- **Neonatal Small Left Colon Syndrome (NSLCS):** Transient functional obstruction, primarily seen in infants of diabetic mothers
- **Neonatal Sepsis:** Can cause systemic ileus leading to abdominal distension and feeding intolerance.
- **Anorectal Malformations:** Imperforate anus or ectopic anus easily identifiable on physical examination.

Medical diagnosis (abbreviated as Dx, D_x, or D_s) is the process of determining which disease or condition explains a person's symptoms and signs. It is most often referred to as a diagnosis with the medical context being implicit. The information required for a diagnosis is typically collected from a history and physical examination of the person seeking medical care. Often, one or more diagnostic procedures, such as medical

tests, are also done during the process. Sometimes the posthumous diagnosis is considered a kind of medical diagnosis.

Complications :

One of the most common and devastating Hirschsprung disease-related complications is Hirschsprung-associated enterocolitis (HAEC), which is defined as an inflammatory disorder of the bowel. In order to [52] impaired mucosal barrier obtain the diagnosis of HAEC, which is the most challenging, a combination of clinical signs and symptoms should be considered. It will result in an inevitably over and under-treatment of patients. The underlying causality of HAEC is still ill-defined. Several hypotheses, including dysbiosis of the intestinal microbiome, function, altered innate immune responses, and bacterial translocation, have been proposed.

Hirschsprung associated enterocolitis (HAEC) is the main cause of mortality of patients with Hirschsprung's disease. Stasis of faeces leads to bacterial overgrowth, particularly of *Clostridium difficile, Staphylococcus aureus,* and anaerobes, within the colon. This often presents as fever, vomiting, diarrhoea, abdominal tenderness, and eventually sepsis if not recognized early enough. The emergency treatment for suspected enterocolitis is with fluid resuscitation, bowel decompression and broad-spectrum IV antibiotics (1). This presentation would also warrant stool culture and abdominal x-ray.[53]

The severity of clinical manifestations in HAEC might vary, and several scoring systems have been designed in an attempt to classify the gastrointestinal and systemic presentations, including a Delphi method to make a consensus among a panel of pediatric gastroenterologists and surgeons, and a clinical grading system based on a prospective trial. The former utilized 16 items, while the latter is based on three main components of diarrhea, abdominal distention, and systemic manifestations to define the grading of HAEC .[54]The preferred management of each patient affected with HAEC is based on the corresponding clinical grade. In grade I or possible HAEC, outpatient management with oral metronidazole accompanied by fluid and electrolytes might be considered. The more severe cases, including definite and severe HAEC, should be admitted to the hospital and treated with intravenous fluid resuscitation and broad-spectrum antibiotics. Rectal irrigation to remove the retained stool and decrease the bacterial load might be considered in those with abdominal distention, irrespective of the HAEC grade. Surgical intervention with a proximal colostomy might be considered in those children with severe HAEC who fail to respond to primary medical management with bowel rest, intravenous fluid resuscitation, rectal irrigations, and broad-spectrum antibiotics.[55]

The only definitive treatment is surgery, and the three main surgical procedures are the Swenson, Soave, and Duhamel pull-through procedures . The details of these are beyond the scope of this article but they are usually performed in the first few months of the neonate's life All involve resecting the aganglionic section of bowel and connecting the unaffected bowel to the dentate line (or just above the dentate line in the pull-through]

CONCLUSION:

Hirschsprung disease is a congenital condition caused by the absence of ganglionic cells , leading to bowel obstruction. Early diagnosis and timely surgical treatment are essential for ensure better effective management . although current therapies provide good outcomes ,some complications may still occur advances in diagnostic techniques and research have improved understanding of the disease. a multidisciplinary approach is important to patient care and quality of life. Hirschsprung Disease is a congenital disorder of the enteric nervous system caused by failure of neural crest cell migration during fetal development, resulting in absence of ganglion cells in the distal bowel. This leads to a functional intestinal obstruction, where the affected segment remains spastically contracted and does not allow normal passage of stool and gas. The disease most commonly involves the rectum and sigmoid colon, but in severe cases it may extend to the entire colon or even parts of the small intestine. Clinically, it usually presents in the neonatal period with failure to pass meconium within 48 hours,

abdominal distension, vomiting, and constipation. If untreated, it can lead to serious complications such as intestinal obstruction, bowel perforation, malnutrition, and life-threatening enterocolitis.

Diagnosis is confirmed by rectal biopsy showing absence of ganglion cells, supported by modern investigations such as anorectal manometry, contrast enema, immunohistochemistry (calretinin staining), and genetic testing. The disease has strong genetic associations, especially mutations in the RET and other neural crest development genes.

The mainstay of treatment is surgical removal of the aganglionic segment followed by pull-through procedures, such as Swenson, Soave, Duhamel, or transanal endorectal pull-through techniques. With advances in minimally invasive and robotic surgery, outcomes have significantly improved, and most patients achieve good long-term bowel function.

In conclusion, Hirschsprung disease is a curable but potentially life-threatening congenital disorder if not diagnosed early. Early recognition, accurate diagnosis, and timely surgical intervention are essential for preventing complications and ensuringnormal growth and quality of life.

References :

1.Amiel J, Lyonnet S. Hirschsprung disease, associated syndromes, and genetics: a review. J Med Genet.

2. Löf Granström A, Wester T. Mortality in Swedish patients with Hirschsprung disease. Pediatr Surg Int. 2017 Nov;33(11):1177-1181.

3.Bhatnagar SN. Hirschsprung's Disease in Newborns. J Neonatal Surg. 2013 Oct-Dec;2(4):51. [PMC free article] [PubMed]

4.Butler Tjaden NE, Trainor PA. The developmental etiology and pathogenesis of Hirschsprung disease. Transl Res. 2013 Jul;162(1):1-15. [PMC free article] [PubMed]

5.Menezes M, Corbally M, Puri P. Long-term results of bowel function after treatment for Hirschsprung's disease: a 29-year review. Pediatr Surg Int. 2006 Dec;22(12):987-90. [PubMed]

6.KA Santos-Jasso. Enfermedad de Hirschsprung. Acta pediatr Mexico.2017;[1]:72-8

7.Weber Estrada N. Enfermedad de Hirschsprung. Rev Med Costa Rica Centroamerica LXI. Med Costa Rica Centroamerica LXI.

8. . .Elhalaby EA, Teitelbaum DH, Coran AG, Heidelberger KP. Enterocolitis associated with Hirschsprung's disease: a clinical histopathological correlative study. J Pediatr Surg. 1995 Jul;30(7):1023–6. doi: 10.1016/0022-3468(95)90334-8. discussion 6-7. [DOI] [PubMed] [Google Scholar]

9. Fujimoto T, Puri P. Persistence of enterocolitis following diversion of faecal stream in Hirschsprung's disease. Pediatric surgery international. 1988;3(2):141–6. [Google Scholar]

10. . Teitelbaum DH, Qualman SJ, Caniano DA. Hirschsprung's disease. Identification of risk factors for enterocolitis. Ann Surg. 1988 Mar;207(3):240–4. doi: 10.1097/00000658-198803000-00003. [DOI] [PMC free article] [PubMed] [Google Scholar]

11. . Pastor AC, Osman F, Teitelbaum DH, Caty MG, Langer JC. Development of a standardized definition for Hirschsprung's-associated enterocolitis: a Delphi analysis. J Pediatr Surg. 2009 Jan;44(1):251–6. doi: 10.1016/j.jpedsurg.2008.10.052. [DOI] [PubMed] [Google Scholar]

12. Reding R, de Ville de Goyet J, Gosseye S, Clapuyt P, Sokal E, Buts JP, et al. Hirschsprung's disease: a 20-year experience. J Pediatr Surg. 1997 Aug;32(8):1221–5. doi: 10.1016/s0022-3468(97)90686-2. [DOI] [PubMed] [Google Scholar

13. . Melendez E, Goldstein AM, Sagar P, Badizadegan K. Case records of the Massachusetts General Hospital. Case 3-2012. A newborn boy with vomiting, diarrhea, and abdominal distention. N Engl J Med. 2012 Jan 26;366(4):361–72. doi: 10.1056/NEJMcpc1103562. [Case Reports Clinical Conference] [DOI] [PubMed] [Google Scholar

14. Butler Tjaden NE, Trainor PA. The developmental etiology and pathogenesis of Hirschsprung disease. Transl Res. 2013 Jul;162(1):1-15. [PMC free article] [PubMed]

15. Weber Estrada N. Enfermedad de Hirschsprung. Rev Med costa Rica Centroamerica LXT 2012;[602]; 251-6

16. Goldman, Lee (2012). Goldman's Cecil Medicine (24th ed.). Philadelphia: Elsevier Saunders. p. 867. ISBN 978-1-4377-2788-3

17. Butler Tjaden NE, Trainor PA. The developmental etiology and pathogenesis of Hirschsprung disease. Transl Res. 2013 Jul;162(1):1-15. [PMC free article] [PubMed]

18. Löf Granström A, Wester T. Mortality in Swedish patients with Hirschsprung disease. Pediatr Surg Int. 2017 Nov;33(11):1177-1181. [PMC free article] [Pub]

19. uri P, Shinkai T (2004). "Pathogenesis of Hirschsprung's disease and its variants: recent progress". *Semin. Pediatr. Surg*

20. Yang J, Duan S, Zhong R, Yin J, Pu J, Ke J, Lu X, Zou L, Zhang H, Zhu Z, Wang D, Xiao H, Guo A, Xia J, Miao X, Tang S, Wang G (2013). "Exome sequencing identified NRG3 as a novel susceptible gene of Hirschsprung's disease in a Chinese population". *Mol. Neurobiol.* ***4***

21. Shin JG, Seo JY, Seo JM, Kim DY, Oh JT, Park KW, Kim HY, Kim JH, Shin HD (2019) Association analysis of NOX5 polymorphisms with Hirschsprung disease. J Pediatr Surg

22. Hartman EE, Oort FJ, Sprangers MA, Hanneman MJ, van Heurn LW, de Langen ZJ, Madern GC, Rieu PN, van der Zee DC, Looyaard N, van Silfhout-Bezemer M, Aronson DC. Factors affecting quality of life of children and adolescents with anorectal malformations or Hirschsprung disease. J Pediatr Gastroenterol Nutr. 2008 Oct;47(4):463-71

23. Webster W. Embryogenesis of the enteric ganglia in normal mice and in mice that develop congenital aganglionic megacolon. J Embryol Exp Morphol. 1973 Dec;30(3):573-85. [PubMed] [Reference list]

24. Shin JG, Seo JY, Seo JM, Kim DY, Oh JT, Park KW, Kim HY, Kim JH, Shin HD (2019) Association analysis of NOX5 polymorphisms with Hirschsprung disease. J Pediatr Surg

25. Kays DW (1996). "Surgical conditions of the neonatal intestinal tract". Clinics in Perinatology. 23 (2): 353–75. doi:10.1016/S0095-5108(18)30246-X. PMID 8780909.

26. Goldberg EL (1984). "An epidemiological study of Hirschsprung's disease". Int J Epidemiol. 13 (4): 479–85. doi:10.1093/ije/13.4.479. PMID 6240474.

27. Menezes M, Corbally M, Puri P. Long-term results of bowel function after treatment for Hirschsprung's disease: a 29-year review. Pediatr Surg Int. 2006 Dec;22(12):987-90. [PubMed] [Reference list]

28. Goldman, Lee (2012). Goldman's Cecil Medicine (24th ed.). Philadelphia: Elsevier Saunders. p. 867. ISBN 978-1-4377-2788-3.

29. "Hirschsprung's disease". Mayo Clinic. Retrieved 30 January 2023.

30 Flori E, Girodon E, Samama B, Becmeur F, Viville B, Girard-Lemaire F, Doray B, Schluth C, Marcellin L, Boehm N, Goossens M, Pingault V (2005). "Trisomy 7 mosaicism, maternal uniparental heterodisomy 7 and Hirschsprung's disease in a child with Silver-Russell syndrome". European Journal of Human Genetics. 13 (9): 1013–8. doi:10.1038/sj.ejhg.5201442. PMID 15915162.

31. Mäkitie O, Heikkinen M, Kaitila I, Rintala R (2002). "Hirschsprung's disease in cartilage-hair hypoplasia has poor prognosis". J Pediatr Surg. 37 (11): 1585–8. doi:10.1053/jpsu.2002.36189. PMID 12407544

32. . de Pontual L, Pelet A, Clement-Ziza M, Trochet D, Antonarakis SE, Attie-Bitach T, Beales PL, Blouin JL, Dastot-Le Moal F, Dollfus H, Goossens M, Katsanis N, Touraine R, Feingold J, Munnich A, Lyonnet S, Amiel J (2007). "Epistatic interactions with a common hypomorphic RET allele in syndromic Hirschsprung disease

33. Saunders CJ, Zhao W, Ardinger HH (2009). "Comprehensive ZEB2 gene analysis for Mowat-Wilson syndrome in a North American cohort: a suggested approach to molecular diagnostics"

34. Bonnard A, Zeidan S, Degas V, Viala J, Baumann C, Berrebi D, Perrusson O, El Ghoneimi A (2009). "Outcomes of Hirschsprung's disease associated with Mowat-Wilson syndrome"

35. Mueller C, Patel S, Irons M, Antshel K, Salen G, Tint GS, Bay C (2003). "Normal cognition and behavior in a Smith-Lemli-Opitz syndrome patient who presented with Hirschsprung disease"

36. "Hirschsprung's disease". Genetic and Rare Diseases Information Center (GARD) – an NCATS Program. 2017. Archived from the original on 24 November 2018. Retrieved 14 December 2017.

37. Pearlson GD, Breiter SN, Aylward EH, Warren AC, Grygorcewicz M, Frangou S, Barta PE, Pulsifer MB. MRI brain changes in subjects with Down syndrome with and without dementia. Dev Med Child Neurol. 1998 May;40(5):326-34. [PubMed] [Reference list]

38. Merrick J, Ezra E, Josef B, Hendel D, Steinberg DM, Wientroub S. Musculoskeletal problems in Down Syndrome European Paediatric Orthopaedic Society Survey: the Israeli sample. J Pediatr Orthop B. 2000 Jun;9(3):185-92. [PubMed] [Reference list]

39 .Arya R, Kabra M, Gulati S. Epilepsy in children with Down syndrome. Epileptic Disord. 2011 Mar;13(1):1-7. [PubMed] [Reference list].

40. Pueschel SM, Louis S, McKnight P. Seizure disorders in Down syndrome. Arch Neurol. 1991 Mar;48(3):318-20. [

41. De Simone R, Puig XS, Gélisse P, Crespel A, Genton P. Senile myoclonic epilepsy: delineation of a common condition associated with Alzheimer's disease in Down syndrome. Seizure. 2010 Sep;19(7):383-9.

4.2Hickey F, Hickey E, Summar KL. Medical update for children with Down syndrome for the pediatrician and family practitioner. Adv Pediatr. 2012;59(1):137-57. [PubMed] [Reference list].

43. Butler Tjaden NE, Trainor PA. The developmental etiology and pathogenesis of Hirschsprung disease. Transl Res. 2013 Jul;162(1):1-15. [

44. Martucciello G. Hirschsprung's disease, one of the most difficult diagnoses in pediatric surgery: a review of the problems from clinical practice to the bench. Eur J Pediatr Surg. 2008 Jun;18(3):140-9. [PubMed] [Reference list]

45. Weidner BC, Waldhausen JH. Swenson revisited: a one-stage, transanal pull-through procedure for Hirschsprung's disease. J Pediatr Surg. 2003 Aug;38(8):1208-11. [PubMed] [Reference list]

46. Rintala RJ. Transanal coloanal pull-through with a short muscular cuff for classic Hirschsprung's disease. Eur J Pediatr Surg. 2003 Jun;13(3):181-6. [PubMed] [Reference list]

47. Hadidi A. Transanal endorectal pull-through for Hirschsprung's disease: experience with 68 patients. J Pediatr Surg. 2003 Sep;38(9):1337-40. [PubMed] [Refere

48. Ekema G, Falchetti D, Torri F, Merulla VE, Manciana A, Caccia G. Further evidence on totally transanal one-stage pull-through procedure for Hirschsprung's disease. J Pediatr Surg. 2003 Oct;38(10):1434-9. [PubMed] [Reference list]

49. Swenson O (1989). "My early experience with Hirschsprung's disease". J. Pediatr. Surg. 24 (8): 839–44, discussion 844–5. doi:10.1016/S0022-3468(89)80549-4. PMID 2671336

50. W. Allan Walker (2004-07-01). Pediatric gastrointestinal disease: pathophysiology, diagnosis, management. PMPH-USA. pp. 2120–. ISBN 978-1-55009-240-0. Retrieved 2010-11-12.

51. Malone PS, Ransley PG, Kiely EM (1990). "Preliminary report: the antegrade continence enema". Lancet. 336 (8725): 1217–1218. doi:10.1016/0140-6736(90)92834-
5. PMID 1978072. S2CID 9203632.

52. Frykman PK, Nordenskjöld A, Kawaguchi A, Hui TT, Granström AL, Cheng Z, Tang J, Underhill DM, Iliev I, Funari VA, Wester T., HAEC Collaborative Research Group (HCRG). Characterization of Bacterial and Fungal Microbiome in Children with Hirschsprung Disease with and without a History of Enterocolitis: A Multicenter Study. PLoS One. 2015;10(4):e0124172. [PMC free article] [PubMed] [Reference list]

53. Bjørnland K, Pakarinen MP, Stenstrøm P, Stensrud KJ, Neuvonen M, Granström AL, Graneli C, Pripp AH, Arnbjörnsson E, Emblem R, Wester T, Rintala RJ., Nordic Pediatric Surgery Study Consortium. A Nordic multicenter survey of long-term bowel function after transanal endorectal pull-through in 200 patients with rectosigmoid Hirschsprung disease. J Pediatr Surg. 2017 Sep;52(9):1458-1464. [PubMed] [Reference list]

54. Cheng Z, Dhall D, Zhao L, Wang HL, Doherty TM, Bresee C, Frykman PK. Murine model of Hirschsprung-associated enterocolitis. I: phenotypic characterization with development of a histopathologic grading system. J Pediatr Surg. 2010 Mar;45(3):475-82. [PMC free article] [PubMed] [Reference list]

55. Gosain A, Frykman PK, Cowles RA, Horton J, Levitt M, Rothstein DH, Langer JC, Goldstein AM., American Pediatric Surgical Association Hirschsprung Disease Interest Group. Guidelines for the diagnosis and management of Hirschsprung-associated enterocolitis. Pediatr Surg Int. 2017 May;33(5):517-521. [PMC free article] [PubMed] [Reference list]