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Hirschsprung's disease: the importance of early diagnosis

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ABSTRACT

Congenital intestinal aganglionosis, also called Hirschsprung disease (HD), is defined as the absence of ganglionic cells in the myenteric (Auerbach) and submucosal (Meissner) plexus, due to a failure in the enteric nervous system development. The extent of intestinal involvement may vary according to the age of embryo development in which this failure occurs. It is not unusual for other malformations to be present, as well as chromosomal trisomies, manly trisomy 21. Enterocolitis is a frequent, life threatening, and feared complication of HD. Moreover, oligohydramnios is a well-known condition frequently associated with malformations, including those related to the gastrointestinal tract. The authors report the case of a newborn that presented a delayed meconium passage. On the third day of life, he presented enterocolitis—the outcome of which was favorable with clinical treatment. While the diagnosis of HD was awaiting confirmation, the enterocolitis relapsed and this time he died due to septic shock. The autopsy findings were compatible with a short segment of congenital intestinal aganglionosis. No other malformation was found. The authors call attention for an early diagnosis of HD whenever the meconium passage does not happen for at least 48 hours and for the risk factors of enterocolitis. This case also demonstrates HD associated with oligohydramnios.

Keywords: Hirschsprung Disease; Enterocolitis Necrotizing; Infant, Newborn; Autopsy.

CASE REPORT

A 17-day-old boy was brought to the hospital after a 1-day history of diarrhea and deterioration in his general status. He was born at 37weeks' gestation through a cesarean section, weighing 3170 g, with a previous diagnosis of oligohydramnios. Until the third day of life, meconium was not passed, and therefore his condition worsened with bloating and recurrent vomiting. The passage of meconium occurred only after an enema on the third postnatal day. The diagnosis of megacolon

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congenitum associated with neonatal sepsis was considered, and the newborn was prescribed a 10-day course of antibiotics. He was discharged on the fifteenth postnatal day for an ambulatory diagnostic investigation. At home, evacuation did not occur, and he began to have diarrhea and foul-smelling vomiting.

The following day, at 17 days old, he was taken back to the hospital, where he arrived floppy, dehydrated, with cold and cyanotic extremities. He had an axillary temperature of 37.5 °C, a pulse of 200 beats per minute, respiratory frequency of 70 respiratory movements per minute, capillary filling time of 6 seconds, immeasurable blood pressure, and weighed 2905 g. The abdomen was distended despite the presence of intestinal sounds. With the diagnosis of septic shock he was promptly treated with volume resuscitation and broad-spectrum antibiotics. As the clinical status progressively worsened, mechanical ventilatory support and vasoactive drugs were required. Regardless of the efforts, the patient remained in shock, presenting metabolic acidosis, intravascular disseminated coagulation and multiple organ dysfunctions. The pediatric surgeon, considering the diagnosis of Hirschsprung disease (HD) and necrotizing enterocolitis performed a loop-transversostomy, aiming to attain urgent digestive tract decompression. The postoperative outcome was troublesome and the newborn died on the third day of hospitalization.

Autopsy Findings

The external examination showed a moderate abdominal distention, as well as a colostomy above the umbilicus, which was apparently free of infection (Figure 1A). At the opening of the skull, enlarged gyri, shallow sulci, and meningeal congestion were evidenced (Figure 1B). The abdominal cavity contained mild yellow-citrine ascites. The small bowel loops and the colon were dilated and covered by a wine-colored serosa (Figures 2A and 2B). The lungs, kidneys, and liver had severe congestion (Figure 2A). The distal duodenum, jejunum, ileum, and colon also showed a wine-colored mucosa with partial loss of wrinkling. Areas of ulceration recovered by fibrin and hemorrhagic content in the lumen (Figure 3) were found. The entire colon presented a 3.5 cm perimeter and a wall thickness of 0.2 cm. In contrast, the distal rectum and the sigmoid presented with a perimeter of 1.0 cm and 0.3 cm in wall thickness, featuring a non-dilated short segment. The colostomy was performed before this segment (Figures 3B and 3C).

Microscopic research of the small and large intestines was held through a representative strip from the distal ileum to the rectum. The ileum, ascending, transverse, and descending colon showed the presence of the submucosal and myenteric plexus, with the ganglionic cells exhibiting the usual morphology normally distributed (Figures 4, 5, and 6A). In contrast, these cells were rarely observed in the transition of the descending colon and the sigmoid (Figure 6B). In the distal sigmoid and rectum, the ganglionic cells were absent

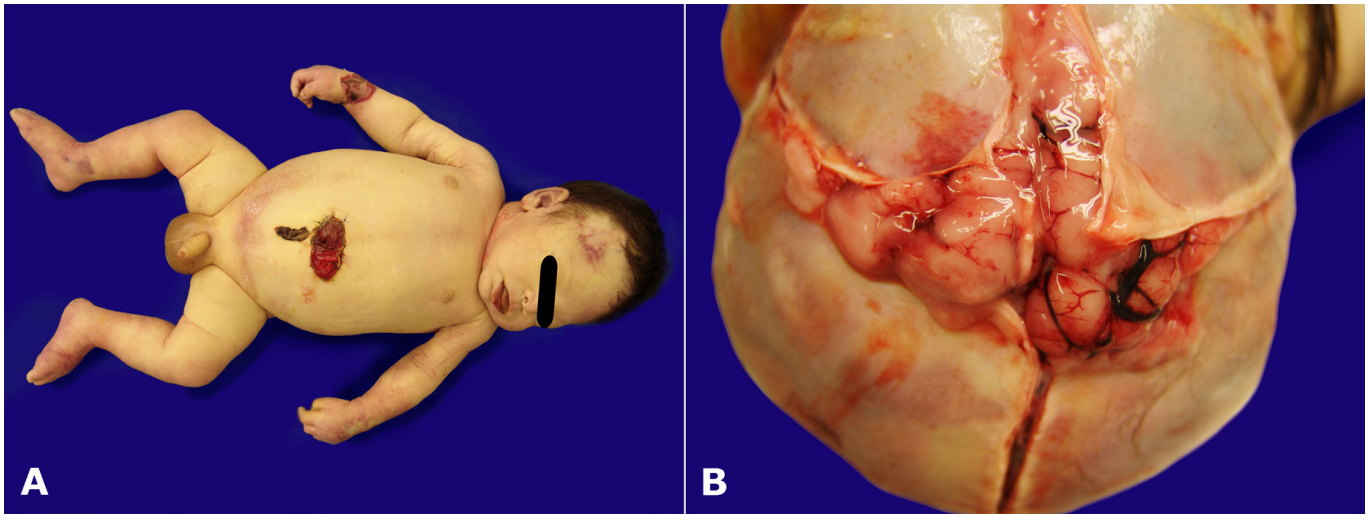

Figure 1 – A - Frontal view of the newborn showing a mild abdominal distention and colostomy above the umbilical cord segment, apparently free of infectious signs; **B** - Cerebral edema, enlarged gyri, shallow sulci, and meningeal congestion.

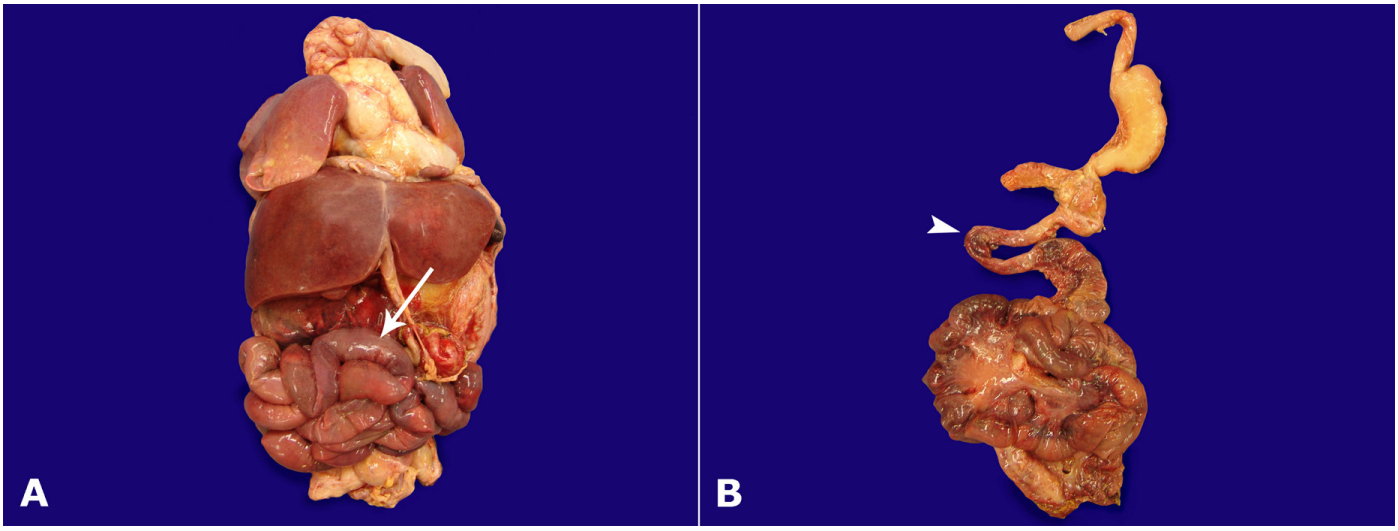

Figure 2 – Gross view of the thoracoabdominal monoblock. **A** - Moderate dilation of the large bowels, wine-colored serosa covering the small and large bowels (arrow); **B** - Dissected gastrointestinal tract showing wine-colored serosa extending from the duodenum (arrowhead) up to the distal segments of the colon.

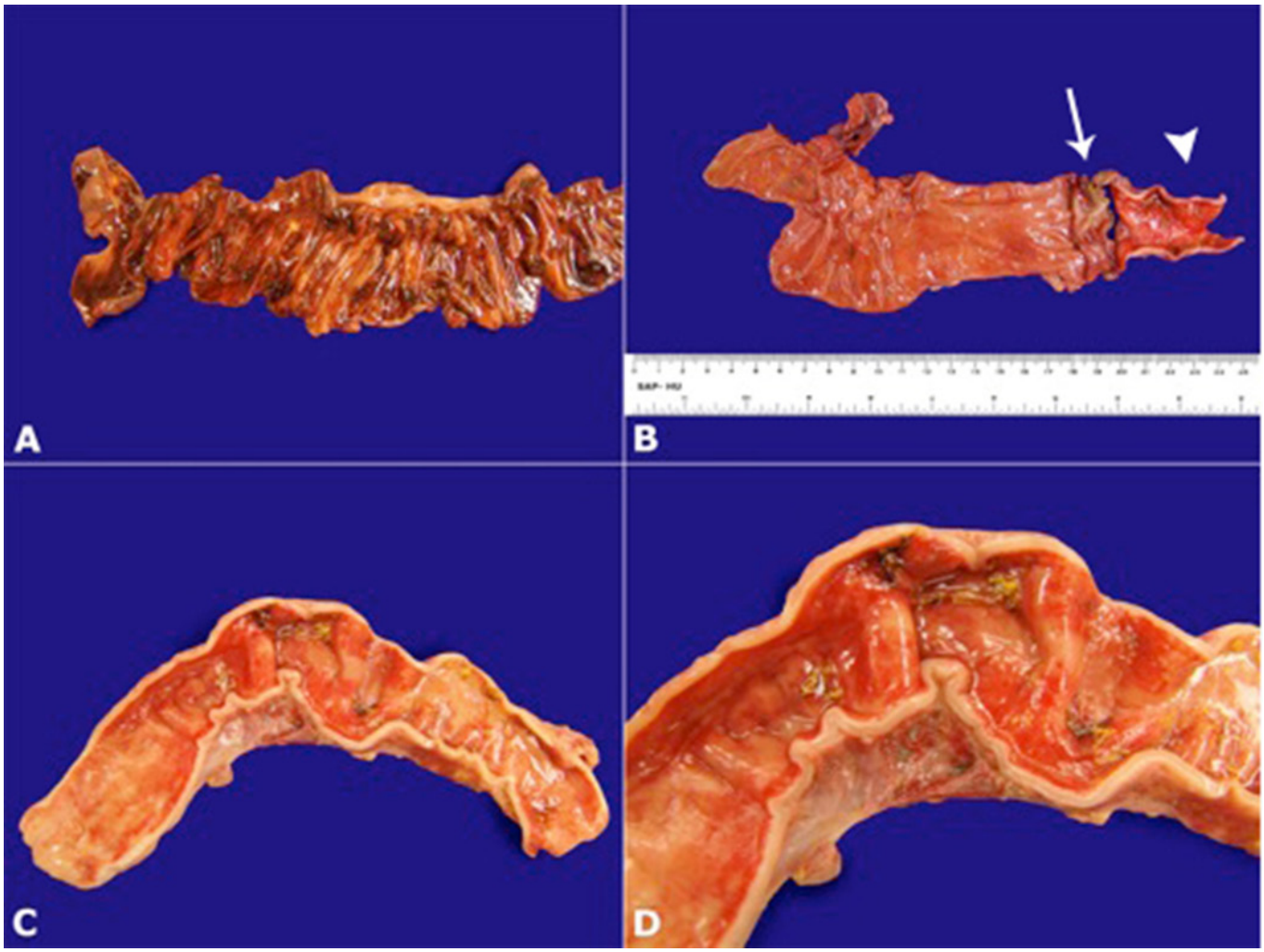

Figure 3 – Gross view of small and large intestines segments. **A** - An ileal segment exhibiting a poor-wrinkled and wine-colored mucosa with ulcerated areas and hemorrhagic content; **B** - Ileal segment, cecum, ascending, transverse, and descending colon. Note the dilation of the cecum lumen up to the transverse colon close to the colostomy (arrow). The descending colon downstream exhibits a discrete and slight reduced diameter (arrowhead); **C** - Sigmoid segment and proximal rectum with reduced diameter and thickened wall; **D** - Detail of the sigmoid colonic mucosa with fibrinous ulcerative areas and wine-colored mucosa.

in the submucosal as in the myenteric plexus, where the nervous fibers were thickened (Figure 7). The immunohistochemical analysis revealed negativity for calretinin in the segment of the sigmoid and rectum, but rare positive calretinin cells could be identified in the transition of the descending colon and sigmoid (Figure 8A). Immunohistochemistry for S100 protein demonstrated Schwann cell proliferation in the aganglionic sigmoid and rectum segments (Figure 8B). Acute fibrinous serositis was also found, therefore characterizing the diagnosis of enterocolitis. Yeast-like structures, PAS positive, compatible with *Candida sp,* were found over the colon serosa (Figure 8B, arrow). Additionally, both intestines presented ischemic areas, and neutrophilic inflammatory infiltration along the mucosa and in patchy areas of the muscular layer (Figure 9A). The remaining organs showed shock-related alterations, characterized by cerebral edema, pulmonary congestion, acute tubular necrosis, acute splenitis, marked hepatic congestion with microvesicular steatosis, and tiny ulcerated gastric mucosal lesions.

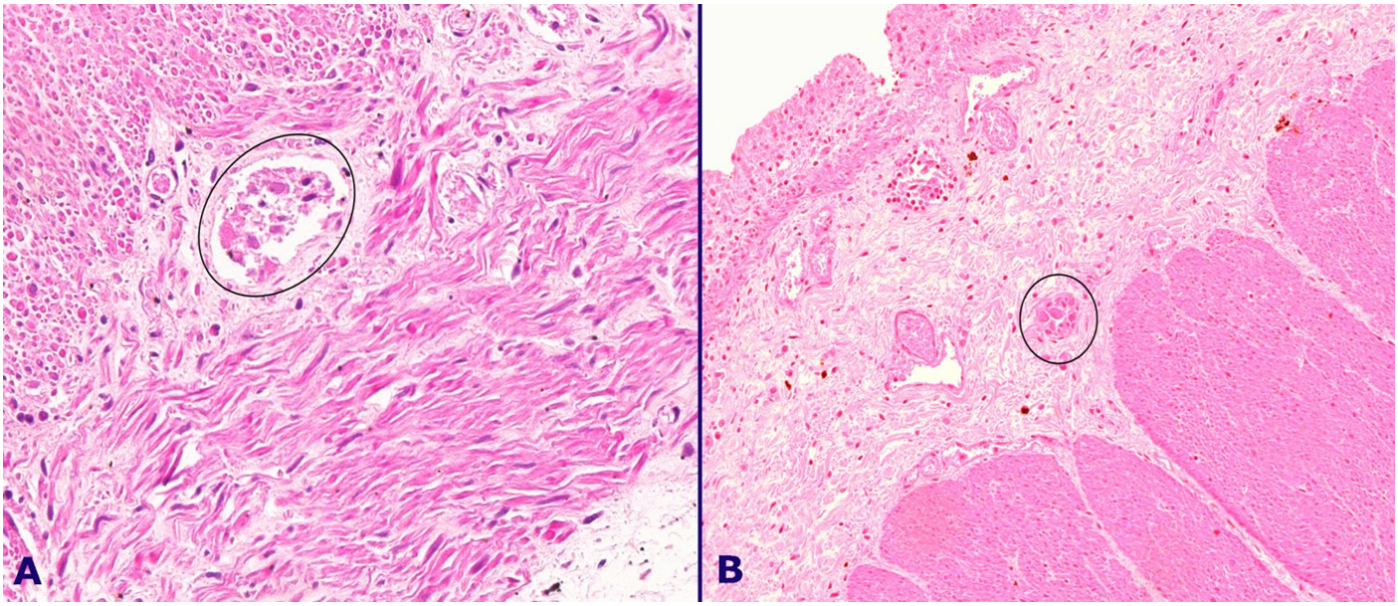

Figure 4 – Photomicrography of the cecum in **A** and ascending colon (H&E 400X) in **B**, showing the presence of ganglionic cells in the submucosa and in the myenteric plexus, respectively (H&E 200X).

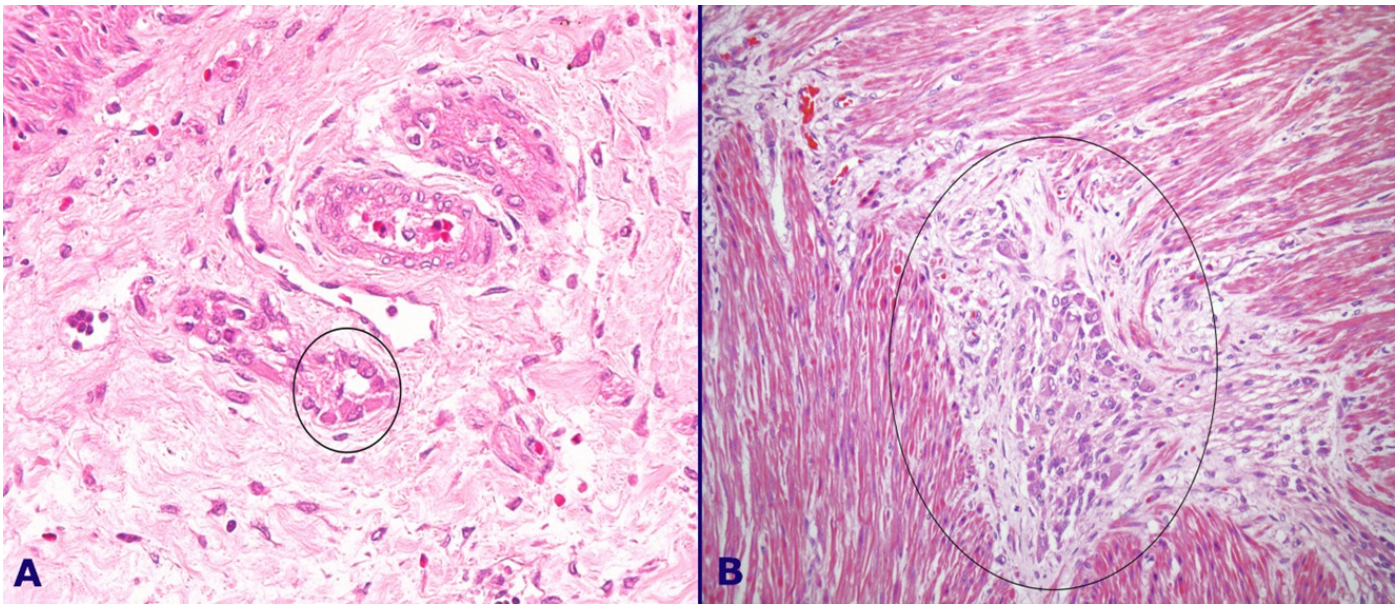

Figure 5 – Photomicrography of **A** - transverse colon and **B** - descending colon, both showing the presence of myenteric ganglionic cells (H&E 400X).

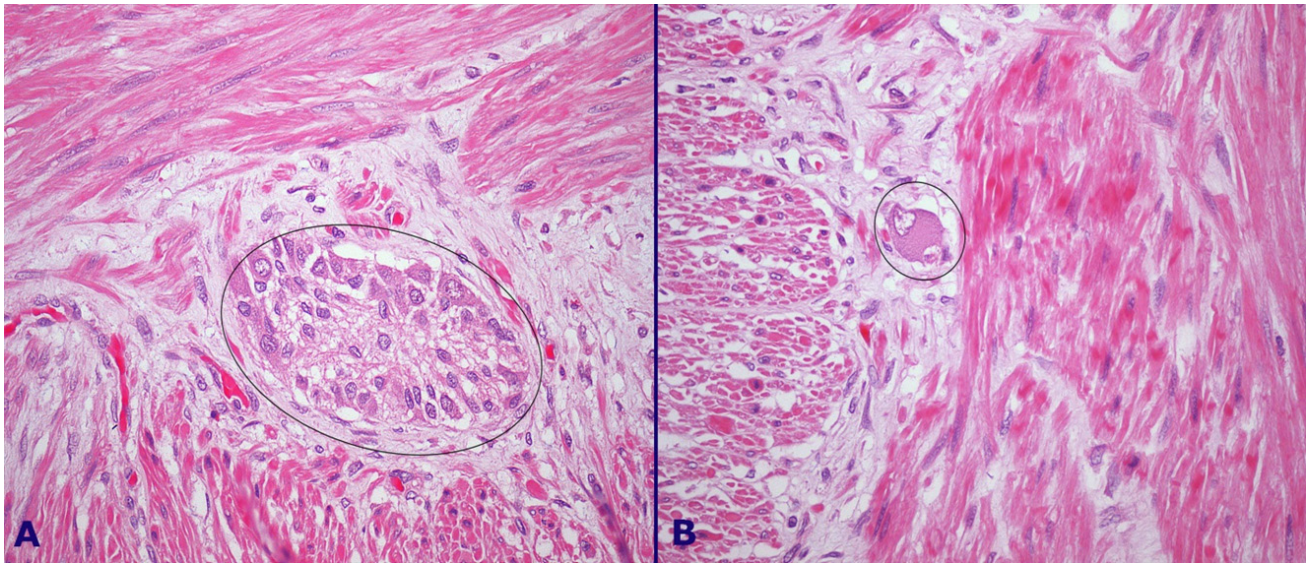

Figure 6 – Photomicrography. **A** - Descending colon showing the presence of myenteric ganglionic cells; **B** - Descending to sigmoid colon transition showing scanty myenteric ganglionic cells (H&E 400X).

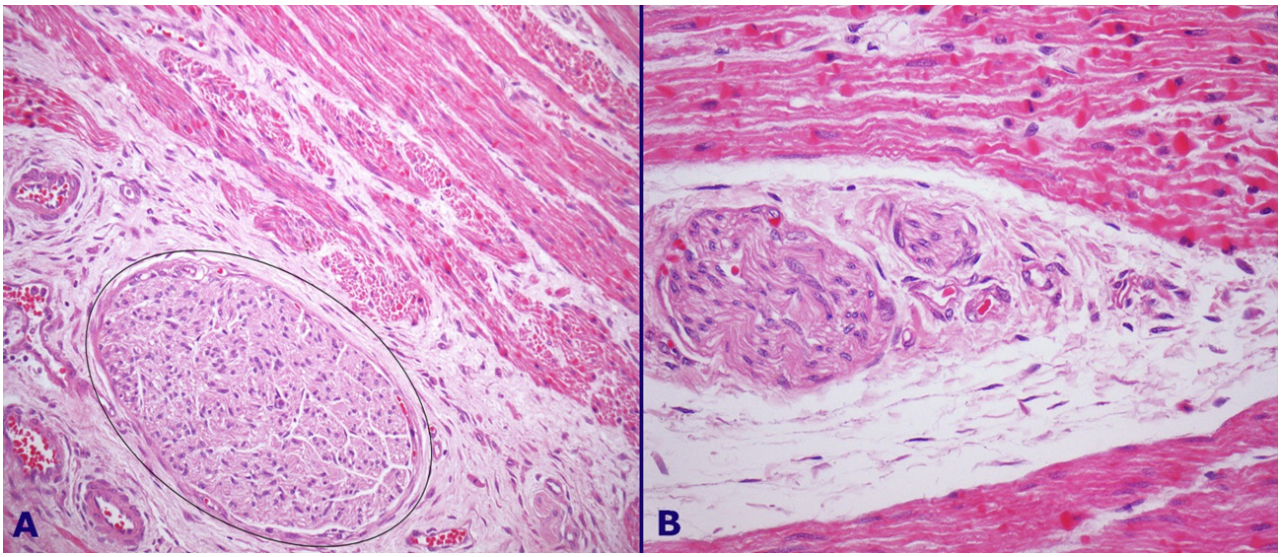

Figure 7 – A and **B** - Photomicrography of the sigmoid (**A**) and rectum (**B**) with absence of ganglionic cells and enlargement of the nervous bundle (H&E 400X).

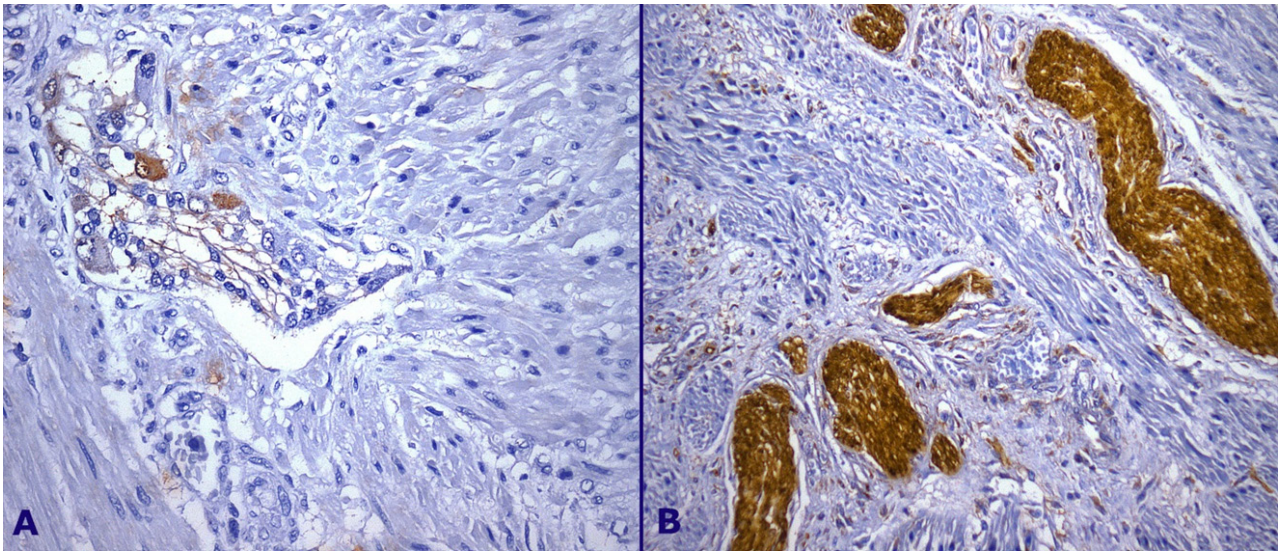

Figure 8 – Photomicrography. **A** - Descending colon and sigmoid transition showing immunohistochemical positive reaction for calretinin (H&E 400X). In the sigmoid and rectum, calretinin reaction was negative; **B** - Immunohistochemical reaction for S100 in the rectum, showing nervous bundle proliferation in the affected region (H&E 400X).

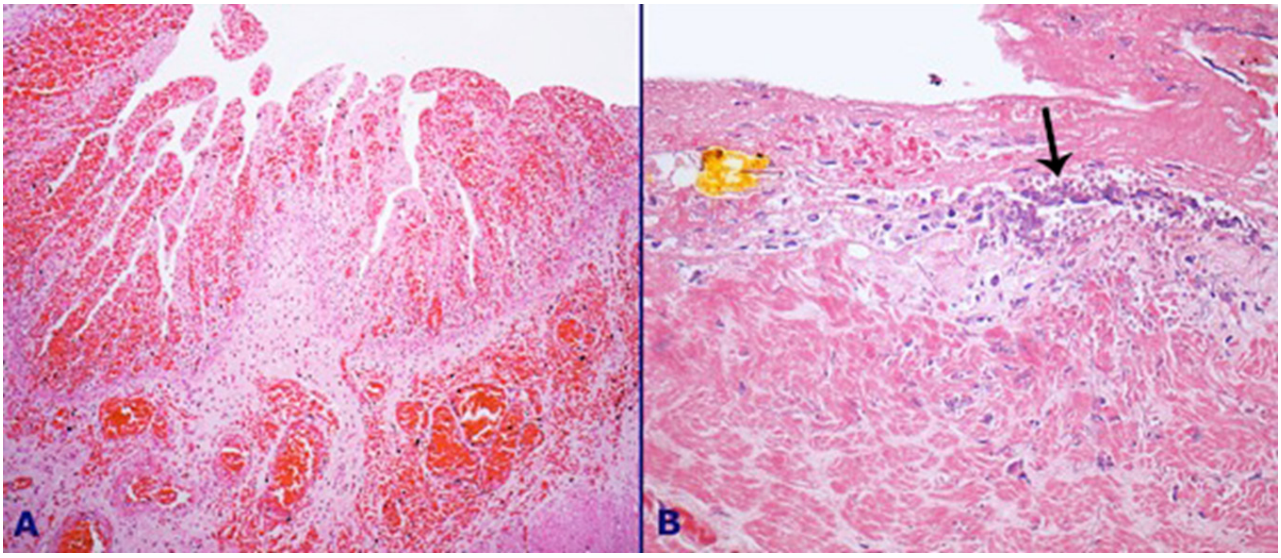

Figure 9 – Photomicrography. **A** - Small bowel ischemic and hemorrhagic mucosa with enteritis (H&E 200X); **B** - Serositis of the colon with the presence of hyphae and spores of *Candida sp* (arrow) (H&E 400X).

DISCUSSION

In 1886, the Danish pediatrician, Harald Hirschsprung, first described what was later named Hirschsprung disease.[1] The pathogenesis of the disease remained unknown for decades until the mid-1940s when the absence of submucosal and myenteric ganglionic cells were considered to be the cause of the disease.[1] Embryological studies show that around the fifth week of gestation neuroblasts derived from the developing neural crest first appear in the esophagus. These cells migrate towards the craniocaudal direction to the rest of the developing gut, until the twelfth week of gestation. Thus, the extent of aganglionosis is defined from the point where migration of neuroblasts, through the intestinal wall, is interrupted. When this stoppage occurs in the seventh week the total colon aganglionosis occurs. Likewise, if the break in neuroblast migrations occurs in the ninth week it results in aganglionosis of the descending colon, sigmoid, and rectum. Migration breaks occurring between the tenth and twelfth week result in the aganglionosis being confined to the sigmoid and rectum.[1,2] After the caudal migration of neuroblasts, they will be distributed within the deeper and upper layers of the intestinal wall, following maturation into ganglion cells. The latter process may also fail, leading to the absence of ganglionic cells in the myenteric and submucosal plexus.[2] Therefore, congenital intestinal aganglionosis was defined as the absence of ganglionic cells in the myenteric (Auerbach) and submucosal (Meissner) plexus, due to a failure in the enteric nervous system development.[3] HD is classified according to the extension of the neuroenteric involvement, as follows: a) ultrashort aganglionosis when the disease is confined to the anal canal; b) short segment, which accounts for 80-90% of cases and is the classic form of HD disease involving the sigmoid and rectum; c) long segment, accounting for 10% of all cases, characterized by the absence of ganglionic cells extending proximally beyond the splenic flexure or transverse colon; d) total colonic aganglionosis, represents 5% of the cases, and refers to aganglionosis of the entire colon; and e) total colonic aganglionosis with occasional extension of aganglionosis into small bowel.[1]

Genetic mutations were described in 10 different genes contributing to HD development. Among them, the most common are: gene *RET* (accounting for 7-35% of the cases), gene *EDNRB* (7% of the cases) and gene *END3* (<5% of the cases), and others like *GDNF* and *SOX103*.[4] The HD is associated with several genetic syndromes such as Down syndrome (trisomy 21), abnormalities of the urogenital tract, hypertrophic pyloric stenosis, intestinal malrotation, Meckel's diverticulum, and anorectal anomalies.[4,5]

The gold standard diagnostic investigation is carried out by rectal suction biopsy, which is performed 3 cm above the pectinate line, allowing examination of the submucosa, which demonstrates the absence of ganglionic cells and the presence of hypertrophic nervous bundles in the submucosa. (The aganglionic cell zone is observed at the same degree on both plexuses).[1,6] Usually, the normal histology reveals 1-5 ganglionic cells in the plexus per each millimeter of normal rectal mucosa. It is noteworthy that in the newborn the ganglionic cells

are often smaller, and the nucleoli and cytoplasmic granules are not prominent, challenging their identification. As the 2 cm intestine wall above the pectinate line is usually a scanty zone in ganglionic cells and nervous fibers, HD may be misdiagnosed; therefore, the pathologic evaluation should consider this particularity during the biopsy evaluation.[6] In these cases, the biopsy may reveal a rectal squamous epithelium of transitional type, indicating the proximity of the pectinate line, and should be noted in the histopathological report.

In challenging cases, the immunohistochemistry is recommended by the reactions with neuron-specific enolase, *RET* oncoprotein, BCL-2, cathepsin D, and PGP 9.5. However, in routine clinical practice, none of the generic neuronal markers offers a significant advantage over thorough histologic research stained with hematoxylin- eosin.[6]

At present, acetylcholinesterase (AChE) expression in the affected tissue is of diagnostic importance in HD. The histochemical reaction measures the activity of acetylcholinesterase, which indirectly means the presence of acetylcholine. AChE is useful in confirming the diagnosis of Hirschsprung disease, by revealing activity of this enzyme in hypertrophied nerve fibers.[7]

The main advantage of this method is that superficial biopsies are sufficient to reveal the activity of the enzyme, confirming the diagnosis HD. However, this method requires frozen tissue samples, and the results may be difficult to interpret. False negative reaction may occur, which results in delayed diagnosis and treatment, particularly in cases of total colonic aganglionosis and prematurity, where AChE might be missing in the nervous bundle hyperplasia.[7] Barshack et al.[8] described the use of calretinin as a diagnostic test adjuvant due to its ability to be expressed in nerve endings and ganglion cells of the myenteric and submucosal plexuses of the entire normal colon and small intestine. This finding was further confirmed by other author's studies.[9,10]

In the case reported here, the diagnosis of HD was suspect from the beginning when the newborn did not show any meconium passage until the third day after birth. The first enterocolitis was promptly treated with a favorable outcome. However, the relapsed enterocolitis was severe, and did not respond satisfactorily to clinical and surgical treatment attempts. At autopsy, the dilation of the small and large bowels was in conformity with this diagnosis. The thorough research for ganglionic cells and nervous bundles along the entire gut confirmed the diagnosis in its short variant. Calretinin was an ancillary diagnostic tool, confirming the absence of ganglionic cells in the sigmoid and rectum as well as the enlarged nervous bundle in this region. In this case, no other malformation, frequently observed in cases of HD, could be detected. It's interesting to note in the present case the previous diagnosis of oligohydramnios, which is well known to be associated with malformations.[11]

In the nineteenth century, Härald Hirschsprung had already recognized the association of enterocolitis in his hallmark description of congenital megacolon,[12] when he noted some pathologic findings of Hirschsprung-associated enterocolitis (HAEC) at autopsies, including crypt abscesses, mucosal ulceration and transmural necrosis. However, in 1962, Bill and Chapman[13] first described HAEC as a clinical syndrome, which was clinically characterized by explosive, watery, diarrhea with foul-smelling stools, vomiting, fever, rectal bleeding, abdominal distention, and prostration. Despite the fact that the occurrence has been reported in different age groups, it is characteristically observed among full-term neonates. The suspicion should be raised whenever the passage of meconium does not occur 24 hours or at least 48 hours after birth. Failure to recognize HD in the early perinatal period places children at greater risk of HAEC.[14] On the other hand, when HD is diagnosed outside the neonatal period, the development of HAEC is less frequent, probably because children in this group may present improved mucosal defenses.[15] Contributing factors for the development of HAEC include: family history, trisomy 21, and previous episodes of HAEC. Some investigators also consider long-segment disease as more prone to HAEC development.[14]

The mean incidence of HAEC is 25% of all the HDs, and the mortality rate varies between 17% and 50% of these cases.[16] Nonetheless, in recent decades, decreased mortality and morbidity due to better diagnosis and early treatment has been seen.

Although debatable, it seems that the HAEC etiology is multifactorial. Basically, the implicating causes include: obstruction, infection, ischemia, and hypersensitivity.

CONCLUSION

HD is not a frequent entity, but should always be considered when facing a delayed meconium passage in neonates, especially if accompanied by oligohydramnios and associated with another syndrome or anomaly. The diagnosis can be performed with a simple biopsy followed by a thorough histological analysis of a hematoxylin and eosin stained slice. If not treated early, HD may lead to developmental delay, a high index of complications almost always associated with severe enterocolitis, and which presents a high mortality rate.

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