ORIGINAL ARTICLE

Acquired isolated hypoganglionosis as a distinct entity: results from a nationwide survey

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Abstract

Purpose Acquired isolated hypoganglionosis (A-IH) is a late-onset intestinal pseudo-obstruction disorder and shows different pathophysiological findings from congenital isolated hypoganglionosis (C-IH). In this study, we retrospectively examined five cases of A-IH and investigated the features of A-IH.

Methods Five cases of A-IH were extracted from a nationwide retrospective cohort study in 10 years, from which totally 355 cases of Allied Disorders of Hirschsprung's Disease (ADHD) were collected.

Results Ages of onset were between 13 and 17 years in three cases, and 4 years and 4 months in ones. Initial symptoms were abdominal distension and/or chronic constipation in 4 cases, whereas one exhibited intestinal perforation. Affected lesions varied from case to case, extending various length of intestinal tracts. All cases underwent multiple operations (average: 4.6 times), such as enterostomy, resection of dilated intestines, and/or pull-through. Pathological findings showed the decreased numbers of ganglion cells and degeneration of ganglion cells, whereas the size of the plexus was normal. Currently, all cases were alive and almost all eat regular food without requiring parenteral feeding.

Conclusion A-IH is rare, but distinct entity characterized by different clinical courses and pathological findings from those of C-IH. The outcome is considered to be favorable after a resection of affected intestine.

Keywords Isolated hypoganglionosis · Acquired · Allied disorders · Hirschsprung's disease · Ganglion cell

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Introduction

Isolated hypoganglionosis (IH) is proposed to be one of the allied disorders of Hirschsprung's disease (ADHD), which is rare and resembles Hirschsprung's disease (HD) and associated with decreased numbers of intestinal ganglion cells [1]. Historically, the existence of this entity has been questioned [2, 3]. Some researchers have doubted the existence of IH because they have never encountered the disease. We previously reported that IH had two distinct entities, with different clinical characteristics and histological findings: congenital IH (C-IH) and acquired IH (A-IH) [4]. A-IH, which is characterized by its late-onset, shows the particular pathological findings that are distinct from those of C-IH. These include the degeneration of ganglion cells, a decrease in ganglion cell numbers, and gliosis in Auerbach's plexus with the preservation of the size of the plexus. These findings strongly suggest that ganglion cells were initially present, and that they has gradually disappeared. In this study, to investigate the incidence, the clinical symptoms and the outcomes of A-IH in Japan, we extracted the cases of pathologically proven A-IH from the data of a nationwide retrospective cohort study of ADHD that was performed in Japan from 2000 to 2009.

Patients and methods

The following diagnostic criteria could be used to define A-IH: (1) the absence of a congenital predisposing factor; (2) the onset of symptoms (e.g., prolonged constipation, abdominal distension without vomiting) after the neonatal period; (3) the pathological findings showing the degeneration of ganglion cells and decrease in their number; (4) a satisfactory outcome after the resection of the involved intestine. In the present nationwide retrospective cohort study, which was supported by the Ministry of Health and Welfare, Japan, preliminary questionnaires were sent to the 161 major institutes of pediatric surgery or gastroenterology (representing the core members of the Japanese Society of Pediatric Surgeons, the Japanese Society of Pediatric Nutrition, Gastroenterology and Hepatology and the Japanese Study Group of Pediatric Constipation). The questionnaires asked about the number of ADHD cases that were treated from January 2000 to December 2009. Replies were obtained from 157 out of the 161 institutes (98%). A total of 355 ADHD cases were collected [5]. We found 5 cases of A-IH regarding the criteria described above. Then, we sent secondary questionnaires that asked about the background characteristics, the clinical and pathological findings, and treatments and outcomes of each case of A-IH. Based on the results of the secondary questionnaire, we collected the clinical and pathological information of 5 cases of A-IH, and investigated the details.

Results

The male to female ratio was 3:2. With the exception of two cases (Case 2: necrotic enterocolitis in neonate, Case 3: influenza encephalitis in early childhood and mental retardation), none of the patients had a specific past medical history before the onset of symptoms. The onset of symptoms

**Table 1** The characteristics and examination results of each case of acquired isolated hypoganglionosis

Case no.	1	2	3	4	5
Sex	Female	Male	Male	Male	Female
Past medical history	None	None	Influenza encephalitis Mental retardation	None	None
Onset of symptoms	School-age	Infant	Early childhood	School-age	School-age
Primary symptoms	Chronic constipation	Abdominal distension	Vomiting Intestinal perforation	Chronic constipation	Abdominal distension Vomiting
Associated malformations	None	None	None	None	None
Chromosomal abnormalities	None	None	None	Not examined	Not examined
Family history	Prader-Willi syndrome	None	None	None	None
Abdominal X-ray image	Abnormal intestinal dilatation	Abnormal intestinal dilatation /air-fluid level	Free air	Abnormal intestinal dilatation	Air-fluid level
Contrast enema	Megacolon	Normal	Not examined	Megacolon	Megacolon
Rectosphincteric reflex	Negative	Not examined	Positive	Positive	Unknown
Rectal mucosal biopsy	Normal	Not examined	Not examined	Normal	Unknown

occurred in infancy ($n=1$, case 2), early childhood ($n=1$, case 3), and childhood (in school-age children; $n=3$, cases 1, 4, and 5). The initial clinical symptoms were chronic constipation ($n=2$, cases 1 and 4), abdominal distension ($n=1$, case 2), and abdominal distension and vomiting ($n=1$, case 5); one case showed intestinal perforation and vomiting (case 3) (Table 1). The abdominal X-ray images showed abnormal intestinal dilatation ($n=3$, cases 1, 2, and 4), air-fluid level ($n=2$, cases 2 and 5), and free air ($n=1$, case 3). Contrast enema showed megacolon ($n=3$, cases 1, 4, and 5) and normal results ($n=1$, case 2); contrast enema was not conducted in case 2 because of the bad condition. Three cases underwent anorectal manometry, which indicated rectosphincteric reflex positivity ($n=2$, cases 2 and 3), and negativity ($n=1$, case 1). Two patients underwent rectal mucosal biopsy; the findings were normal in both cases (Table 1). Initially, except for case 3 with immediate surgical intervention due to intestinal perforation, all four cases were managed with conservative medical treatment; however, their conditions did not improve. Finally, all five patients underwent multiple operations (average: 4.6 operations per case) because multiple intestinal segments were affected. The procedures included creation of enterostomy, resection of the involved segments of the dilated intestine, and/or pull-through because the involved segments spread from the stomach to the rectum (partially or totally) in each case (Table 2). Hematoxilin and eosin (H&E) staining revealed that all five cases had ganglion cell degeneration and decreased ganglion cell numbers with a normal size Auerbach's plexus in the resected intestine (Fig. 1); however, it should be noted that in 2 of the 5 cases the numbers and sizes of ganglion cells were normal at the first resection. Immunohistochemical staining, which was performed using HuC/D, S-100 and SOX10 antibodies, was effective in identifying the remaining ganglion cells, the preservation of the size of Auerbach's plexus, and increased glial cell numbers in the plexus (Fig. 2). Currently, all five

Table 2 The surgical treatments and outcomes of each case

Case no.	1	2	3	4	5
Involved segment	Sigmoid colon-rectum	Ileum	Stomach-anus	Ileum, ascending colon, rectum	Duodenum, ileum, sigmoid colon-rectum
Total number of operation	7	3	5	3	6
Pull-through	2	0	0	1	2
Resection of dilated intestine	0	0	0	0	1
Creation of enterostomy	3	2	1	0	0
Resection of dilated intestine and enterostomy	0	0	1	0	0
Others	2	1	3	2	3
Histological findings from the resected intestine	Hypoganglionosis/sigmoid colon and rectum	1st: Normal/ileum 3rd: Decrease of ganglion cells/ileum	1st: Normal/ileum 3rd~: Decrease of ganglion cells and gliosis/ileum	Decrease of ganglion cells, immaturity/sigmoid colon & rectum	Chronic inflammation with decrease of ganglion cells, degeneration of ganglion cells/ileum
Outcome	Survived	Survived	Survived	Survived	Survived
Current nutrition	Ingestion of general diet	Ingestion of general diet	Ingestion of defined formula diet with parenteral nutrition	Ingestion of general diet	Ingestion of general diet
Remaining enteric fistula	Yes	No	Yes	No	No
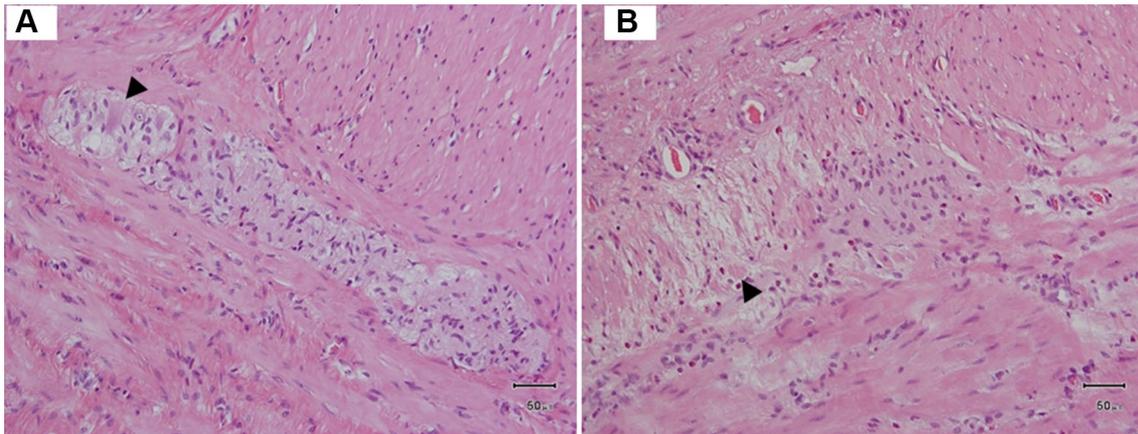
Fig. 1 The typical pathology of acquired hypoganglionosis (Case 5). **a** The colon. The plexus shows a mixture of almost normal ganglion cells and gliosis. **b** The ileum. The ganglion cells have disappeared and glial cells increased. Arrow-heads indicate degenerated ganglion cells; hematoxylin & eosin staining
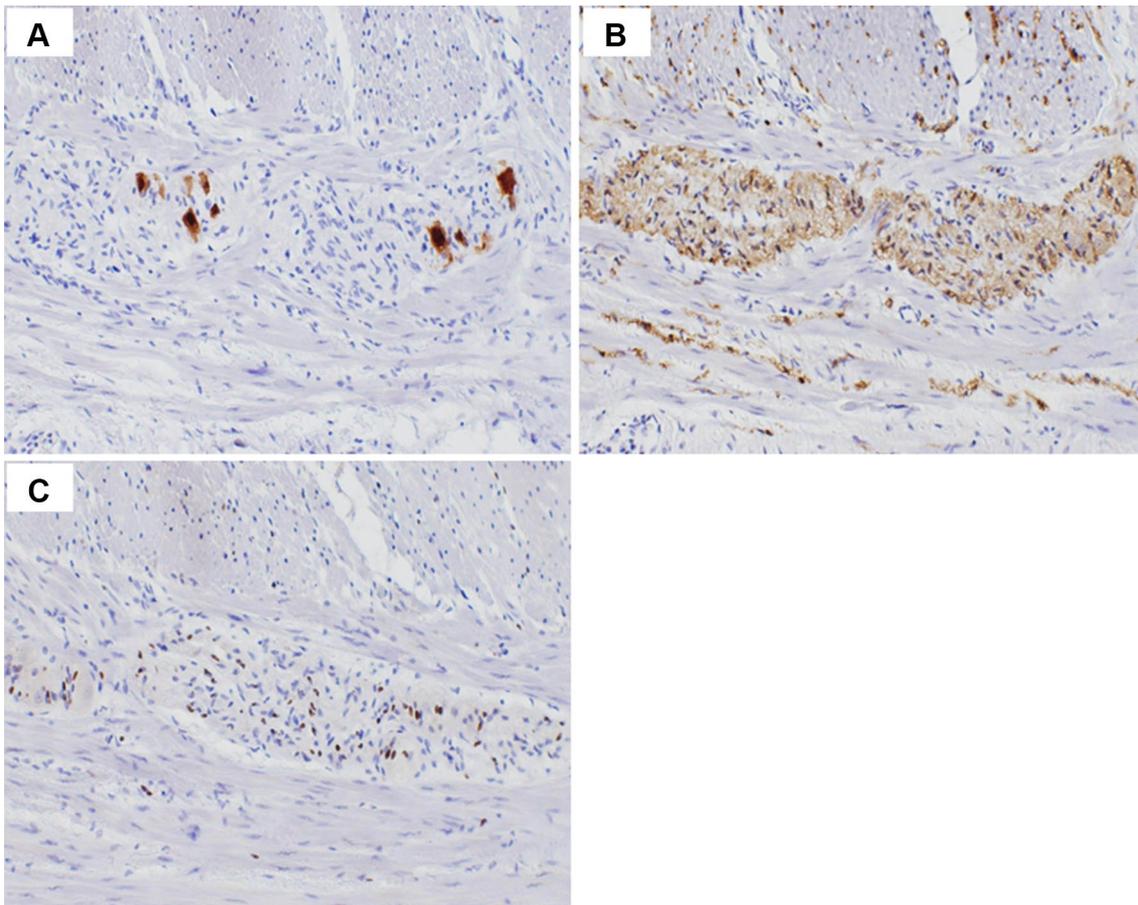
Fig. 2 The immunohistochemical findings in a patient with acquired hypoganglionosis (Case 5). **a** HuC/D immunostaining. **b** S-100 protein immunostaining. **c** SOX10 immunostaining. HuC/D immunostaining highlights the indistinguishable ganglion cells in the increased glial cell numbers in the plexus. S-100 protein is pan-neuronal marker and shows the size of enteric plexus. Glial cells were positively stained by SOX10

cases are alive, and 2 enteric fistulas remain. All but one of the patients are capable of ingesting a general diet without the need for parenteral feeding (Table 2).

Discussion

Isolated hypoganglionosis (IH) remains a diagnostic challenge [2, 3] and preoperative correct diagnosis of IH is difficult because the clinical symptoms of IH resemble those of Hirschsprung's disease (HD). Both patients with HD and those with IH present delayed passage of meconium, abdominal distension, and vomiting. Hypoganglionosis as an isolated disease represent only 5% of neuronal intestinal malformations [6]. Meanwhile, we previously reported IH had two entities, with different clinical characteristics and histological findings: congenital IH (C-IH) and acquired IH (A-IH) [4]. The present study revealed that incidence of A-IH was very low (5 cases from a 10-year nationwide survey), in comparison with the other categories of ADHD [5]. The definitive diagnosis of A-IH requires pathological examination of resected bowel [7]; however, if the symptoms are not so severe, physicians may be hesitant to perform surgical resection. Thus, it is possible that potential patients with A-IH are present among patients with chronic constipation.

The onset of the symptoms of A-IH patients in the present study occurred at various ages in childhood after the neonatal period (from infants to school children); the main symptom was constipation, which was prolonged and gradually progressive. In contrast, the onset of symptoms in patients with C-IH is in the early neonatal period, and the symptoms of C-IH include severe abdominal distension and intestinal obstruction after ileostomy in the neonatal period [5, 8]. Thus, the onset and symptoms of A-IH seem different from those of A-IH.

The intestinal segments that were involved in the cases with A-IH in the present study were wide-ranging. In line with the observations of a previous report [4], the lesions were seemed like "skip segments", spreading from the stomach to rectum (Table 2). A previous systematic review showed that the involved intestinal segments of C-IH were localized, from total intestinal to rectosigmoidal (especially colonic) [6]. In contrast, Watanabe et al. reported the operative findings of 90 cases of C-IH and noted the presence of dilated intestine with caliber changes starting at the small intestine in 63 cases [8], indicating that the involved location spreads sub-totally from the small intestine to the rectum in patients with C-IH.

In the description regarding hypogenetic-type C-IH reviewed by Friedmacher et al. [7], the number of ganglion cells decreases and the size of plexus is also small. In the present study, all of the patients with A-IH showed ganglion cell degeneration and decreased ganglion cell numbers, while the size of Auerbach's plexus was preserved. These findings are similar to the previous report in which the size of Auerbach's plexus was normal, whereas ganglion cell degeneration, decreased ganglion cell numbers, and the proliferation of glial cells were prominent [4]. The report also noted that these findings were evidence that ganglion cells were initially present, and that they gradually disappeared. In the present study, in 2 out of the 5 cases the size and the number of ganglion cells were normal at the first resection, and then the ganglion cell numbers decreased. Therefore, it is possible that some factors may affect the ganglion cells [4]. Chagas disease, an infection caused by the protozoan parasite *Trypanosoma cruzi*, is known to cause the degeneration and decrease of ganglion cells. Previous reports on colonic involvement in Chagas disease patients have described that particular pathological features such as the degeneration of the intrinsic myenteric neurons and a decrease in their numbers, as well as reduced numbers of nitric oxide-containing myenteric neurons, deficiency of the interstitial cells of Cajal, T lymphocyte-induced ganglion cell damage, increased fibrosis, and increased numbers of mast cells [9]. The results of the previous reports [4] and the present study suggest that factors which are causative of A-IH, such as severe chronic constipation, chronic enterocolitis due to chronic constipation, peritonitis due to intestinal perforation, or infection, might exist, in other words, the degeneration of ganglion cells and decrease in their number might occur secondary to prolonged constipation, systemic shock or intestinal ischemia on the involved intestines.

Immunohistochemical examinations are useful for the definitive pathological diagnosis of ADHD [10]. S-100 protein is pan-neuronal marker that is not found in ganglion cell, which also shows the pattern of distribution and the size of enteric plexus, SOX10 is positive in the nuclei of the enteric glia but negative in the nuclei of ganglion cells [11]. HuC/D protein is routinely used as a neuronal marker in both the central and the peripheral nervous system. HuC/D staining offers a practical advantage, making qualification of individual neurons easy [12]. In the present study, although H&E staining plays an important role in the identification of ganglion cells and glial cells in the plexus, the use of HuC/D, S-100 protein and SOX10 were also helpful for identifying the numbers of remaining ganglion cells, the preservation of the size of Auerbach's plexus, and increased glial cell numbers in the plexus of A-IH.

Although the A-IH patients required multiple operations which were similar to those required by C-IH patients, their postoperative outcomes were far better. In C-IH, dysmotility does not improve over time, re-enterostomy was required in most cases, and the combination of continuous parenteral nutrition and partial enteral nutrition was necessary for the patients to survive [4]. On the other hand, while the A-IH

cases in the present study required multiple operations, satisfactory outcomes were eventually obtained, with the oral intake of a general diet without the need for parenteral nutrition in all but one of the patients.

In conclusion, the findings of the present study prove that A-IH is a rare, but distinct entity. The pathological characteristics include a decrease in ganglion cell numbers and gliosis, and the preservation of the size of Auerbach's plexus. The onset of symptoms occurs in patients of various ages. The clinical outcome after the resection of the involved intestine, which usually shows dilatation, is considered to be favorable. Patients with A-IH may require multiple operations due to the varying extent of the associated lesions.

Compliance with ethical standards

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. And, for this type of study formal consent was not required. This retrospective study was also approved by the ethics committee for clinical research of Kyushu University Hospital (No. 28–155).

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