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Epithelial and ganglionic distribution at the distal rectal end in anorectal malformations: could it play a role in anastomotic adaptation?

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ABSTRACT

Purpose: In anorectal malformations (ARMs), the epithelium of the distal rectal end is not well described. We histomorphologically evaluated epithelial and ganglionic distribution in the distal rectal end of ARMs resected during anorectoplasty to assess similarities and differences with normal anal canal structure.

Methods: In this single-center retrospective study, specimens from 60 ARM patients (27 males, 33 females) treated between 2008 and 2019 were evaluated.

Results: Epithelium type and alignment sequence as well as ganglionic distribution were similar in the distal rectal end and in a normal anal canal. Stratified columnar epithelium (anal transitional zone, ATZ) was seen in 49/60 (81.7%) cases and in all ARM types, including the no-fistula type. Anal crypts were identified in the stratified columnar epithelium (ATZ) of 46/49 (93.9%) patients. Regarding distal rectal end-resecting anorectoplasty, in 90% of patients, resection was performed distal to the Herrmann line. Ganglion cell distribution was exclusively proximal to the Herrmann line.

Conclusion: Epithelial and ganglionic distribution was similar in the distal rectal end of ARMs and in a normal anal canal. The ATZ is the epithelial boundary between the rectum and skin in a normal anal canal. ATZ preservation could reproduce anal canal structure in ARM reconstruction.

Keywords: Anorectal malformation, Epithelium, Anal transitional zone, Ganglionic distribution, Distal

rectal end

Introduction

Anorectal malformations (ARMs) are common congenital abnormalities involving incomplete development of the hindgut, cloaca, and ectoderm during the fetal period. Various types of malformations seen in male and female babies result in a defective anal canal structure [1]. Although remarkable improvements in surgical techniques in the last decade have generated good outcomes, completely satisfactory results for defecation function after ARM reconstruction have not yet been achieved [2, 3]. To pursue better outcomes for anorectoplasty, many discussions focus on whether the distal rectal end should be preserved or resected for reconstruction. Knowing and comparing the differences between a normal anus and a reconstructed anus can thus inform discussion of this problem.

Few studies have assessed the epithelial and ganglionic characteristics of the distal rectal end of ARMs. We investigated the types of epithelia that line the inside of the specimens resected from the distal rectal end of ARMs and examined the distribution of ganglion cells from an epithelial perspective. The aim of this study was to identify similarities and differences between the distal rectal end of ARMs and normal anal canals.

Materials and methods

Fig. 1 illustrates the definition, structure, and nature of embryonic development of a normal anal canal.

There are several definitions of the anal canal; however, in this article, we defined the anal canal as the so-called surgical anal canal. The anal canal is a complex functional structure consisting of an external anal sphincter muscle, internal anal sphincter muscle, and epithelium. The anal canal is autonomously motored by ganglion cells. It is the foreshore boundary between the gastrointestinal tract and the surface of the body.

Three types of epithelium cells line the inner lumen of the anal canal: simple columnar epithelium (colorectal zone), stratified columnar epithelium (anal transitional zone, ATZ), and stratified squamous epithelium (squamous zone), with the transition from proximal to distal. The borders between each epithelium are called the Herrmann line (between the colorectal zone and the ATZ) and the dentate line (between the ATZ and the squamous zone); both borders are clearly recognized visually and microscopically [4]. Ganglion cell distribution in the normal anal canal is also an important factor to be considered. A natural hypoganglionic zone is found above the dentate line [5, 6]. Embryologically, the anal canal is a composite of the endoderm (the hindgut and cloaca) and ectoderm. The internal anal sphincter, ganglion cells, and epithelium above the dentate line develop from the endoderm. We emphasize here that the ATZ develops from the endoderm [7, 8].

This single-center retrospective study was approved by the local institutional ethics committee (R31-46).

Patients in whom ARM reconstructive surgery was performed by posterior sagittal anorectoplasty (PSARP) or anterior sagittal anorectoplasty (ASARP) at Kobe Children's Hospital between 2008 and 2019 were enrolled in this study. Informed consent was obtained from all patients.

Specimens were collected from cases that underwent a distal rectal end-resecting anorectoplasty. The clinical types of ARM (by location of fistula opening) were investigated according to the Krickbeck classification [1]. Cases wherein a distal rectal end-resecting anorectoplasty was performed but for which, no specimen was submitted and cases for which specimens were collected but histomorphological inspection was not possible were excluded from the study. The distal rectal end was dissected to the stump of the rectal end to a feasible extent. The distal rectal specimen was resected up to about 3 centimeters in order to optimize it for reconstruction and to eliminate surplus tissue and was fixed in formalin solution. Paraffin-embedded tissue was cut longitudinally. The types of epithelium and existing ganglion cells in each epithelium region in the resected rectal end were evaluated histomorphologically using hematoxylin and eosin staining.

Results

Seventy-seven patients underwent an anorectoplasty between 2008 and 2019 at our institution. Seventy-one cases underwent a distal rectal end-resecting anorectoplasty. No specimens were submitted in eight of these cases, and specimens could not be evaluated in three cases, owing to severe damage during the operation. Overall, 60 cases were evaluated histomorphologically. Sex distribution and types of ARMs are shown in **Table 1**.

The types of epithelium seen in the specimens of the distal rectal end were simple columnar epithelium, stratified columnar epithelium, and stratified squamous epithelium. These three types of epithelium are the same as those seen in a normal anal canal, and they correspond to the colorectal zone, ATZ, and squamous zone, respectively. The alignment sequence of each epithelium was also the same as that of a normal anal canal. Simple columnar epithelium (colorectal zone) was recognized in 37 of 60 cases (61.7%) (**Fig. 2a**). Stratified columnar epithelium (ATZ) was recognized in 49 of 60 cases (81.7%) (**Fig. 2b**). The anal crypt was identified in 46 of 49 cases (93.9%) in the stratified columnar epithelium (ATZ) region. The Herrmann line was clearly identified (**Fig. 2c**) in all specimens having both simple columnar epithelium (colorectal zone) and stratified columnar epithelium (ATZ).

The epithelial combinations seen in each specimen are shown in **Table 2**. In six cases (10%), only simple columnar epithelium was observed. In 54 of 60 cases (90%), epithelium extending distally beyond the Herrmann line was seen. Thus, in these cases, sufficient dissection toward the distal rectal end was made during reconstructive surgery. Stratified squamous epithelium (squamous zone) was seen in only cutaneous or vestibular fistula since other clinical types did not have squamous epithelium ahead of the opening of the fistula.

The epithelial findings of each clinical type are presented in **Table 3**. Stratified columnar cells (ATZ) were seen in all clinical types.

The distribution of ganglion cells is shown in **Table 4**. We could not find any ganglion cells in the stratified columnar epithelium (ATZ) or stratified squamous epithelium (squamous zone). However, some ganglion cells, either Meissner's or Auerbach's plexus, were seen in 64.9% of specimens in the simple columnar epithelium region (colorectal zone). However, the density of ganglion cells seen near the distal end of the simple columnar epithelium region (colorectal zone) appeared to be less than that in a normal colon (**Fig.**

3). A continuous muscle coat was seen in all specimens.

Discussion

ARMs are one of the most common congenital anomalies of the digestive system; however, the cause or mechanism is not fully known. Understanding normal anal canal structure and embryology is crucial for discussing and understanding ARM treatment. ARMs are developmental malformations of the endoderm (hindgut and cloaca) and ectoderm. The distal rectal end of ARMs is a result of the malformation of the endoderm (hindgut and cloaca). Embryologically, proximal to the dentate line, the ATZ develops from the endoderm [7, 8]. Inevitably, the ATZ should be seen in the distal rectal end, and our result supports this hypothesis.

However, it remains unclear whether the distal rectal end should be preserved or resected for reconstruction in anorectoplasty. Histomorphological and immunohistochemical studies of the distal rectal end largely focus on the internal sphincter muscle and nervous system. Few studies report impairments and dysplasia in both the neurological and muscular structures of the distal segment of ARMs. They conclude that the distal segment should not be used for reconstruction [9-11].

Assessment of functional outcome after distal rectal end-preserving anorectoplasty has, however, been reported. Holschneider et al. have reported that specific neuronal intestinal malformations do not correlate significantly with constipation [12]. Manometry and voluntary bowel function studies report good internal sphincteric function after anorectoplasty preserving the distal rectal end [13, 14]. Even if the muscular and nervous systems were impaired, we have demonstrated the presence of epithelium and ganglion cell distributions similar to those in a normal anal canal and continuous muscle layer transit from the circular muscle coat of the colorectal wall in the distal rectal end and that the structural components of the endoderm-originated anal canal, the “ectopic half anal canal,” potentially exist at the distal rectal end. As fecal continence and constipation tend to disappear in adolescence [15], some adaptation to these functional outcomes can be expected in the long term.

Few studies have noted the importance of epithelium for reconstructing ARM. Our results show that stratified columnar epithelium (ATZ) exists at the distal rectal end in any type of ARM. We suggest that the stratified columnar epithelium (ATZ) plays an important role in the adaptation of anastomosis in anorectoplasty. Preserving stratified columnar epithelium (ATZ) and aganglionic region will reproduce the alignment sequence of the epithelium and the nature of the end of the normal anal canal. Kamal reported

good external appearance of the reconstructed anus with distal rectal end-preserving anorectoplasty [16].

The existence of stratified columnar epithelium (ATZ) could indicate different epithelia being adjoined forcibly by anastomosis.

A 100% occurrence of the stratified columnar epithelium (ATZ) was not noted, owing to varying surgical procedures and specimen conditions. At least 90% of our patients underwent sufficient resection distally beyond the Herrmann line. Some had no stratified columnar epithelium (ATZ) left in the specimen, owing to loss of the epithelial layer by perioperative handling of the specimen; however, the presence of a stratified columnar epithelium (ATZ) was highly predictable. Therefore, the presence of a higher amount of stratified columnar epithelium (ATZ) is expected at resection.

We assert that stratified columnar epithelium (ATZ) is present at the distal rectal end in any type of ARM.

The distribution of ganglion cells is also the same as that in a normal anal canal. Preserving stratified columnar epithelium (ATZ) could reconstruct the missing piece of the anal canal in ARMs.

Declarations

Funding: No funding was received for this study.

Compliance with Ethical Standards

Disclosure of potential conflicts of interest: The authors declare that they have no conflicts of interest.

Ethics approval: All procedures performed in this study involving human participants were in accordance with the ethical standards of the institution at which the study was conducted (R31-46) and were performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments.

Consent to participate: For this study, informed consent was obtained from all patients.

Consent to publish: Patients signed informed consent regarding publishing their data and photographs.

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Tables

Sex	Male	27
	Female	33
Type of ARM (location of fistula opening)	Cutaneous fistula	9
	Vestibular fistula	17
	Vaginal fistula	1
	Cloacal fistula	7
	Urethra fistula	17
	Bladder fistula	3
	No fistula	6

Table 1. Sex distribution and types of anorectal malformation (ARM)

Table 2. Epithelium combinations seen in specimens. ATZ, anal transitional zone

		Combination						
Proximal	Simple columnar epithelium (colorectal zone)	⊙	⊙	⊙				37/60 (61.7%)
↓ Type of epithelium	Stratified columnar epithelium (ATZ)		⊙	⊙	⊙	⊙		49/60 (81.7%)
↓ Distal	Stratified squamous epithelium (squamous zone)			⊙ ^a		⊙ ^a	⊙ ^a	19/26 (73.1%)
Number of cases		6	24	7	11	7	5	60

^a Stratified squamous epithelium was only seen in cutaneous and vestibular fistula

Table 3. Epithelium seen in each type of anorectal malformation. ATZ, anal transitional zone

		Cutaneous (N=9)	Vestibula (N=17)	Vagina (N=1)	Cloaca (N=7)	Urethra (N=17)	Bladder (N=3)	No fistula (N=6)	
Proximal	Simple columnar epithelium (colorectal zone)	4/9	8/17	1/1	6/7	14/17	2/3	2/6	37/60 (61.7%)
↓									
Type of epithelium	Stratified columnar epithelium (ATZ)	7/9	14/17	1/1	5/7	14/17	3/3	5/6	49/60 (81.7%)
↓									
Distal	Stratified squamous epithelium (squamous zone)	6/9	13/17	-	-	-	-	-	19/26 (73.1%)

Table 4. Ganglion distribution seen in each epithelium. ATZ, anal transitional zone

		Cutaneous (N=9)	Vestibula (N=17)	Vagina (N=1)	Cloaca (N=7)	Urethra (N=17)	Bladder (N=3)	No fistula (N=6)	
Proximal	Simple columnar epithelium (colorectal zone)	1/4	4/8	0/1	5/6	11/14	2/2	1/2	24/37 (64.9%)
↓ Type of epithelium	Stratified columnar epithelium (ATZ)	0/7	0/14	0/1	0/5	0/14	0/3	0/5	0/49 (0.0%)
↓ Distal	Stratified squamous epithelium (squamous zone)	0/6	0/13	-	-	-	-	-	0/19 (0.0%)

Figure legends

Fig. 1 Definition, anatomy, and embryology of the anal canal

Fig. 2a Simple columnar epithelium (colorectal zone) near the end of the rectum

Fig. 2b Stratified columnar epithelium (anal transitional zone, ATZ) is recognized at the end of the rectum; the anal crypt (white arrow) is recognized in the majority of ATZs

Fig. 2c The boundary between the colorectal zone and the anal transitional zone (ATZ); asterisk indicates the colorectal zone, black triangle indicates the ATZ, and white arrow indicates the boundary between the colorectal zone and ATZ (the Herrmann line)

Fig. 3 White triangle indicates ganglion cell that is recognized only in the colorectal zone

Sex	Male	27
	Female	33
Type of ARM (location of fistula opening)	Cutaneous fistula	9
	Vestibular fistula	17
	Vaginal fistula	1
	Cloacal fistula	7
	Urethra fistula	17
	Bladder fistula	3
	No fistula	6

Table 1. Gender distribution and types of anorectal malformation (ARM)

		Combination						
Proximal ↓ Type of epithelium ↓ Distal	Simple columnar epithelium (colorectal zone)	⊙	⊙	⊙				37/60 (61.7%)
	Stratified columnar epithelium (ATZ)		⊙	⊙	⊙	⊙		49/60 (81.7%)
	Stratified squamous epithelium (squamous zone)			⊙ ^a		⊙ ^a	⊙ ^a	19/26 (73.1%)
	Number of cases	6	24	7	11	7	5	60

Table 2. Epithelium combinations seen in specimens. ATZ: anal transitional zone

^a Stratified squamous epithelium was only seen in cutaneous and vestibular fistula

		Cutaneous (N=9)	Vestibula (N=17)	Vagina (N=1)	Cloaca (N=7)	Urethra (N=17)	Bladder (N=3)	No fistula (N=6)	
Proximal	Simple columnar epithelium (colorectal zone)	4/9	8/17	1/1	6/7	14/17	2/3	2/6	37/60 (61.7%)
↓ Type of epithelium	Stratified columnar epithelium (ATZ)	7/9	14/17	1/1	5/7	14/17	3/3	5/6	49/60 (81.7%)
↓ Distal	Stratified squamous epithelium (squamous zone)	6/9	13/17	-	-	-	-	-	19/26 (73.1%)

Table 3. Epithelium seen in each type of anorectal malformation. ATZ: anal transitional zone

		Cutaneous (N=9)	Vestibula (N=17)	Vagina (N=1)	Cloaca (N=7)	Urethra (N=17)	Bladder (N=3)	No fistula (N=6)	
Proximal	Simple columnar epithelium (colorectal zone)	1/4	4/8	0/1	5/6	11/14	2/2	1/2	24/37 (64.9%)
↓ Type of epithelium	Stratified columnar epithelium (ATZ)	0/7	0/14	0/1	0/5	0/14	0/3	0/5	0/49 (0.0%)
↓ Distal	Stratified squamous epithelium (squamous zone)	0/6	0/13	-	-	-	-	-	0/19 (0.0%)

Table 4. Ganglion distribution seen in each epithelium. ATZ: anal transitional zone









