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ORIGINAL ARTICLE

Morphology of the region anterior to the anal canal in males: visualization of the anterior bundle of the longitudinal muscle by transanal ultrasonography

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Abstract
Purpose The anterior bundle of the longitudinal muscle in the region anterior to the anal canal in males has been described, although the anatomical details remain unclear. The present study was undertaken to clarify the precise morphology of the anterior bundle of the longitudinal muscle and its relationship to the surrounding structures, and to visualize the anterior bundle of the longitudinal muscle via transanal ultrasonography.
Methods Histological examination was carried out using seven male cadavers; an additional three male cadavers were used for transanal ultrasonography, and the ultrasonography images were compared with the actual sagittal sections. In addition, transanal ultrasonography images of 50 male patients at Tokatsu-Tsujinaka Hospital were studied.
Results The region anterior to the anal canal consisted of smooth muscles and skeletal muscles. The anterior bundle of the longitudinal muscle was situated between the bulbospongiosus and the external anal sphincter, and consisted of smooth muscle. The bundle was identified in the transanal ultrasonography of cadavers by comparison with the actual sections. Transanal ultrasonography images of living bodies showed the anterior bundle of the longitudinal muscle as a hypoechoic layer of approximately 17.7 mm in length.
Conclusions The detailed anatomical findings of the anterior bundle of the longitudinal muscle suggested "an alternate arrangement of smooth muscles and skeletal muscles" in the region anterior to the anal canal and facilitated the visualization of the anterior bundle of the longitudinal muscle in transanal ultrasonography.

Keywords Anal canal · Longitudinal muscle · External anal sphincter · Perineal body · Rectourethralis muscle · Ultrasonography

Introduction

The morphology of the region anterior to the anal canal in males is very important for radical prostatectomy to determine the excision line [4, 5, 8, 11, 15, 16]. This region is situated between the anal canal and the genitourinary organs; it is often recognized to be a particularly complex region in males compared to females [12, 13]. In the male region anterior to the anal canal, the characteristic structure termed, "the anterior bundle of the longitudinal muscle" (or the additional longitudinal muscle bundle), has been suggested to be of surgical importance, based on the results of previous studies [1, 19].

Previous studies reported that the anterior bundle of the longitudinal muscle covers the ventral surface of the external anal sphincter, and terminates at the perineal body. However, the specific range of the anterior bundle and its precise boundaries with the surrounding perineal structures remain unknown.

As for the examination of the perineal structures, the useful device transanal ultrasound has received clinical attention [2, 3, 6, 7]. The basic muscular structures of the anal canal, such as the external anal sphincter and the internal anal sphincter, have already been shown using transanal ultrasonography. However, ultrasonography studies

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showing the anterior bundle of the longitudinal muscle in living bodies have hardly been reported.

Therefore, the present study was undertaken to clarify the anatomical details of the anterior bundle of the longitudinal muscle and also to visualize the anterior bundle via ultrasonography examination of living bodies based on the anatomical findings.

Materials and methods

Histology of cadaveric specimens

Seven male cadavers (age range 77–93 years; mean age 87.0) were used for the histological examination. The rectum, the anus, adjacent muscular tissues and connective tissues were obtained en bloc from the cadavers. The complete block was frozen at −80 °C and cut into 8-mm thick sagittal sections. Small blocks for histological analysis were obtained from the sagittal sections. The blocks were fixed in 10% formalin, dehydrated and embedded in paraffin. After sectioning in 5-μm-thick sections, the histological sections were stained with Elastica van Gieson (EVG). To confirm the distribution of smooth muscle and skeletal muscle, immunohistological staining of the sections was conducted. By incubating tissues in methanol containing 0.3% H_2O_2 for 30 min, endogenous peroxidase activity was inactivated. Nonspecific binding was blocked with PBS containing 0.05% Tween 20 and 5% goat serum at room temperature for 30 min. The sections were incubated with the following primary antibodies: anti-smooth actin (ready-to-use, Actin, Smooth Muscle Ab-1, Clone 1A4, Thermo Fisher Scientific, Fremont, CA, USA) and anti-skeletal myosin (ready-to-use, Myosin, Skeletal Muscle Ab-2, Clone MYSN02, Thermo Fisher Scientific) overnight at room temperature. The sections were washed and incubated with biotinylated goat anti-mouse IgG (1:200, Vector Laboratories, Burlingame, CA, USA) as the secondary antibody for 30 min at room temperature. Signal amplification was performed using the VECTASTAIN ABC Kit (Vector Laboratories) and TSA Biotin Systems (PerkinElmer, Tokyo, Japan) according to the instructions of the manufacturers. The immunocomplexes were detected with 3,3-diaminobenzidine (DAB) (Wako, Osaka, Japan) and were counterstained with hematoxylin for 1 min.

Prints of the immunostained specimens were made on A4 size paper. The stained areas were taken out using tracing papers and scanned. The areas taken out were colored in green (smooth muscle) or red (skeletal muscle). Then, the colored images were superimposed to assess the relationship between smooth muscle and skeletal muscle. This method was the same as that described by Muro et al. [10].

Comparison between gross anatomy and ultrasonography in cadaveric specimens

Three male cadavers (age range 72–80 years; mean age 76.7 years) were used for morphological comparison between the gross anatomical observation and ultrasonography examination. First, transanal ultrasonography examinations were conducted on these cadavers. Linear images of the region anterior to the anal canal were obtained. The ultrasound devices used were the SSD-4000 (Aloka Co., Tokyo) and a transanal linear probe (7.5 MHz). These devices were applicable only for laboratory use and not for clinical use. Ultrasonography was conducted by an experienced specialist (Y. Nakajima) who is skilled in transanal ultrasonography. After obtaining the ultrasonography imaging data, the cadaveric specimens were cut in the sagittal plane. Then, the ultrasonography images and the actual section views were compared morphologically.

Ultrasonography in living bodies

The ultrasonography imaging data of 50 Japanese male patients (age range 22–76 years; mean age 43.1 years) were collected. These patients had visited Tokatsu-Tsujinaka Hospital (Shimbashi, Tokyo, Japan) before July 31, 2013, and underwent transanal ultrasonography for examination of an anal fistula occurring in the region posterior to the anal canal. The ultrasound devices used were an SSD-4000 (Aloka Co., Tokyo), a transanal linear probe (7.5 MHz) and a transanal radial probe (10 MHz). These devices were only applicable for clinical use and were the same model as that used for cadavers. Ultrasonography was conducted by an experienced specialist (Y. Nakajima) who was skilled in transanal ultrasonography.

In the collected data, the linear images of the region anterior to the anal canal and radial images of the anal canal were analyzed based on the anatomical findings. The maximum length of the anterior bundle of the longitudinal muscle (AB) and the maximum lateral width of the rectourethralis muscle (Ru) were measured in the ultrasonography images by two persons (K. Akita and S. Muro). The imaging data of three patients were excluded because the region anterior to the anal canal was damaged by the fistula.

Statistical analysis

Statistical analyses were performed by using JMP 12.0.1 (SAS Institute Inc., Cary, NC, USA). Analysis of interobserver reliability, regarding the measurement of the maximum length of AB and the maximum lateral width of Ru, yielded an interclass correlation coefficient of 0.99.

Cadavers

All of the cadavers used in the present study were obtained from the dissecting room of the School of Medicine, Tokyo Medical and Dental University. The cadavers were donated to the Tokyo Medical and Dental University, Department of Anatomy, for use in clinical studies, within the guidelines of the Act on Body Donation for Medical and Dental Education law of Japan. Cadavers were fixed by arterial perfusion with 8% formalin and preserved in 30% alcohol to resist fungus growth and to maintain the softness of the tissues.

Ethical approval

Study approval was obtained from the Board of Ethics of the Faculty of Medicine, Tokyo Medical and Dental University (No. 1624).

Results

Histology of cadaveric specimens

The sagittal section of the region anterior to the anal canal as observed in a male cadaveric specimen is shown in Fig. 1. The anal canal (AC), the bladder (Bl), the prostate (Pr), the urethra (Ur) and the corpus spongiosum of the penis (CSP) were viewed. The inferior surface of CSP was covered by the bulbospongiosus (Bs). Adjacent to the anal canal, the internal anal sphincter (IAS), the longitudinal muscle (LM) and the external anal sphincter (EAS) were identified. The most inferior part of EAS, i.e., the subcutaneous part of EAS (sEAS), protruded anteriorly (Figs. 1, 2a, c). The rectourethralis muscle (Ru) was identified in the region surrounded by AC, Pr and CSP (Figs. 1, 2a, b). This muscle protruded from LM and consisted of smooth muscle (Fig. 2b).

The anterior bundle of the longitudinal muscle (AB) extended from LM anteroinferiorly, descended between EAS and Bs, covered the anterior surface of EAS and terminated in loose connective tissue (Figs. 1, 2a, b, d, e). AB consisted of smooth muscle (Fig. 2b, e). The region anterior to the anal canal consisted of smooth muscles and skeletal muscles (Fig. 2f). These muscles bordered each other. In other words, the smooth muscles and the skeletal muscles were alternately situated.

Comparison between gross anatomy and ultrasonography in cadaveric specimens

In the sagittal section of a male cadaveric pelvis, as shown in Fig. 3a, IAS, EAS and Bs were identified as the brown surfaces of the section in the region anterior to the anal canal (Fig. 3b). LM ran downward between IAS and EAS as a white layer, whereas AB ran downward between EAS and Bs as a white layer.

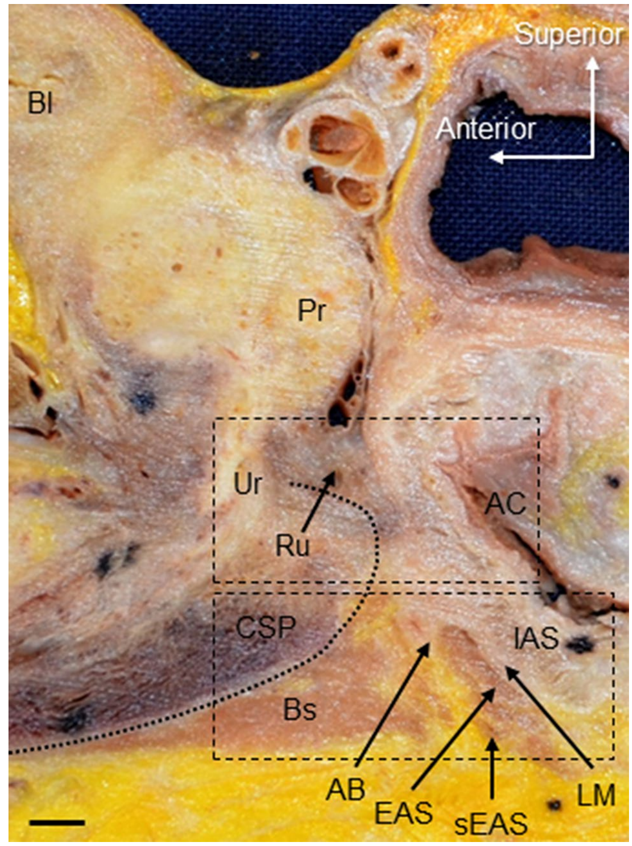

Fig. 1 Sagittal section of a male cadaveric specimen. In the region anterior to the anal canal, the muscles of the anal canal and other perineal muscles were seen. The areas within *dashed line squares* were obtained for histological examination. *Curved dotted line* the shape of CSP, *AB* anterior bundle of the longitudinal muscle, *AC* anal canal, *Bl* bladder, *Bs* bulbospongiosus, *CSP* corpus spongiosum of the penis, *EAS* external anal sphincter, *IAS* internal anal sphincter, *LM* longitudinal muscle, *Pr* prostate, *Ru* rectourethralis muscle, *sEAS* subcutaneous part of EAS, *Ur* urethra

The transanal ultrasonography image, which was obtained from the same cadaver before the sagittal sectioning, was compared with the actual section view. Figure 3c shows the linear image of the region anterior to the anal canal in the same cadaveric specimen as in Fig. 3a, b. In the ultrasonography image, IAS, LM and AB were identified as hypoechoic layers (Fig. 3c). EAS was seen as a thick speckled hypoechoic layer.

Ultrasonography in living bodies

Transanal ultrasonography images of living bodies were analyzed based on the anatomical findings described above

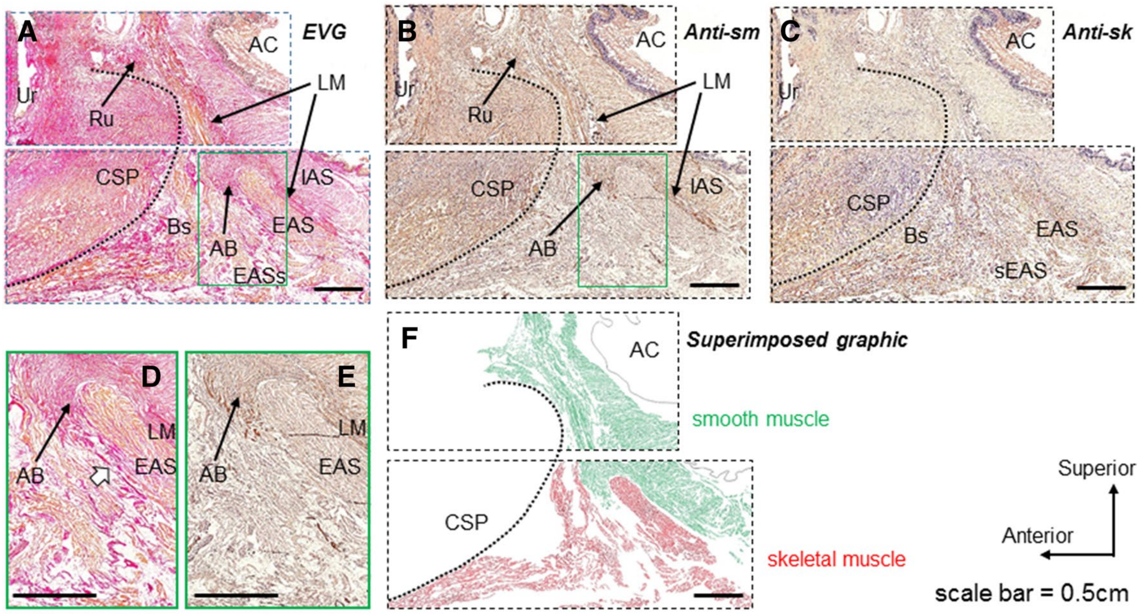

Fig. 2 Histological sections obtained from the areas within dashed line squares in Fig. 1. **a** Sections stained with Elastica van Gieson (EVG). AB descended between EAS and BS, and covered the anterior surface of EAS. **b** Sections stained with anti-smooth muscle antibody (Anti-sm). IAS, LM, AB and Ru consisted of smooth muscle. **c** Sections stained with anti-skeletal muscle antibody (Anti-sk). EAS, sEAS and Bs consisted of skeletal muscle. **d** Enlarged view of a portion of B. AB continued to lose fibrotic tissue (indicated by *white arrow*). **e** Enlarged view of a portion of **c**. The loose fibrotic tissue which continued from AB (indicated by the *white arrow* in **d**) did not contain smooth muscle. **f** Superimposed graphic indicating the smooth muscle in *green*, skeletal muscle in *red*. Smooth muscle and skeletal muscle were alternately arranged in the region anterior to the anal canal. *Curved dotted line* the shape of CSP; *white arrow* connective tissue continuous with AB; the abbreviations are explained in Fig. 1

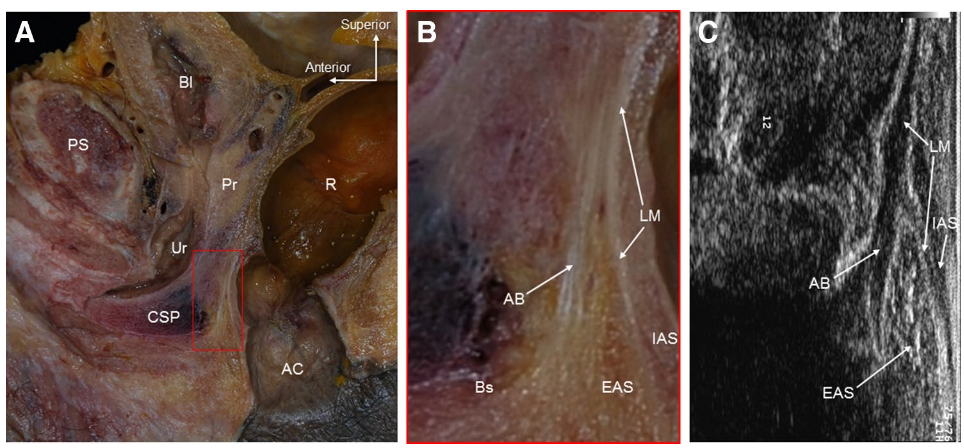

Fig. 3 **a** Sagittal section of a male cadaveric specimen. **b** Enlarged view of the *red line square* area in (**a**) IAS, LM, EAS, Bs and AB were observed. **c** Linear image of the region anterior to the anal canal corresponding to the area in (**b**) IAS, LM and AB were observed as hypoechoic layers. EAS was observed as a thick speckled hypoechoic layer. The abbreviations are explained in Fig. 1

(Fig. 4). In the linear image of the region anterior to the anal canal, Ur was identified as the hypoechoic region of the curve. Pr and CSP were observed adjacent to Ur (Fig. 4a). The inferior surface of CSP was covered by Bs which was shown as a speckled hypoechoic area. Adjacent to the anal canal, IAS and LM were observed as hypoechoic layers, whereas EAS and sEAS were seen as speckled hypoechoic areas.

Ru was identified as a hypoechoic area protruding from LM anteriorward between Pr and CSP (Fig. 4a). Ru was also seen in the radial image as an area protruding from LM (Fig. 4b). The lateral width of Ru (indicated by the red double-headed arrow in Fig. 4b) was 7.3 ± 1.8 mm (3.6–11.6 mm) on average.

AB was observed as a hypoechoic layer which continued from LM and extended downward between Bs and EAS (Fig. 4a). The length of AB (indicated by the yellow double-headed arrow in Fig. 4a) was 17.7 ± 5.1 mm (10.9–42.5 mm) on average. It was also seen anterior to

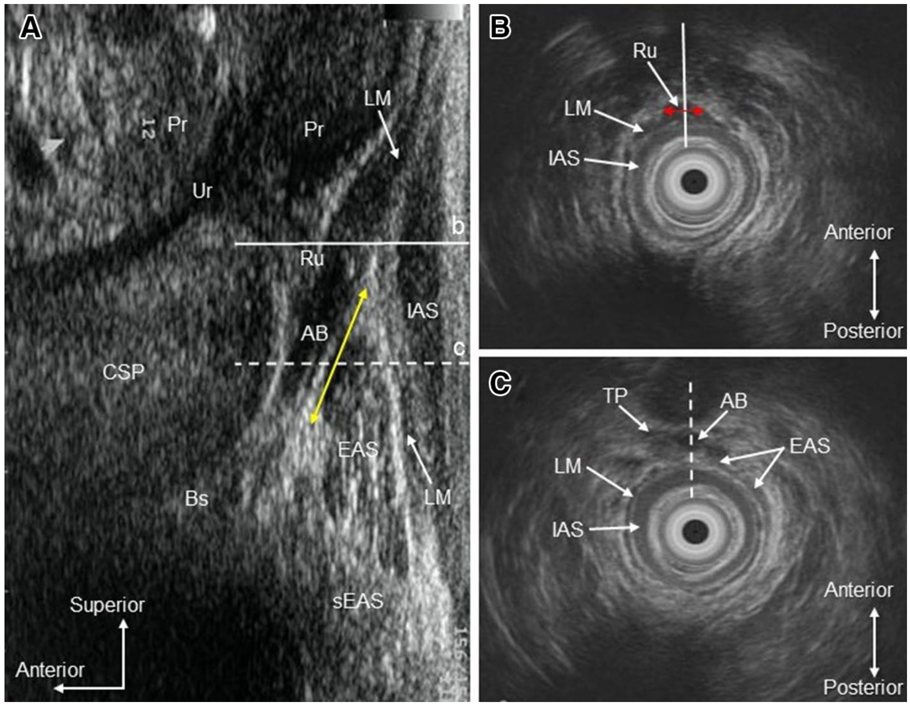

Fig. 4 Transanal ultrasonography of a male living body. **a** Linear image of the region anterior to the anal canal. The muscles of the anal canal and other perineal muscles were identified. Ru was observed as a hypoechoic area which protruded anteriorly from LM between Pr and CSP. AB was shown as a hypoechoic layer which continued from LM, extended downward between EAS and Bs and covered the anterior surface of EAS. The length of AB (indicated by the *yellow double-headed arrow*) was 17.7 ± 5.1 mm (10.9–42.5 mm) on average. **b** Radial image on the level of the *white line* (*line* **b**) in **a**. IAS and LM were observed as concentric hypoechoic layers. Ru was seen as a hypoechoic protruding area from LM. The lateral width of Ru (indicated by the *red double-headed arrow*) was 7.3 ± 1.8 mm (3.6–11.6 mm) on average. **c** Radial image on the level of the *white dashed line* (*line* **c**) in **a**. IAS and LM were observed as concentric hypoechoic layers. EAS was seen as a concentric speckled hypoechoic layer. AB was observed anterior to EAS as a hypoechoic area. *Yellow double-headed arrow*: the length of AB; *red double-headed arrow*: the lateral width of the Ru; *TP* transverse perinei muscle; the other abbreviations are explained in Fig. 1

EAS in the radial image (Fig. 4c). The transverse perinei muscles were observed bilateral to AB.

Discussion

Anatomy of the region anterior to the anal canal

In the present study, the anatomical details of the anterior bundle of the longitudinal muscle (AB) were clarified (Fig. 5). Three characteristic features of AB were observed: (1) AB was continuous with the longitudinal muscle (LM), (2) AB was closely sandwiched between the bulbospongiosus (Bs) and the external anal sphincter (EAS), and (3) AB terminated in loose connective tissue.

Previous macroscopic anatomical studies reported the presence of the anterior bundle of the longitudinal muscle (or the additional longitudinal muscle bundle) in the region anterior to the anal canal [1, 14, 19]. Some reports have used different terms for this structure, such as "the perineal body" or "the rectoperineal muscle" [4, 18]. The

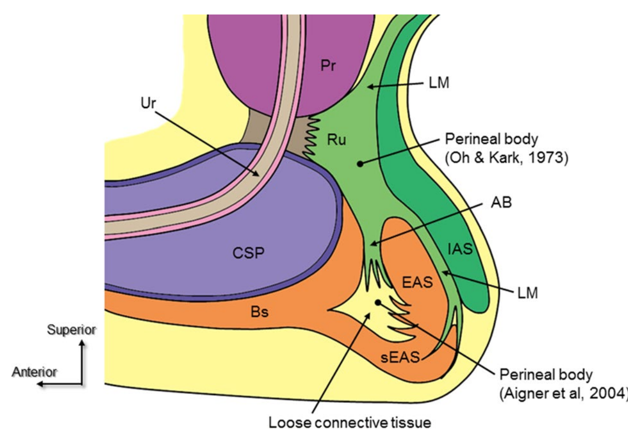

Fig. 5 Region anterior to the anal canal. This region was mainly occupied by smooth muscles (IAS, LM, Ru, AB) and skeletal muscles (EAS, sEAS, Bs). These muscles were alternately arranged. AB continued from LM, ran downward between Bs and EAS and terminated in loose connective tissue. The abbreviations are explained in Fig. 1

present study histologically showed more detailed structure of AB and the structural relationship with the surrounding muscles. In addition, immunostaining clarified the precise distribution of smooth muscle and skeletal muscle in the region anterior to the anal canal. The region was mainly occupied by skeletal and smooth muscular tissues. The smooth muscles and the skeletal muscles were arranged in an alternate manner (Fig. 5). AB was situated as a part of the arrangement of smooth muscles and skeletal muscles.

Our findings may help to understand the morphology of the perineal body (PB). PB has been described to be an attachment point of perineal muscles as a central tendon [18]. Its exact location is still controversial (Fig. 5). Some reports have indicated that PB is in the area superior to AB [13], while other reports have described PB to be in the area inferior to AB [1, 19]. In contrast, one histological study reported that PB could not be identified [15]. However, in the present study, no firm fibrotendinous structure was histologically identified in the region anterior to the anal canal in male cadavers. This region mainly consisted of smooth and skeletal muscular tissues. These findings may shed doubt on the concept of the perineal body in males.

Such a reciprocal relationship of smooth muscles and skeletal muscles is probably important in order to discuss the structure and the function of the anal canal, as some previous studies have indicated [10, 17]. The present study clarified the reciprocal relationship of the smooth muscles and skeletal muscles in the region anterior to the anal canal (Fig. 5). In this region, the smooth muscles and skeletal muscles were found to be arranged in an alternate manner. This finding suggests the concept of a stable region anterior to the anal canal in contrast to the dynamic region posterior to the anal canal.

Transanal ultrasonography

The present study visualized AB and the surrounding structures in transanal ultrasonography. The ultrasonography images and the actual section views of cadaveric specimens were compared. Then, based on the findings, the ultrasonography images of living bodies were examined.

In the previous studies, AB was not identified in transanal ultrasonography [2, 3, 6, 7, 9]. The present study clarified the demonstration of AB in transanal ultrasonography, both in cadavers and in living bodies. The transanal ultrasonography showed AB as a hypoechoic layer (approximately 17.7 mm in length) which continued from LM and extended downward between Bs and EAS.

This visual appearance of AB in ultrasonography images most likely reflected its histological features. AB was histologically continuous with LM and consisted of smooth muscular tissue. These features probably resulted in the hypoechoic layer continuing from LM in ultrasonography images. In terms of the correspondence between histology and ultrasonography, it may be helpful, for the interpretation of ultrasonography images, to understand the above-mentioned “an alternate arrangement of smooth muscles and skeletal muscles” in the region anterior to the anal canal (Fig. 5).

Combined examination of gross anatomy, histology and ultrasonography

In the present study, gross anatomical and histological examinations of cadavers clarified the nature of the tissue in the anterior region to the anal canal. Furthermore, ultrasonography examinations of living bodies showed the spatial structure of the region. The combined examination of gross anatomy, histology and ultrasonography is likely useful to study such a complicated region as the perineal central region.

In addition, ultrasonography examination of living bodies may compensate for one of the limitations of cadaveric study. It seems that the muscle shape of cadavers differs from that of living bodies, because the muscles of cadavers have lost their tonicities. Ultrasonography examination on living body likely shows the shape of muscles maintaining their tonicity.

Conclusion

The present study clarified the anatomical details of the anterior bundle of the longitudinal muscle and confirmed “an alternate arrangement of smooth muscles and skeletal muscles” in the region anterior to the anal canal. In addition, the anterior bundle of the longitudinal muscle was visualized in the transanal ultrasonography of living bodies, as based on the anatomical findings.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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