Review

Fascial Organisation and Lymphatic Systems Around the Pelvic Floor: A Literature Review

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Abstract. *This review summarises the anatomy and lymphatic systems around the pelvic floor. We investigated the lymphovascular network in the anorectal region, focusing on the hiatal ligament, which comprises smooth muscle fibres derived from the longitudinal muscle and connecting the anal canal and coccyx, and the endopelvic fascia, which seems to comprise collagen and elastic fibres. During rectal surgery, endopelvic fascia is recognized as a sheet of fascia covering the levator ani muscle. Endopelvic fascia is extensively attached to the smooth muscle fibres diverging from the longitudinal muscle of the rectum. Analysis of the lymphovascular network using submucosal India ink injection and indocyanine green fluorescence imaging suggests a functional lymphatic flow between rectal muscle fibres and hiatal ligament and endopelvic fascia. Precise analysis of the lymphatic systems of fascial organization around the pelvic floor may be useful in formulating therapeutic strategies for low rectal cancer.*

To date, only few studies have attempted a macroscopic analysis of the lymphatic systems in the anorectal region (1). Several microanatomical studies of this region have revealed muscular and fascial structures, including the levator ani muscle (LAM), hiatal ligament (HL), endopelvic fascia (EF), and Denonvilliers' fascia (DVF) (2-9), but details of the lymphatic drainage in this region have not been reported.

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Understanding the lymphatic system in the anorectal region can provide important information regarding therapeutic strategies for low rectal cancer. Abdominoperineal resection (APR) showed poorer outcomes than sphincter-preserving procedures in previous studies (10, 11). Although the reason for the difference in outcome between abdominoperineal resection and sphincter-preserving procedures is controversial and a selection bias may have existed in those studies, several therapeutic strategies, such as enlarging the resection area (12), preoperative chemotherapy (13) and chemoradiotherapy (14, 15) have been evaluated. On the other hand, local excision has been attempted for early low rectal cancer because low anterior resection and intersphincteric resection (ISR) have the risks of anastomotic leakage and permanent stoma, especially in male patients (16). Although the quality of life following local excision is satisfactory, its oncological safety remains controversial. Moreover, the recurrence rate after local excision is higher than that after total mesorectal excision (17, 18). Although local excision with adjuvant radiotherapy and chemoradiotherapy are currently performed (19, 20), the appropriate indications for these modalities remain debatable. Thus, investigating the lymphatic system of the anorectal region may provide useful information for developing therapeutic strategies for early and advanced low rectal cancer. This study reviewed previous analyses of the anatomical structures and lymphatic systems of the anorectal region, especially of the pelvic floor and the surrounding structures, including the LAM, HL, EF, DVF, and lateral ligament. We also reviewed our previous study, which investigated the lymphovascular network and tissue drainage in the anorectal region using India ink injections, immunohistochemistry, and intraoperative indocyanine green (ICG) – fluorescence imaging (FI) (21). This literature review aimed to clarify the anatomical structures around the pelvic floor. We discussed the scope of future studies required to develop adequate therapeutic strategies for low rectal cancer.

Fascial Structures and Lymphatic Distributions

HL and EF. The HL is usually a thick white bundle on the surface of the LAM in the posterior portion of the anal canal (2), which mainly consists of smooth muscles (3, 4, 7). Kinugasa *et al.* reported a similar structure, known as the “ventral layer of the anococcygeal ligament,” comprising abundant smooth muscle and elastic fibres and connecting the anal canal and coccyx between bilateral slings of the LAM (3). This structure seems to be corresponding to “anococcygeal raphe” that Shafik *et al.* reported (2). Tsukada *et al.* reporting the detailed muscular anatomy around the LAM described the presence of a thick tissue on the surface of the LAM, comprising smooth muscle fibres and continuing to the longitudinal muscle (7). This thick fascia filled the gap between the LAM and a longitudinal muscle, which corresponded to the HL. Thus, HL may comprise smooth muscle fibres derived from the longitudinal muscle. Generally, EF represents a sheet of fascia lining the pelvic walls and floor and covering the obturator internus, piriformis, LAM, and coccygeus muscles (22). However, its interpretation varies among colorectal surgeons, gynecologists, and urologists. Moreover, EF has many synonyms, including lateral pelvic fascia, superior pelvic fascia, parietal pelvic fascia, levator fascia, and parapelvic fascia (22). These variations in interpretation and the several synonyms have caused confusion.

Colorectal surgeons usually identify EF intraoperatively as the fascia on the surface of LAM. In the pelvic floor, this fascia is regarded as the parietal peritoneum, whereas the prostatic fascia corresponds to the visceral peritoneum (23, 24). This review focuses on the fascia covering the LAM, which is defined as the EF by colorectal surgeons.

Elastic and collagen fibres are the main components of the EF (25). In the region around the LAM, the border between the fibres of the EF and smooth muscle fibres from the rectal longitudinal muscle is unclear. Several studies have reported a structure corresponding to the EF that covers the LAM. Arakawa *et al.* reported that the LAM was covered with fascia composed of smooth muscles, which merged to form the external rectal muscularis propria (6). Macchi *et al.* reported that the longitudinal anal muscle originates from the LAM and the longitudinal muscle of the rectum and interposes between the internal and external anal sphincters (5). Immunohistochemical staining demonstrated mixed skeletal muscle and smooth muscle fibres around the LAM. Tsukada *et al.* reported that smooth muscle fibres continued from the longitudinal muscle and were directly attached to the LAM as an indentation in the lateral and anterolateral portions (7).

Thus, the original EF seems to comprise collagen and elastic fibres; the attachment of smooth muscle from the longitudinal muscle to the EF may spread relatively

extensively on the surface of the LAM. Conversely, HL consists mainly of smooth muscle; therefore, HL and EF seem to be separate structures. As described above, previous studies on HL and EF only examined muscular structures because of their importance in low rectal cancer surgery, especially in sphincter-preserving procedures such as ISR. These studies demonstrated an interaction between smooth muscle fibres from the longitudinal muscle, HL, and EF. Analyses of the microanatomical lymphovascular network in these areas could explain the low rectal cancer spread around the pelvic floor, further facilitating the development of therapeutic strategies and surgical procedures that improve oncological safety. During ISR, the HL is cut and retained in the unresected area. Moreover, smooth muscle fibres from the rectal longitudinal muscle seem to attach to the LAM in an indented manner or mix with the EF. Therefore, tumours may spread extensively around the LAM through these smooth muscle fibres.

DVF. Denonvilliers first described the DVF in 1836 (26). The DVF separates the rectum from the bladder and seminal vesicles in men and the cervix and vagina in women (27). In women, the DVF is occasionally referred to as the rectovaginal septum (28). The DVF is traditionally regarded as a fusion fascia (29); however, Kim *et al.* described the DVF as a tension-induced structure, rather than a fusion fascia (8). These authors suggested that the DVF develops in later stages of foetal development, from mechanical stress, due to the increased volume of surrounding organs.

Many studies have thoroughly examined the microanatomical fascial structures of the DVF. The structure mainly comprises multiple fascial condensations of collagen and smooth muscle fibres (9). Kinugasa *et al.* reported that the DVF is single-layered in the centre and divides into 2-3 layers laterally on both sides, ending in neurovascular bundles and extending to the posterolateral side, separating the neurovascular bundle from the mesorectum (30). Similarly to the HL and EF, the lymphatic systems of the DVF have not been studied.

Lateral ligament. The lateral ligament comprises a broad band of dense connective tissue that passes toward and outward from the lateral walls of the rectum toward the base of the bladder at the termination point of the ureters (31). However, the existence of the lateral ligament is controversial because this ligament was not detected in some cadaveric specimens (32, 33). Goligher identified the lateral ligament after dissecting the anterior and posterior portions of the rectum (34). Anatomical analyses of the lateral ligament, including its lymphatic drainage, have been performed. According to the studies, the middle rectal artery runs from the lower mesorectum to the lateral pelvic wall

through the lateral ligament (31), and the lymphatic flow seems to run alongside this artery (1). However, Sato *et al.* reported that the middle rectal artery was observed in only 22% of cases (35). Pak-art *et al.* reported that no artery was found histologically in any lateral ligament (36). Moreover, studies on the lymphatic flow of the lateral ligament were mainly based on the macroscopic anatomy and on speculations from the clinical metastatic features (1, 31). Yanagita *et al.* suggested the existence of lymph node metastatic pathways between the pararectal and lateral regions, using the isotope method (37). However, this study did not indicate whether the pathway involved the lateral ligament or whether it involved the lateral ligament and other pathways.

Functional Analyses of Lymphatic Flow

Lymphatic tracer injection. Lymphatic tracer injection has been used for decades. Jamieson *et al.* injected Prussian blue dye into cadavers and analysed the macroscopic distribution of the lymphatic ducts of the colon (38). Submucosal injections of carbon particles and India ink have been used as preoperative markings in laparoscopic colorectal surgery (39, 40). Recently, this method was applied for the detection of sentinel, regional, and metastatic lymph nodes (41-43). Additionally, Gao *et al.* examined the barrier of lymphatic flow between the visceral and parietal fascia of the colon by injecting carbon nanoparticles subserosally during surgery (44). Thus, the lymphatic flow in the colon has been well studied.

However, studies that examined the lymphatic systems in the anorectal region using lymphatic tracers are lacking due to observation difficulties. Macroscopic examination of the lymphatic systems in the lower rectum and anal canal using lymphatic tracers was first established by Blair *et al.* (1). These authors demonstrated the lymphatic flow pattern in the anorectal region, such as the lymphatic flow running with the superior rectal vessels, with the middle rectal vessels to the hypogastric nodes, and with the inferior rectal vessels to the hypogastric nodes. Block *et al.* injected a sky blue solution into the rectal wall and demonstrated extensive lymphatic communication between the lower rectum and surrounding tissues, such as the route along the LAM (45). Presumptions about the lymphatic flow in the anorectal region were made in these studies based on the distribution of dyed lymphatic ducts from macroscopic analyses. Kumashiro *et al.* injected carbon particles into pedal lymphatic ducts and demonstrated lymphatic pathways between the inguinal, lateral, and para-aortic regions (46). The authors also made speculations based on the distribution of black-dyed lymphatic nodes. Lymphatic flow in the anorectal region is assumed to be complicated; therefore, only examining dyed lymph nodes seems insufficient. Microanatomical examinations of the actual

flow of lymphatic tracers into lymphatic ducts in the pelvic floor, including those in the HL, EF, LAM, lateral region, and inguinal region, are essential to analyse functional lymphatic systems. However, such studies have not yet been conducted.

Isotope methods. In 1977, Bartholdson *et al.* reported radioisotope injection into the rectal submucosa (47). The authors used gold 198 (^{198}Au) and reported that <1% of the isotope drained into the pelvic lymph nodes when the radioisotope was injected into the upper and middle rectum. Takemura *et al.* injected technetium-99m ($^{99\text{m}}\text{Tc}$) into the submucosa on the dentate line and demonstrated that the lateral pelvic lymph nodes (LPLNs), especially obturator lymph nodes, had higher accumulation of the radioisotope than the mesenteric lymph nodes (48). Funahashi *et al.* demonstrated two types of lymphatic drainage routes in advanced low rectal cancer using $^{99\text{m}}\text{Tc}$. These routes were the lateral and mesorectal drainage routes (49). Recently, Yanagita *et al.* reported the utility of the radioisotope method by the submucosal injection of $^{99\text{m}}\text{Tc}$ to detect metastatic LPLNs in low rectal cancer cases (37). Thus, radioisotope methods have been used to examine lateral lymphatic drainage from the lower rectum. However, recently, ICG-FI emerged as an alternative examination method because of its ease of use.

ICG-FI. Currently, ICG-FI is the most commonly used method for intraoperative lymph node mapping in colorectal cancer. Few ICG injection-related adverse effects have been reported (50), and their management is easier than that of radioisotope-related adverse effects. Furthermore, recent laparoscopic systems with near-infrared camera systems permit relatively easy performance of ICG-FI by colorectal surgeons.

ICG-FI is mainly used for lymph node mapping in the mesocolon and mesorectum (51-55). A systematic review and meta-analysis demonstrated that the median sensitivity and specificity of malignant lymph node detection using ICG-FI in colorectal cancer were 73.7% and 100%, respectively (50).

Lymph node mapping using ICG-FI for low rectal cancer has also been attempted. Several studies have examined the lymphatic flow from the lower rectum to LPLNs (56, 57). Noura *et al.* reported that sentinel LPLNs were successfully identified in 92% of cases (56). Zhou *et al.* reported a significantly larger number of harvested LPLNs in a group of patients who underwent ICG-FI compared to another group of patients that did not undergo ICG-FI (57). Thus, intraoperative ICG-FI seems useful for detecting lymphatic pathways between the lower rectum and the lateral region. Furthermore, real-time ICG-FI could detect the actual lymphatic flow pathways between the lower rectum and the lateral region.

Table I. Review of anatomical studies of pelvic floor around the anorectal region.

Author (ref.)	Years	Study design	Materials	Methods	Staining	Key findings
Shafik <i>et al.</i> (2)	1999	Descriptive study	Cadavers (n=23)	Microscopic examination	Hematoxylin-eosin staining and Verhoe-van Gieson staining	•Posterior portion: presence of the "anococcygeal raphe", thick fascial bundle connecting between rectum and coccyx
Kinugasa <i>et al.</i> (3)	2011	Descriptive study	Cadavers (n=20) and fresh cadavers (n=6)	Macroscopic and microscopic examination	Masson-Trichrome staining and aldehyde–fuchsin staining	•Posterior portion: presence of the "ventral layer of the anococcygeal ligament", comprising abundant smooth muscle and elastic fibres and connecting the anal canal and coccyx between bilateral slings of the LAM
Muro <i>et al.</i> (4)	2014	Descriptive study	Cadavers (n=14)	Macroscopic and Microscopic examination	Hematoxylin-eosin staining, Elastica van Gieson staining and immunohistochemistry, for smooth and skeletal muscle	•Posterior portion: presence of a thick white fibrous bundle extending from the rectum to the coccyx, comprising smooth muscle fibres
Macchi <i>et al.</i> (5)	2008	Descriptive study	Cadavers (n=16)	Microscopic examination	Hematoxylin-eosin staining, azan-Mallory staining, Weigert's staining, and immunohistochemistry for smooth and skeletal muscle	•Presence of the "longitudinal anal muscle" which mainly composed of outer striated muscle fibres and small numbers of inner smooth muscle fibres, originate from the LAM and the longitudinal muscle of the rectum
Arakawa <i>et al.</i> (6)	2004	Descriptive study	Cadavers (n=46)	Microscopic examination	Hematoxylin-eosin staining, Azan staining, aldehyde–fuchsin staining, and immunohistochemistry for smooth muscle	•Variations in the interface between the LAM and the rectal muscularis propria •Existence of the covering fascia of the LAM changed abruptly into smooth muscles, which merged into the external rectal muscularis propria.
Tsukada <i>et al.</i> (7)	2016	Descriptive study	Cadavers (n=5) and specimens of APR (n=13)	Microscopic examination	Hematoxylin-eosin staining, Elastica van Gieson staining, and immunohistochemistry for smooth and skeletal muscle	•Posterior portion: presence of a thick tissue on the surface of the LAM, comprising smooth muscle fibres and continuing to the longitudinal muscle •Lateral and anterolateral portion: smooth muscle fibres continued from the longitudinal muscle and were directly attached to the LAM as an indentation
Sato <i>et al.</i> (21)	2021	Descriptive study	•Fresh cadaver (n=1) and specimens of APR (n=6) •Patients underwent surgery for low rectal cancer (n=4)	•India Ink injection and microscopic examination •Intraoperative ICG-FI	Hematoxylin-eosin staining, silver impregnation staining, and immunohistochemistry for lymphatic duct and blood vessel	•Posterior portion: functional lymphovascular flow between the rectal longitudinal muscle fibres and the HL •Lateral portion: functional lymphovascular flow from the anal canal to the surface of the LAM through longitudinal muscle fibres extending to the EF

Table I. Continued

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Author (ref.)	Years	Study design	Materials	Methods	Staining	Key findings
Raychaudhuri <i>et al.</i> (22)	2008	Literature review	–	–	–	•EF represents a sheet of fascia lining the pelvic walls and floor and covering the obturator internus, piriformis, LAM, and coccygeus muscles
Cör <i>et al.</i> (25)	2003	Descriptive study	Female patients underwent gynaecologic surgical procedures and biopsies from the EF (n=54)	Microscopic examination	Hematoxylin-eosin staining, Masson-Trichrome staining, Weigert's staining, and immunohistochemistry for collagen type I and type III	•Elastic and collagen fibres are the main components of the EF •Quantity of elastic and collagen fibres in the EF does not play a significant role in continence

LAM: Levator ani muscle; APR: abdominoperineal resection; ICG-FI: indocyanine green fluorescence imaging; HL: hiatal ligament; EF: endopelvic fascia.

However, examination of lymphatic flow between the anorectal region and pelvic floor structures, such as the HL, EF, and LAM, using intraoperative ICG-FI has not been reported. To our knowledge, this is the first study to examine these areas using ICG-FI. ICG-FI seems to be the most reasonable method for examining lymph flow around the pelvic floor because this area is clearly visualised during laparoscopic surgery. Further studies on the pelvic floor using ICG-FI are required.

Our Novel Findings of the Anorectal Lymphatic System

Our observational study attempted to demonstrate functional anorectal lymphovascular networks (21). We performed histological examinations using a combination of immunohistochemistry, India ink injection in fresh cadavers and abdominoperineal resection specimens, and intraoperative ICG-FI in low rectal cancer cases.

Submucosal India ink injection on the dentate line and immunohistochemical analyses demonstrated the distribution of lymphatic ducts and vasculature, suggesting tissue drainage and a functional lymphovascular network around the LAM. India ink deposition was detected in the venous lumina and surrounding stroma of the rectal wall, and in the HL and smooth muscle fibres from the longitudinal muscle extending to the EF. These results are presented as figures in our published article (21).

ICG-FI demonstrated tissue fluid flow around the pelvic floor. ICG fluorescence was detected in both the caudal portion of the HL (Figure 1A, B) and the lateral and anterolateral sites of the EF (Figure 1D-F), with band-like structures connecting the LAM. Figure 2 shows the schemas of lymphovascular flow around the anal canal

illustrated from the results of our analyses. The illustrated lymphovascular flow may overlap the fascial structures of the pelvic floor from previous studies that reported that smooth muscle fibres from the longitudinal muscle may spread on the surface of the LAM attaching with EF and that HL may comprise smooth muscle fibres derived from the longitudinal muscle (Figure 2C).

Summary and Expected Future Perspectives

This literature review summarised the anatomy and lymphatic systems around the pelvic floor. Table I shows the key anatomical studies of pelvic floor around the anorectal region. Although our data do not completely prove the existence of functional lymphatic flow in the anorectal region, the localisation of lymphatic vessels in this area and the migration of lymphatic tracers in the interstitial tissue strongly suggest the presence of lymphatic flow. Moreover, the ICG-FI suggests lymphatic flow between rectal muscle fibres, HL, and EF.

Considering from this review, enlarging the resection area of APR including the EF and HL seem to be effective. In addition, this review may provide important information about the oncological safety of ISR. Generally, during ISR, smooth muscle fibres from the longitudinal muscles are not resected. However, Denost *et al.* reported that the resection line of transanal ISR is posterior to the pelvic sheath, and that transanal ISR enables EF resection (58). In low rectal cancer cases where the invasion depth may reach the longitudinal muscle, HL and EF resection, as well as preoperative therapy, may be useful. Likewise, the higher recurrence rates observed in local excision than in total mesorectal excision may be explainable by the widespread lymphovascular network between the rectal wall and EF, and HL.

Figure 1. ICG-FI findings in a 57-year-old man with low rectal cancer. (A, B) Images of the posterior wall before the dissection of the HL (surrounded by dotted lines). (C, D) Images of the posterior wall after HL dissection. (E, F) Images of the left lateral and antrolateral walls. (B) Lymphatic flow (green) is observed in the deep part of the HL. (D, F) Lymphatic flow is observed from the muscular layers of the anal canal to the surface of the LAM (endopelvic fascia) through the longitudinal muscle fibres (white arrow). (B, D, F) Near-infrared images. EF, Endopelvic fascia; HL, hiatal ligament; ICG- FI, indocyanine green fluorescence imaging; LAM, levator ani muscle; R, rectum. Figures are cited from (21).

Figure 2. Schemas of lymphovascular flow around the anal canal. (A) Sagittal section of the posterior wall. (B) Frontal section of the lateral and antrolateral walls. (C) Axial section around the anal canal and LAM in a male cadaver. Communications between blood vessels (red solid lines) and initial lymphatics (green solid lines with round dots) from the LM to the HL; EF and longitudinal fibres are demonstrated. Resection lines of ISR (blue dotted line) and standard APR (red dotted line) are shown in (A) and (B). APR, Abdominoperineal resection; CM, circular muscle; DL, dentate line; EAS, external anal sphincter; EF, endopelvic fascia; HL, hiatal ligament; IAS, internal anal sphincter; ISR, intersphincteric resection; LAM, levator ani muscle; LM, longitudinal muscle; MR, mesorectum; R, rectum; SM, submucosa. Figures are cited from (21).

We suggest that future research should include a combination of ICG-FI and histological examination using fresh cadavers. Although these methods have not been used to examine the anorectal region in previous studies, they have been used to examine superficial lymphatics in fresh cadavers (59). We believe that these methods can be used to observe functional lymph flow in the anorectal region in detail. A notable region of interest of functional lymphatic flow around the anorectal region is the region from the lower rectum to the inguinal region because tumour invasion of the dentate line and lymphovascular tumour invasion are considered risk factors for inguinal lymph node metastasis (60, 61). However, microanatomical inguinal lymph node metastatic pathways have not been reported in anorectal lesions. The lymphatic system around the DVF is also an important region of interest because low rectal cancer in the anterior portion has poor oncological outcomes (62).

Conclusion

This review summarized the fascial structures and lymphovascular network around the pelvic floor. Lymphatic flow between rectal muscle fibres, HL, and EF are suggested. The results provide important information to discuss therapeutic strategies for low rectal cancer. Existence of widespread lymphovascular network around the pelvic floor suggests that only conventional surgical treatment may not improve oncological outcomes because the network exceeds the resection lines of conventional APR and ISR. Therefore, enlarging the resection area of APR to include the EF and HL within the resection area, or introducing preoperative therapy seem to be effective.

Although the lymphatic systems of the pelvic region remain unclear, our novel findings help researchers analyse this complex area (21). The analysed methodology may be applied to other areas in the pelvis and may contribute to future research. There are many regions of interest for analysing functional lymphovascular networks in the pelvis, and continuous research is expected to take place.

Conflicts of Interest

The Authors declare no conflicts of interest.

Authors' Contributions

Kentaro Sato, Takuya Miura, Yoshiyuki Sakamoto, Hajime Morohashi, and Kenichi Hakamada contributed to the study concept and design. Kentaro Sato wrote the paper and prepared original draft. Takuya Miura, Yoshiyuki Sakamoto, Hajime Morohashi, and Kenichi Hakamada reviewed and edited the manuscript. Kenichi Hakamada supervised the study. All Authors read and approved the final manuscript.

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