To determine whether the clitoral infrafrenulum layer exists. This descriptive observational study was conducted using 15 fresh human female cadavers. Macro and micro stratum-by-stratum anatomical dissections of the clitoral frenulum were performed bilaterally. Target tissue biopsies were obtained for microscopic examination and the specimens were stained with eosin and hematoxylin. The skin folds with erectile tissue between them create the superficial stratum of the clitoral frenulum. The inferior edge of the skin fold is firmly fused with the superficial infrafrenulum fascial bundle, which creates the deep stratum. Histological examination confirmed that the clitoral frenulum comprises two layers: 1) the superficial stratum consists of the dermis, sub-dermis with cutis appendages (sebaceous and apocrine glands) and the squamous mucosa with erectile tissue between the skin folds; 2) the infrafrenulum fascia bundle consists of fibro-connective-adipose tissue with intermit-tent layers of adipose cells and fibrous bands. The clitoral infrafrenulum fascial bundle exists and creates the deep stratum of the clitoral frenulum. Histologically, the clitoral frenulum consists of two layers, the superficial and deep strata. Both strata form the clitoral frenulum stabilizing mechanism. This anatomical discovery has potential for immediate clinical implementation. Clin. Anat. 00:1–6, 2018. © 2018 Wiley Periodicals, Inc.

Key words: clitoral frenulum; clitoral frenulum anatomy; labium minus; clitoris; female genitalia; vulva; female; female organs

INTRODUCTION

The dedicated work of anatomists throughout the centuries has left little room for new discoveries, as exemplified by current scientific anatomical research. Without this work there would have been no progress in the fields of physiology or surgery. There is ample scientific-clinical evidence for the mutual dependency between anatomy and other medical fields, with anatomy leading and the rest following.

Traditional anatomical textbooks or atlases describe the clitoral frenulum as the anterior lower branch of the labium minus (de Graaf () [translated by Jocelyn and Setchell (1972)], Dunglison (1859), Clemente (1987), Netter (1989), Standring (2008), Rock and Jones (2003). These brief anatomical descriptions of the clitoral frenulum can be encapsulated as: the labium minus splits cranially into the superior (the preputium of the clitoridis) and the inferior frenulum of the clitoridis (the clitoral frenulum). The inferior branch of the labium minus (nympha), called the frenulum clitoridis, is attached to the posterior surface of the clitoral glans. An anatomically accurate description of any structure is of paramount significance for surgeons, and the rationale for conducting this anatomical investigation on the clitoral frenulum arises from three clinical considerations:

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Received 18 April 2018; Revised 10 May 2018; Accepted 16 May 2018
Published online in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ca.23215

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1. Intraoperative anatomical observations are not fully consistent with anatomical descriptions of the clitoral frenulum structure (Dunglison, 1859; Clemente, 1987; Netter, 1989; Standring, 2008).

2. Anatomical clitoral frenulum hypertrophy is a well-known phenomenon (Sydney, 1956) and surgeries for it are often performed for esthetic reasons or medical indications.

3. Significant anatomical deformities of the clitoral frenulum and abnormal clitoral over-mobility are often followed by surgical division of the frenulum in clinical settings.

The objectives of this investigation were to provide a detailed description of the anatomical structure of the infrarenulum and its potential role in stabilizing the clitoris, particularly the clitoral glans. The study protocol was designed to determine:

1. whether the clitoral frenulum is just the labium minus branch;
2. whether an anatomical infrarenulum structure exists;
3. whether this structure is consistently present;
4. the characteristic microscopic features of the clitoral frenulum.

**MATERIALS AND METHODS**

Fifteen fresh Caucasian female cadavers were included in this study. The Ethics Committee of Warsaw Medical University, Poland, approved the study protocol (AKBE 146/12). The demographic data of each subject were limited to identification number, subject’s initials, and age. No additional demographic or medical history information was available.

**Inclusion/Exclusion Criteria**

Within 24 hours of death and after forensic autopsies had been completed by the departmental staff, anatomical macro- and micro-dissections of the clitoral frenulum were performed. Subjects who demonstrated a disseminated illness, evidence of prior operation on the clitoral frenulum, contagious disease, disfiguration or pathologically increased size of the external genitalia, a tumor, or enlarged inguinal lymphatic nodes, and those who had been raped or involved in incest, were excluded. Refrigerated cadavers were also excluded. “Rigor mortis” of the subject did not interfere with dissections of the clitoral frenulum, the clitoral prepuce or the labium minus.

**Anatomical Dissection**

Macro- and micro-stratum-by-stratum dissections of the clitoral frenulum were performed using a surgical 3.5 to 4.0 x magnifying loop. A suture was placed at the attachment of the clitoral frenulum to the posterior clitoral glans, and a second suture was applied at the anterior labial bifurcation. The sutures were retracted in opposite directions to visualize the demarcation line of the clitoral frenulum. A superficial skin incision was made on the demarcation line and continued from the clitoral glans attachment to the anterior labium minus bifurcation. After the skin had been split from the clitoral frenulum structure, the superficial layer of the clitoral frenulum was removed and the deeper anatomical structure was retracted to allow the infrarenulum area to be visualized (Fig. 3). The superficial stratum of the frenulum was excised and the deep stratum of the frenulum clitoridis was exposed for examination (Fig. 1). After the scientific examinations of the deep layer of the clitoral frenulum were complete, a sharp incision through the infrarenulum fascial bundle sheet was made to determine the role of this structure in stabilizing the clitoris. Target biopsies were obtained and the tissue specimens were stained with eosin/hematoxylin. The initial microscopic examinations were conducted in Poland and the specimens were subsequently re-examined by a certified pathomorphologist in the U.S.A.

Electronic and manual literature searches were conducted from 1662 to January 2018 using Medical Subject Headings (MeSH) related to the frenulum clitoridis. All these headings were selected and used to search the ISI Web of Science (including conference proceedings that had been published in peer review journals), PubMed, ACOGNET, ProQuest, OVID, Cochrane Collection, the Lancet Online Collection, MD Consultants, New England Journal of Medicine, American College of Physician Online Resources, Highwire Journal, and Citation Index Reference.
RESULTS

Electronic and manual searches of the literature failed to identify a single anatomical article indicating the existence of a deep stratum of the clitoral frenulum. The present anatomical study used a sample of 15 subjects aged between 27 and 83 years (mean 50 ± 23 years). Eight women were of reproductive age and seven were post-menopausal. The clitoral frenulum was dissected bilaterally, so altogether 30 dissections were completed.

The findings verified accurate descriptions in the literature of the superficial stratum of the clitoral frenulum as the anterior lower branch of the labium minus (two separate small band-like structures with the superior edge free). The study revealed that the clitoral frenulum varies greatly in size (Fig. 2). The proximal part of the superficial clitoral frenulum stratum inserts into the posterior clitoral glans and the distal part fuses with the labium minus bifurcation (Figs. 1–2B, 2C). The inferior edge of the clitoral frenulum is firmly fused to the infrafrenulum structure (Fig. 1). Removal of the superficial stratum revealed a whitish triangular sheet with its apex pointed posteriorly (Fig. 3). Grossly, this structure resembles a fascia (Fig. 3). The infrafrenulum fascial bundle is oriented in the diagonal-longitudinal plane and corresponds to the superficial stratum of the clitoral frenulum alignment (Figs. 1 and 3). The infrafrenulum fascial bundle inserts into the surrounding tissues: 1. the entire superior edge of the triangle is intimately fused with the inferior part of the superficial stratum of the clitoral frenulum; 2. the proximal superior edge of the triangle attaches to the posterior part of the clitoral glans and the distal edge to the anterior labium minus bifurcation; 3. the anterior (median) and posterior surfaces of the triangular sheet interlock with the sizes of the clitoral frenula are present. The right-side. (E) of the clitoral frenulum is thinner and longer (the black arrow) than the right-side (F) the red arrow. (G) The left-side of the labium minus directly inserted to the posterior clitoral glans (the red arrow). The right-side clitoral frenulum is fragmented (black arrow). [Color figure can be viewed at wileyonlinelibrary.com]
surrounding tissues; 4. the antero-lateral-inferior edges of the fascial bundle sheet fuse with the tissue in the intralabial crease. Progressing downwards, the rope-like structure becomes thinner. The anterior and posterior surfaces of the fascial sheet fuse with adjacent tissues (Fig. 3). Its triangular tip fuses with the tissue of the intralabial crease (Fig. 3). Laterally, the deep stratum of the clitoral frenulum is fused with the labium minus and interlocks with adjacent tissues; cranially, it attaches to the posterior clitoral glans; and caudally, the clitoral frenulum inserts into the peri- and para-urethral areas (Fig. 3). Posteriorly, the deep stratum of the frenulum is connected to the anterior-superior surface of the vestibular bulbs (Fig. 3). The mean length of the triangular band-like structure was 5.1 cm ± 0.2 cm (mean ± standard deviation). The mean lengths of the two triangular arms were 1.1 cm ± 0.4 cm, the mean width was 0.3 cm ± 0.1 cm, and the mean thickness was 0.5 cm ± 0.2 cm (Fig. 4).

The superficial and deep layers of the clitoral frenulum are part of the clitoral stabilizing mechanism. Dividing or removing the entire deep layer of the infrarfrenulum fascial bundle sheet or a portion of it caused the clitoral glans to move upwards to an abnormal semi-everted position, exposing the clitoral root area. Also, the clitoris became mobile in all directions and did not return to its original location and orientation. The clitoral stabilizing mechanism can be damaged by dividing or removing a piece or resecting the entire infrarfrenulum layer (Fig. 5).

Histologically, the clitoral frenulum comprises two layers. The superficial stratum consists of the dermis, Fig. 4. The infraclitoral tissues and infrarfrenulum tissues are presented. (A) The red arrow indicates divided infrarfrenulum fascial bundle anatomical structure. (B) The clitoral frenulum with the infrarfrenulum fascial bundle (the black arrow). (C) The infraclitoral fascial bundle and the infrarfrenulum fascia bundle (D) were resected (the red arrow). [Color figure can be viewed at wileyonlinelibrary.com]

Fig. 5. Complications resulted from dividing and resecting the infrarfrenulum fascial bundle of the clitoral frenula. The posterior clitoral glans was partially resected. The infrarfrenulum fascial bundle of the clitoral frenula was bilaterally resected causing clitoral glans migrates upwards with retracting the clitoral prepuce (the black arrow) and clitoral glans (the yellow arrow). The red arrows indicate excisions of the superficial and deep layers of the clitoral frenulum bilaterally. [Color figure can be viewed at wileyonlinelibrary.com]
sub-dermis, and cutis appendages (sebaceous and apocrine glands) (Fig. 6A). There is squamous mucosa with erectile tissue between the skin folds (Fig. 6B). The newly-discovered deep stratum of the clitoral frenulum (the infrafrenulum fascia bundle) consists of fibro-connective-adipose tissue with intermittent layers of adipose cells and fibrous bands (Fig. 7).

DISCUSSION

This investigation established the existence of the deep stratum of the clitoral frenulum (Figs. 1 and 3) and determined the characteristic microscopic features of the infrafrenulum fascial bundle sheet structure (Figs. 6 and 7). It also established the anatomical role of this structure in maintaining the clitoral glans in its natural position and preventing it from becoming mobile. The biomechanical clitoral frenulum stabilizing mechanism is altered when the deep stratum of the frenulum is divided.

Clinical data indicate that severe complications can occur when the infrafrenulum fascial bundle is inadvertently severed during frenulum reduction surgery, causing clitoral hypermobility (Fig. 5). Complications resulting from dividing or resecting the infrafrenulum fascial bundle during clitoral frenuloreduction are identified as partial resection of the posterior clitoral glans and bilateral resections of the infrafrenulum fascial bundle of the clitoral frenula, causing upwards migration of the clitoral glans (yellow arrow) with retraction of the clitoral prepuce (black arrow) (Fig. 5). These anatomical discoveries have potential for immediate clinical implementation in reductive or reconstructive surgeries on the clitoral frenulum and help to prevent severe surgical complications.

Histological examination proved the existence of two layers of the clitoral frenulum. The superficial stratum (the dermis with appendages) consists of two-cutis folds with the squamous mucosa between

![Fig. 6. The clitoral frenulum superficial stratum histology is depicted. (A) The cutis layer of the clitoral frenulum: dermis, skin appendages (sebaceous and apocrine glands and subcutaneous layer). (B) Squamous mucosa with erectile tissue (red arrows). [Color figure can be viewed at wileyonlinelibrary.com]](image)

![Fig. 7. The deep stratum of the clitoral frenulum histology is depicted. The microscopic characteristic features consist of fibro-connective-adipose tissue with intermittent layers of adipose cells and fibrous bands (black arrows). [Color figure can be viewed at wileyonlinelibrary.com]](image)
the results are subject to the researcher and adipose tissue with intermittent layers of adipose cells and fibrous bands (Fig. 7).

Any anatomical study has inherent limitations since the results are subject to the researcher’s interpretation. The weakness of studies on fresh cadavers is the likelihood of topographic postmortem distortions. Also, the absence of accepted terms can influence the meaning of descriptions.

The strength of this study is the anatomical discovery and documentation of the clitoral infrarrenulum fascial bundle. The sample size was sufficient to establish the existence of the deep stratum of the clitoral frenulum and its role in providing mechanical stabilization for the clitoris. There is no similar study in the literature to compare with the current results.

Descriptions of the frenulum of the clitoridis in traditional textbooks failed to present the deep stratum of the clitoral frenulum and presented only its superficial stratum. Other researchers noticed disparities in anatomical descriptions of female genital structures; however, they did not examine the frenulum of the clitoridis (O’Connell et al., 1998, 2005, 2008).

Stojanovic and Djordjevic (2015) reviewed current knowledge of clitoral anatomy and its relevance to clitoral reconstruction in female-to-male sex reassignment surgery. Pauls (2015) reported that the clitoris has an intricate anatomy; therefore, understanding the clitoral frenulum as the anatomical structure stabilizing the clitoris is important, since the clitoris is considered to be central to female orgasmic responses. Saylor et al. (2018) emphasized the need for a clear understanding of female anatomy when male-to-female transgender surgery is performed. The surgical precursors of the anatomical structures of the female genitalia were itemized by Saylor et al. (2018) as follows: the clitoris is crafted from the glans penis so it retains its innervation; the labia minora are formed using excess penile skin tissue; and the long male urethra is shortened to that of its feminine counterpart. The main goal of these reconstructive methods is to create a functionally and esthetically acceptable vagina and vulva, together with expected voiding function and satisfactory sexual function.

CONCLUSIONS

The clitoral infrarrenulum fascia bundle structure exists and forms the anatomical deep stratum of the clitoral frenulum. The histological distinction between the superficial and the deep strata of the clitoral frenulum provides evidence that both these layers are present within it.

ACKNOWLEDGMENTS

The author thanks Prof. Dr. Pawel Krajewski, chairman of the Department of Forensic Medicine, Warsaw Medical University, Poland for selecting and providing subjects for the study and for helping me to obtain Ethics Committee approval. I also express my deepest gratitude to Zbigniew Antosz, M.D., chairman of the Department of Pathomorphology, Regional Hospital, Tychy, Poland, for preparing the histological material and performing the initial histological examination, and to Keren Davis, M.D, chairman of the Department of Surgical Pathology at St. Antony Hospital, St. Petersburg, Florida, U.S.A. for verifying the characteristic microscopic features of the clitoral frenulum.

AUTHOR CONTRIBUTIONS

AO conceived, designed and executed the project, collected, analyzed and interpreted the data, and drafted the manuscript.

CONFLICT OF INTEREST

The author has no conflicts of interest to report.

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