Innervation of the Labia Minora of Prepubertal Girls

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Abstract.

Introduction: Surgical and histologic sources of information give little reference to innervation, vascular, and epithelial details of the labia minora. Little is known about areas of nerve density, epithelial qualities, and vascular compartments of the labial minora that contribute to sexual arousal and orgasm. Surgical procedure development and counsel about surgical risks related to labioplasty and surgical flaps created from labial tissue may be based on inadequate information.

Methods: Labial samples from 10 normal girls (aged 2–9 years) who underwent surgery for labial fusion utilized waste tissue strips for immunohistochemical identification of S-100 and neuronal nitric oxide synthase (nNOS) in the labia minora.

Results: Vascular and lymphatic plexus lie within the reticular dermis, which contains a dense mesh of nerve fibers with a higher concentration of nerve fiber at the level of the subepithelial plexus. Dense innervations are located at the epidermis, extending along the basal and spinous layers of the epithelium of labia minora. Nerve bundles in the papillary dermis are associated with sebaceous and eccrine glands and nerve terminals located throughout the epithelium. The introital epithelium of the labia minora is highly innervated with widespread and intense staining, detected in the introital border of the labia minora versus the external one. The dermis appeared to display S-100 and nNOS immunolabelling. S-100 was also immunopositive in the epidermis.

Conclusion: Labia minora is highly innervated along its entire edge. Related vascular compartment tissue involved in engorgement during sexual arousal makes this tissue important for sexual response. Labioplasty risks removal of tissue with an important contribution to sensory sexual arousal. Movement of labial tissue during genitoplasty may have different sensory outcomes dependent on which labial surface is used.

Key Words. Labia minora—Vulva—Sexual sensitivity—Labioplasty

Introduction

Labioplasty or plastic surgical revision for reduction in size of the labia minora has become increasingly popular in the last years. Initially devised for functional purposes in cases of extreme asymmetry, or hypertrophy causing discomfort, problems with clothing concerning concealment of the genitalia, and difficulty with maneuvers such as intermittent catheterization; it has become a more common procedure, considered esthetic particularly among an adolescent population. Advertisement in popular magazines along with esthetic plastic surgical options may give added inducement. Social practices such as shaving the pubic hair make the labia more visible adding to the impression that the labia are larger. A very important consideration is the request for labioplasty to help resolve psychological or behavioral issues.

There is enormous variety and diversity of the length of labia minora between women. Changes in size, configuration and pigmentation generally begin in puberty. Labia minora hypertrophy can be congenital or acquired by lymphangioma, chronic irritation, inflammatory conditions (Crohn’s, endometriosis), exogenous androgenic hormones or stretching with weights. There is also an association with vulval varicosities. Labial hypertrophy may be unilateral or bilateral. Hypertrophy has been documented in children with myelodysplasia. This has been attributed to dermatitis from the use of diapers for incontinence. Woman with vulvar pain syndrome and a sexual abuse history may present for this surgery, inappropriately.

The main reasons patients seek such a procedure may be categorized as esthetic (personal opinion or partner opinion), functional impairment (pain and discomfort, interference with intromission during sexual intercourse or catheterization), a combination of esthetic and physical discomfort. Labial asymmetry and excision biopsy of pigmented lesions (melanoma risk) are also considered. Excision for ulcerations...
associated with Crohn’s disease may be necessary for diagnosis, but such lesions have been found to resolve spontaneously. A rare cause for excision biopsy is ulceration due to endometriosis. A very important consideration is the request for labioplasty for the solution of psychologic and behavioral consequences. Surgery in this circumstance is unlikely to deliver desired results.

For these reasons, scrutiny of genital plastic surgery is valid. A lack of standards of care, evidence-based outcome norms, standardization in diagnosis, nomenclature, and surgical training standards have been cited. Knowledge about the function of the labia minora in the lay population is likely to be incomplete. Review of surgical and histology sources of information give little reference to innervation, vascular and epithelial details. Little is known about the chief areas of nerve density, epithelial qualities and vascular compartments of the labial minora that contribute to the process of sexual arousal and orgasm. Thus, counsel about risks is difficult or incomplete. Plastic surgical procedure development may also be improved by such information, to be used in cases where such a surgery is actually warranted. In this study, we wanted to further elucidate innervation and vascularity associated with the labia minora in young females.

### Materials and Methods

Samples were derived from ten girls from ages two to nine years who have undergone surgery of the vagina for labial fusion. All specimens were obtained by the senior investigator, a pediatric urologist, after informed consent had been obtained from the parent or legal guardian. The specimens were waste tissue strips obtained at surgical separation of the labial fusion. Each specimen was marked with a suture on the posterior end of the strip. This study was approved by the hospital IRB.

Areas of innervation were globally assessed using the standard silver stain technique of Bielchowsky. Immunocytochemistry was then used to probe for markers specific to the neuronal phenotype as well as sites of vascularity. These included S-100 and nitric oxide synthase, respectively. We used general stains including Masson’s and H&E to establish general architecture/histologic features.

The tissue was fixed in 4% paraformaldehyde, stored in 0.1 M phosphate buffer and 10% sucrose for 24 hours, frozen, and cut into 30μm thick sections on a cryostat. The sections were washed twice for 5 minutes in phosphate-buffer saline (PBS), pH 7.4 incubated in blocking solution (10% methanol, 3% H₂O₂ in PBS) for ten minutes, rinsed twice in PBS followed by the gelatin blocking step (0.75% gelatin in PBS) for ten minutes and rinsed twice in PBS. Matching series of sections from each specimen were incubated for 48 hours at 4 °C, with the following antibodies in a humidified chamber: rabbit anti-neuronal nitric oxide synthase (nNOS) 2μg/ml (Zymed Laboratories Inc.) and rabbit anti S-100 dilution 1:400 (Dako Cytomation, Denmark). Following incubation in primary antibody, the sections were then processed according to a standard procedure for the rabbit IgG Vectastain ABC Kit (Vector Laboratories). Sections were then placed with agitation in two 5-minute washes of PBS, pH 7.4 followed by a 5-minute wash in 0.05 M TRIS buffer (Sigma Chemicals Corp, St. Louis, MO), pH 7.6. The sections were then immersed in 0.05% 3,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma) with 0.03% H₂O₂ in 0.05 M TRIS buffer pH 7.6 and reacted for 10 minutes, rinsed with 0.05 M TRIS buffer pH 7.6 followed by distilled water. The reaction product appeared as dark brown stain. In the negative control primary antibody was absorbed by preincubation with the respective synthetic peptide. Some sections were stained with hematoxilin and eosin, Masson’s technique, and with slightly modified Bielschowsky silver stain. All sections were dehydrated through graded alcohols, cleared in xylene and coverslips applied with Permount.

### Results

The labia minora are paired, hairless folds of skin that border the vestibule. Sections of labia minora stained with Masson’s and hematoxilin and eosin revealed that it was lined by stratified squamous epithelium. The epidermis contains the typical four layers: a basal layer which rests on the basal lamina, a spinous layer, a granular layer, and the stratum corneum, the superficial cornified layer. There were two differentiated parts in the dermis, the papillary and the reticular. The papillary dermis was composed of fine collagen fibers, together with elastic and reticular fibers. Sometimes the connective tissue papillae project into the epithelium. The tip of these projections appears as isolated structures surrounded by epithelium. The reticular dermis lies below the papillary dermis was composed of collagen fibers and elastic fibers. The labia minora was richly provided with sebaceous glands and with eccrine sweat glands, which open directly onto the skin. The vascular network extended through all the dermis creating the erectile-like tissue (Fig. 1, a, b). The vascular and lymphatic plexus lie within the reticular dermis, which also contained a dense mesh of nerve fibers (Fig. 2). The innervation of the labia minora was studied by using a standard histologic technique (Bielchowsky technique). The higher concentration of nerve fiber was at the level of the
subepithelial plexus (Fig. 2). Nevertheless a large amount of fibers were detected superficially, with a dense innervation located at the exterior epithelium, extending along the basal and spinous layers of the epithelium of labia minora (Fig. 2 and 3c,d). Even though the higher concentration of nerve fibers was in the reticular dermis (Fig. 2), there were also nerve bundles in the papillary dermis (Fig. 3a) and associated to sebaceous and eccrine glands (Fig. 3b) and nerve terminals located throughout the epithelium (Fig. 2 and 3c,d). The introital epithelium of the labia minora was highly innervated as shown inFig 4, in fact there was a difference, characterized as more widespread and intense staining, between the innervation detected in the introital border of the labia minora versus the external one (Fig. 2 a and 4). The innervation was characterized also by the presence of the general nerve marker S-100 and neuronal nitric oxide synthase (nNOS). The dermis appeared to display S-100 and nNOS immunolabelling (Fig. 5 a,b). S-100 was also immunopositive in the epidermis. Many thick nerve bundles were observed in the reticular and papillary dermis (Fig. 5 c,d). Thin nerve fibers were found throughout the dermis and epidermis as well (Fig. 5 a, b). Our silver staining along with IHC revealed distinctions heretofore unrecognized/not described. Areas of innervation identified by silver staining were also characterized by immunolabelling for S-100 and nNOS. Both immunolabels were ubiquitously found in regions of the dermis, suggesting loci that correspond to highly vascular and innervated areas. However, only S-100 staining occurred in the epidermis.

Discussion

A search for documentation of the labia minora histology particularly detailing the histology of the vascular compartment and the neural distribution as it might relate to labioplasty surgical design found scant information. The most detailed description11 of the labia minor cites a horny, cornified, granular epidermis in the outer section that merges with noncornified stratified squamous epithelium in the vestibule. These areas have been described as richly supplied with sebaceous glands, devoid of apocrine glands and containing scant eccrine glands. Using the Lavrentyev modification of the Bielschowski-Gross method of

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**Fig. 1.** Sections from labia minora showing blood vessels located in the dermis that constitute the erectile tissue. (a) (CT) connective tissue. (ET) Erectile tissue. (E) Epidermis Masson’s 4X, and (b) dermis of the labia minora observed at higher magnification showing the blood vessels of the erectile tissue H&E 20X.

**Fig. 2.** Sections of the labia minora stained with Bielchowsky silver technique. Showing the dense mesh of nerve fibers located in the (RD) reticular dermis, (PD) papillary dermis, (E) epidermis, (EB) external border of the labia minora, (IB) introital border of the labia minora.
silver staining, Malinovsky’s work\textsuperscript{12} extended these observations to descriptions of sensory nerve endings in this tissue. Sensory apparatus included free endings.

Both labia majora and labia minora respond with engorgement during clitoral stimulation.\textsuperscript{13} Yang\textsuperscript{14} recently demonstrated the labia minora as a sexually responsive vascular compartment (non-erectile/specialized genital tisssue) noting genital changes that occur during the female sexual response, using a gross anatomical and histological study of the vascular tissue of the vulva, supplemented with magnetic resonance imaging (MRI). During MRI imaging with erotic video stimulation, the labia minora increased in width and enhancement in pre and post menopausal women. Masson’s trichrome demonstrated the vascular tissue of the labia minora as different from the erectile tissue of the clitoris.\textsuperscript{14}

Nitric oxide synthase (NOS) is present in the deep arteries, veins, and capillaries of the human vagina.\textsuperscript{15} NOS isoforms are largely distributed in the cavernous tissue of the clitoris and human vagina, and labia minora, particularly in the nerve bundles and vascular and sinusoidal endothelium,\textsuperscript{15–19} as well as in the vagina and uterus and clitoris of experimental animals.\textsuperscript{20–22} These results imply that NO may play a role in controlling blood flow and capillary permeability, the mechanisms of sexual lubrication due to cyclic guanosine monophosphate (cGMP) action, induced by NO. The homeostasis of this system needs cGMP breakdown. It appears that physiologic response to sexual arousal in the female follows the same biochemical pathway as in the male. cGMP/NO appears to be a key pathway mediating clitoral smooth muscle relaxation.\textsuperscript{23} The final effect of NO

**Fig. 3.** Bielchowsky silver staining showing (a) the nerve bundles located in the papillary dermis (20×), and (b) the nerve fibers associated to sebaceous glands and eccrine glands located in the dermis (10×). (c) Nerve fibers in the dermis and epidermis (10×). (d) Nerve fibers located in the epidermis. Note the nerve fibers surrounding the connective tissue papillae that is projecting into the epithelium (20×).

**Fig. 4.** Cross section of the labia minora. (EB) external border of the labia minora, (IB) introital border of the labia minora. Note that the introital epithelium is highly innervated compare to the external border of the labia minora (5×).
synthesis, cGMP production, and smooth muscle relaxation regulated by NO-type 5 phosphodiesterase, is in fact the vascular engorgement of the clitoris and the anterior wall of the vagina; however, dense nNOS immunoreactivity on the genital skin covering the clitoris and labia was observed, which was not present in male specimens. Our study documents nNOS localization to the reticular dermis, and the papillary dermis of the labia minora. The distribution of nNOS is related to the blood vessels of nonerectile specialized genital tissue (tissue that becomes engorged). A probable explanation could be sensory responsibilities of nNOS or its local vasomotor effects.18

Malinovsky et al12 studied the types of sensory nerve endings present in both normal labia minora and hypertrophied labia minora in adult women and found no significant difference. Impregnated with silver nitrate by Lavrentyev’s modification of the Bielschowski-Gross method, sensory nerve endings were divided into the following groups: free endings and arborizations, spray-like endings, seven types of clew or ball-like nerve endings, and Pacinian corpuscles. Clew-like endings predominate. Great individual variability was seen in capsule thickness of the clew and in distribution of the types of endings. Rare occurrence of Meissner’s endings in the labia minora in woman were notable. These authors suggested that varying rate of occurrence of the nerve endings in the external genitals could affect general sensitivity in this region, including sexual sensitivity.12

The present study accords further insights into vascularity and innervation in this tissue. Neural staining for S-100 identified many thick nerve bundles in the reticular and papillary dermis. Of even greater import, intense staining occurred throughout the length and border of the epidermis in the labia minora. Dense innervation was observed at the exterior epithelium, extending along the basal and spinous layers of the epithelium of labia minora with higher concentration of nerve fibers in the reticular dermis. We believe that this density within the labia minora is indicative of high sensory value erotically. Findings indicating a substantial difference between the innervation detected in the introital border of the labia minora versus the external one are new information. We did not note a difference of innervation along the length of the labia as it became closer to the clitoris. The innervation was characterized by the presence of the general nerve marker S-100 and nitric oxide synthase nNOS indicating the involvement of neural sensory activation with specialized genital tissue that includes vascular engorgement. Complementary to this, staining for nNOS suggest an interplay between vascular engorgement and sensory activation. A study of sexual sensitivity in adult sexually active women, ranked labial sensation higher for orgasmic intensity than the introitus of the vagina.24 When considering these

Fig. 5. (a,b,c,d) Labia minora positive stained for S-100 and n-NOS in the dermis and the epidermis. Bundles of nerves immunostained with (b) S-100 and (d) nNOS.
findings, we suggest that this tissue is sensorially valuable for sexual arousal; also that these findings are important to the surgeon who considers surgical labiaplasty or labial surgical margins for excision.

While the present study provides additional insight into the vascularity and innervation of the labia minora, there are several limitations to this study. These include small sample sizes, representative of only prepubertal females, as well the presence of labial fusion which may or may not influence vascularity and innervation because of inflammation. Additional studies including tissue from pubertal and mature females including receptor status and vascular staining might enhance our understanding of the relationship of estrogens to sexual sensitivity and arousal mechanisms. The limitations of this study also include the fact that prepubertal specimens may not represent the exact architecture or histology of the same tissue after puberty, or after menopause. The same tissue should be studied from specimens representative of these life stages. As well, the entire labia is not excised, merely very thin strips of the edge of the labia. Study of innervations may be enhanced by full dissection of cadaveric specimens, or larger portions of labia obtained during labiaplasty for labial hypertrophy. There was no statistical analysis of the specimens collected. Each specimen did however contain similar distribution of nerve density of fibers, and fiber bundles in same locations.

Conclusion

The labia minora is highly innervated along its entire edge as shown by neural staining and nNos. Vascular compartment tissue illustrated is involved in the process of engorgement during sexual arousal, and makes this tissue an important part of the genital sensory dermatome involved in sexual response. Labiaplasty has the potential and risk for removal of tissue with an important contribution to sensory sexual arousal.

References