Nasolacrimal duct opening to the inferior nasal meatus in human fetuses

By

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Summary: The purpose of this study is to describe the Hasner’s membrane which is the main factor of congenital nasolacrimal duct obstruction. Hasner’s membrane at the nasal end of the fetal nasolacrimal duct (NLD) is considered to rupture at and after birth. However, topographical anatomy around the membrane as well as a mechanism of rupture seems to be still obscure. We observed frontal or sagittal sections of 20 late-stage fetuses (28–33 weeks) and found the on-going rupture in 2 specimens. The present sections demonstrated that 1) the nasal dilation was not a simple ball-like structure but extended posteriorly and laterally; 2) dilation of the NLD consistently involved the lacrimal sac; 3) Hasner’s membrane and ductal mucosal layer contained no macrophages and no or few arteries and nerves. The posterior extension of the NLD end ranged from 1–2 mm, while the lateral extension 3-5 mm although a site of the thinnest membrane varied in location between specimens. Moreover, the thickest NLD due to dilation was in the slightly orbital or upper side of the nasal end. Therefore, before surgical treatment of Hasner’s membrane, evaluation using medical images seems to be necessary. Since the nasal epithelium on Hasner’s membrane was most likely to destroy earlier than the NLD mucosal lining, observations of the membrane from the nasal cavity seemed helpful for diagnosis at which site would be broken and when.

Introduction

The lacrimal gland is responsible for the majority of tear production. The tears then drain into the lacrimal sac via the superior and inferior canaliculi. The superior and inferior canaliculi fuse to form a common canaliculus that drains into the lacrimal sac. The NLD exits the lacrimal fossa and provides drainage into the inferior meatus via the valve of Hasner1). Congenital NLD obstruction (CNLDO) is one of the most frequent issues in pediatric ophthalmology and rhinology2). The most common CNLDO manifestation is a persistent Hasner’s membrane3). It is important to know the change of Hasner’s membrane during fetal life to understand the CNLDO.

Close to the conjunctiva of eye, the nasolacrimal duct initially appears as the lacrimal cord of ectoderm-derived epithelial cells, extends from the orbital end and reaches the inferior nasal meatus by 10 weeks3, 4). At the nasal end, the epithelium of NLD attaches tightly to the nasal mucosal membrane of endodermal-origin and they together provides a thick “Hasner’s membrane”5, 6) or “Valve of Hasner”7, 8). The name “valve” tends to be used for a remnant of the membrane in adults.

At birth, Hasner’s membrane is sometimes or often (6–20%; [9]) maintained without rupture, i.e., CNLDO or dacrocystitis neonatorum. Cassady (1952) and Busse (1979) demonstrated histology of the distal NLD obstruction by Hasner’s membrane in full-term stillborn infants5, 6). Conversely, in late-stage fetuses, Hasner’s membrane usually seems to close the nasal end of NLD. Thus, the nasal end is most likely to be obstructed to make a ballooning of duct filled with a secretion from the lacrimal gland, the conjunctiva and/or the duct itself. However, we had several questions: 1) the dilation due

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Materials and Methods

This study was performed in accordance with the provisions of the Declaration of Helsinki 1995 (as revised in Edinburgh 2000). We examined the paraffin-embedded, frontal or sagittal sections of 20 fetuses (230–280 mm crown-rump length or CRL or approximately at 28–33 weeks). All fetuses were part of a collection kept at the Department of Anatomy, Akita University, Akita, Japan. They had been donated to the Department by the families concerned during the period 1975–1985, and preserved in 10% w/w neutral formalin solution for more than 30 years. The available data were limited to the date of donation and the number of gestational weeks. There were no related documents giving details of the family name, the name of the attending obstetrician or hospital, or the reason for abortion. The use of the specimens for research was approved by the ethics committee of Akita University (No. 1378). The head specimens were incubated at room temperature in Plank-Rychlo solution (AlCl$_3$/6H$_2$O, 7.0 w/v%; HCl, 3.6; HCOOH, 4.6) for 1–2 weeks. After routine procedure for paraffin embedded specimens, semiserial sectioning (7–10 µm thickness; 0.1 or 0.5 mm interval) was performed and stained with hematoxylin and eosin (HE) or Masson trichrome.

Using the 20 specimens, prior to the present study, we examined whether the sections was available for immuno-histochemistry. We found a fact that, in spite of the severe decalcification, immunostainings of macrophages, arterial endothelial cells and nerves were able to be evaluated in 4 of the 20 (all sagittal sections). The primary antibodies were 1) rabbit monoclonal anti-human CD68 (dilution 1:100; Dako, Glostrup, Denmark); 2) rabbit polyclonal anti-human S100 protein or S100 (dilution, 1:100; Dako N1573; Dako, Glostrup, Denmark) and; 3) mouse monoclonal anti-human alpha SMA (1:100; Dako M0851, Glostrup, Denmark). After incubation for 30 min in Histofine Simple Stain Max-PO (Nichirei, Tokyo, Japan) for the diaminobenzidine (DAB) reaction with horseradish peroxidase (HRP), dark brown coloration (DAB reaction) were obtained. Negative control sections were incubated with normal rabbit or mouse IgG (10 µg/ml) instead of primary antibodies. Sections stained using the DAB method were counterstained with hematoxylin. Observations and photography were usually performed with a Nikon Eclipse 80, but photos at ultra-low magnification (less than x1 at the objective lens) were taken using a high grade flat scanner (Epson GTX970) with translucent illumination. Dako N1573 antibody for smooth muscle actin is useful for a marker of endothelial cells of arteries$^{10}$, CD34 is the best vascular marker in fetuses$^{11}$, but because of severe decalcification, the antibody was not available for present specimens.

Results

We observed frontal or sagittal sections of 20 late-stage fetuses (28–33 weeks) and found the rupture in 2 specimens: a fetus at 31 weeks (Fig. 1) and another at 30 weeks. The present sections demonstrated that 1) the nasal dilation was not a simple ball-like structure but extended posteriorly (Figs. 1–3) and laterally (Fig. 4); 2) the dilation of NLD consistently involved the lacrimal sac (Figs. 2 and 5); 3) Hasner’s membrane as well as the ducal mucosal layer contained no macrophages and few arteries and nerves (Figs. 4C and 5C–E). In contrast, CD68-positive macrophages were densely distributed in bone tissues near the NLD. The dilated lacrimal sac was separated from the eye by a thick fascia (Fig. 2DE).

The posterior extension of the NLD end ranged from 1–2 mm, while the lateral extension 3–5 mm although a site of the thinnest membrane varied in location between specimens. At the thinnest part of Hasner’s membrane, the covering nasal epithelium appeared to destroy earlier than the other, ducal epithelium (Figs. 2F and 3E). The nasal end of NLD was wavy and made a series of double or triple lumens taking a grape-like appearance and separated by thin membranes (Fig. 1BC). However, the septa appeared to be in the absorption process without macrophage (Fig. 5B). Sometimes, the largest dilated part of NLD was not in the nasal end but in the slightly orbital or upper side (Fig. 4A). The submucosal loose tissue along NLD ranged from 0.2–0.5 mm in thickness near the lacrimal sac, but it was less than 0.1 mm near the nasal end. Thus, the periosteum was almost attached to the mucosal layer (Figs. 1BC and 2EF). The submucosal tissue the NLD end a venous plexus (Fig. 2F), but Hasner’s membrane did not. In the anterior side of the nasal end, a bundle of veins drained from the palate: the veins were separated from the duct by a thin bony plate (Figs. 4A and 5A). The left-right difference in the nasal end of NLD was often seen: e.g., large (Fig. 2A left) and small (Fig. 2B right); thin and ruptured (Fig. 1C) and thick and unruptured (Fig. 1B). In contrast to the dilated NLD, drainage routs from paranasal sinuses were narrow, poor in macrophages and with thick submucosal tissue (Figs. 3DF, 4A and 5A).

Consequently, 1) the dilation with secretion involved not only the NLD but the lacrimal sac; 2) no macrophages contributed onto rupture of Hasner’s membrane; 3) destruction of the membrane appeared to start from the nasal epithelial side.
Discussion

There are some therapeutic options for the CNLDO. If conservative treatments have failed, pressurized probing and syringing of the nasolacrimal duct is the method of choice\(^2\). But for some children who show persisting symptoms, other surgical therapy, for example dacryocystorhinostomy (DCR), has to be considered.

The current endoscopic DCR techniques were developed in the 1980s and 1990s\(^12\)-\(^14\). The endoscopic DCR has become a well-accepted and a safe surgical technique for management of NLD obstruction with high success rates of around 90% in adult\(^15\)-\(^17\). The endoscopic DCR to CNLDO has been operated for infants recently\(^18\), \(^19\). Although in young patients the nasal anatomy is more complex and narrow than in adults, the endoscopic DCR

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Fig. 1. Frontal section of a specimen of 31 weeks (260 mm).
Panels B and C are higher magnification views of squares in panel A (HE), respectively. Hasner’s membrane (arrows in panels B and C) is ruptured in the left nasolacrimal duct (open star in panel C) but still appears to be hard in the right (panel B). Circles indicate the mucosal layer, while triangles indicate the nearby periosteum. At the inferior end, the right nasolacrimal duct makes triple lumens (panel B). Panels B and C were prepared at the same magnification (scale bars in panels A and B). See also the common abbreviation in the final page.
Fig. 2. Frontal sections of a specimen of 31 weeks (260 mm).

The specimen same as in Fig. 1. HE staining. Panel A (or C) shows the most anterior (or posterior) site in the figure. Interval between panels are 0.5 mm (A–B, B–C). Panel C is 3.0 mm anterior to Fig. 1A. Panels D–F are higher magnification views of squares in panel C, respectively. The superior and inferior lacrimal canaliculi (ULC, LLC) are not dilated (panel A), but dilation occurs in the lacrimal sac (LS in panel B) as in the nasolacrimal duct (panel C and Fig. 1). Circles indicate the mucosal layer, while triangles indicate the nearby periosteum. Near the lacrimal sac, instead of the periosteum, the nasolacrimal duct is covered by a thick fascia (panels D and E). A venous plexus is adjacent to the duct (panel F). Panels A–C (or D–F) were prepared at the same magnification (scale bars in panels A and D). EB, ethmoidal; bone; OOM, orbicularis oculi muscle. Other abbreviation, see the common abbreviation.
The present study demonstrated that macrophages did not contribute to destruction of the membrane. This result was not described in the classical studies. Likewise, absorption of the septa between multiple lumens of the NLD end did not require macrophages. Such a process is known in fusion of the elderly thyroid colloidal lumens. Involvement of the lacrimal sac into dilation of NLD by secretion substances was one of striking features in this study. Possibly due to active and one-way transport through lacrimal canaliculi, the lacrimal sac might not interfere with the ballooning. Although injuries of the lacrimal drainage apparatus in congenital dacryostenosis might not be reported (i.e., [9]), we should note such a possibility. Excess expansion of the lacrimal sac could make a failure of normal development of the medial canthal ligament and Horner’s muscle that occurs just in the same stage.

Because of thin submucosal tissue with no or few

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**Fig. 3.** Frontal sections of a specimen of 30 weeks (254 mm).

Panel A is 1.5 mm anterior to panel B. HE and Masson trichrome. Panel C (a near section of panel A) is a higher magnification view corresponding to a square in panel A, while panel F is a higher magnification view of a square in panel B. Panel D shows a site between panels C and F and panel E is a higher magnification view of the center of panel D. Bilateral Hasner’s membranes are in a situation near rupture (arrow in panels A, D and E). In panel E, circles indicate the mucosal layer, while a triangle indicates the nearby periosteum. In the posterior end, the nasolacrimal duct makes double lumens (stars in panel F). Panels A and B (or C, D and F) were prepared at the same magnification (scale bars in each panel). See also the common abbreviation.
macrophages, a mass effect or mechanical stress from the filled substances was most likely to simply cause a rupture of Hasner’s membrane. Actually, the destruction seemed to start from the nasal epithelial side of the membrane. However, there might be no effect of air flow from the inferior nasal meatus. During expansion of the NLD, the nasal epithelium seemed to be less resistant to mechanical stress than the NLD epithelium or inner lining of the membrane possibly due to under-developed junction structures\textsuperscript{22}.

Fig. 4. Sagittal sections of a specimen of 29 weeks (250 mm).
Panel A (or F) displays the most medial (or lateral) site in the figure. Intervals between panels are 1.5 mm (A–B), 0.2 mm (B–C) and 1.0 mm (C–D, D–E, E–F), respectively. Panel F appears to show a morphology near the rupture. Stars indicate a corresponding site between panels. A tooth in panel A is next anterior to the tooth in panel F. Thus, the nasal end of the duct extends laterally. Panel C (immunohistochemistry of CD68) exhibits few macrophages along the duct mucosal layer. All panels were prepared at the same magnification (scale bar in panel A). See also the common abbreviation.
Panel A (Masson trichrome) displays dilatations of the lacrimal sac (LS) and the nasal end of the duct (square). Note veins concentrated in the anterior side of the duct. Panels B–E (adjacent sections) are higher magnification views of a square in panel A. Macrophages do not line the mucosal membrane of the duct but along nearby bone tissues (CD68; panel C). Arterial endothelial cells (SMA; panel D) as well as nerves (S100; panel E) are few in number along the nasal end of duct. Panels B–E were prepared at the same magnification (scale bars in panels A and B). See also the common abbreviation.
Fortunately, breeding was unlikely from the mucosal venous plexus at the rupture: the venous plexus was usually 1 mm distant from the membrane. The posterior extension of the NLD end ranged from 1–2 mm, while the lateral extension 3–5 mm although a site of the thinnest membrane varied in location between specimens. Moreover, the largest dilated part of NLD was likely to be in the slightly orbital or upper side of the end. Therefore, surgical treatment of Hasner’s membrane, evaluation using medical images seems to be necessary. Because of wavy course and 2–3 composite lumens like a grape, deep insertion of a straight needle seemed to be danger. The nasal epithelium covering Hasner’s membrane was most likely to destroy earlier than the NLD mucosal lining. Therefore, observations of membrane from the nasal cavity seemed helpful for diagnosis of at which site and when would be broken.

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Figure legends

concha, inferior nasal concha or turbinate; LS, lacrimal sac; MR, medial rectus muscle; MX, maxilla; NLD, nasolacrimal duct; sinus, drainage ducts from the paranasal sinus;

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