The Arachnoid Sleeve Enveloping the Pituitary Stalk: Anatomical and Histologic Study

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BACKGROUND: The arachnoid membrane in the suprasellar region may affect the growth pattern of sellar and suprasellar tumors however, the topographic relationships between the pituitary stalk and the surrounding arachnoid membranes remained unclear.

OBJECTIVE: The aim of this study was to evaluate the anatomical and histological characteristics of the arachnoid membranes.

METHODS: Microsurgical dissection and anatomical observation were performed in 16 formalin-fixed adult cadaver heads. In the other 5 adult cadaver heads, histologic sections of sellar-suprasellar specimens were studied under light microscopy.

RESULTS: An arachnoid sleeve enveloping the pituitary stalk of variable length presented in all specimens, which was formed by direct upward extension of the basal arachnoid membrane covering the diaphragma sellae. In the majority of specimens, the arachnoid sleeve was reinforced by the arachnoid trabeculae originating from the basal arachnoid membrane, the Liliequist membrane, and the medial carotid membrane.

CONCLUSION: The relationship between the pituitary stalk and the surrounding arachnoid membrane is important in evaluating the growth patterns of the sellar and suprasellar tumors, and their topographical relationships.

KEY WORDS: Anatomy, Arachnoid membrane, Histology, Pituitary stalk, Subarachnoid space

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In surgery of tumors in sellar and suprasellar regions, the arachnoid membrane has long been considered an effective barrier limiting the cisternal extension of the tumors.[1-5] Valuable information about the nomenclature, architecture, neurovascular relationships of the arachnoid membranes, and cisterns in suprasellar region has been provided by different authors based on intraoperative observation and anatomical study.[2,4,6-13] However, authors who dealt with this issue seldom made detailed description of the topographic relationships between the pituitary stalk and the surrounding arachnoid membranes, which are fundamentally important for a better understanding of anatomical location and growth pattern of the tumors relative to the sellar and suprasellar critical neurovascular structures. Furthermore, we could not find answers in the literature to the following questions: (1) Does the pituitary stalk directly descend through a focal defect on the basal arachnoid membrane covering diaphragma sellae to reach the pituitary gland? (2) Is the inferior part of pituitary stalk enveloped in a downward arachnoid sleeve formed by basal arachnoid membrane covering diaphragma sellae as seen in other cranial nerves? And if so, where does the arachnoid sleeve terminate? (3) Is the pituitary stalk a structure in subarachnoid space, or with part in subarachnoid space and part in extraarachnoid space? We attempted to answer these questions by evaluating the anatomical and histologic characteristics of the arachnoid membrane related to the pituitary stalk.

ABBREVIATIONS: ASPS, arachnoid sleeve enveloping the pituitary stalk

MATERIALS AND METHODS

Of 16 adult cadaveric formalin-fixed heads used for anatomical dissection, red latex was injected into arterial vessels in 10. The cranial vault and brain tissue 2 cm above the axial plane across the nasion and the inion were removed. Then, the brain tissue over and around the supratentorial basal cisterns was subpially removed in piecemeal fashion, with special attention to not stretch the underlying leptomeninges. By stepwise opening the pia mater covering the carotid cistern, chiasmatic cistern, and interpeduncular cistern, the arachnoid membrane was investigated, with close relationship to the pituitary stalk. A Leica M651 surgical microscope (Samsung Co., Seoul, Korea) was used for microsurgical dissections, with a Samsung SCC-101BP digital video camera (Leica Co., Heerbrugg, Switzerland) attached for photographic documentation of relevant structures.

In another 5 adult cadaveric formalin-fixed heads used for histologic study, the sellar-suprasellar regions were removed from each head en bloc. The bone in each sample was decalcified. Serial histologic sections were obtained in sagittal and coronal directions at 5-μm intervals. All these sections were stained with Masson trichrome stains and immunohistochemically labeled for vimentin. Observation was made under the Olympus-DP71 light microscope (Olympus Co., Tokyo, Japan).

RESULTS

Cadaveric Dissection

Basal Arachnoid Membrane Covering Diaphragma Sellae

The basal arachnoid membrane covering the diaphragma sellae constituted the inferior wall of the chiasmatic cistern. It was continuous with the basal arachnoid membrane covering the tuberculum sellae, anterior clinoid processes, and limbus sphenoidale anteriorly; the superior wall of the cavernous sinus and temporal fossa laterally; and the dorsum sellae, posterior clinoid processes, and clivus posteriorly. The pituitary stalk penetrated the basal arachnoid membrane to reach the pituitary gland. At the penetrating site, the basal arachnoid membrane extended upward along the pituitary stalk, which formed an arachnoid sleeve enveloping the pituitary stalk (ASPS) in all specimens (Figure 1).

We observed 2 basic types of the relationships between the lower end of ASPS and the pituitary stalk. In 10 specimens, being supradiaphragmatic in 7 and protruding into the sella turcica in 3, the basal arachnoid membrane did not contact the superior surface of the pituitary gland at the opening of diaphragma sellae. There was an interval, no more than one-third the full length of the pituitary stalk, between the basal arachnoid membrane and the superior surface of the pituitary gland at the opening of diaphragma sellae. This made the lower part of the pituitary stalk, being free of arachnoid, envelop in variable length (type I; Figure 1, A-D). The distance between the lower end of the ASPS and the superior surface of the pituitary gland was nearly equal on the anterior and posterior surfaces of the pituitary stalk in 6 specimens (type IA; Figure 1, A and B), and longer on the posterior surface than that on the anterior surface of the pituitary stalk in 4 (type IB; Figure 1, C and D).

In the other 6 specimens, being supradiaphragmatic in 2 and protruding into the sella turcica in 4, the basal arachnoid membrane directly contacted the superior surface of the pituitary gland at the opening of diaphragma sellae, and the ASPS enveloped the pituitary stalk of nearly full length (type II; Figure 1, E and F).

In all specimens, stripping the ASPS upward could be accomplished at its lower part (Figure 1, B and F), but could not proceed beyond the middle part of the pituitary stalk without tearing the ASPS because of the tight adherence.

In 7 specimens, the basal arachnoid membrane between the posterior surface of the pituitary stalk and dorsum sellae sent out a few arachnoid trabeculae to attach to the posterior and lateral surfaces of the middle and lower parts of the ASPS (Figure 2, A and B), its presence being associated with the absence of the diencephalic membrane of the Liliequist membrane, or the presence of higher protruding dorsum sellae.

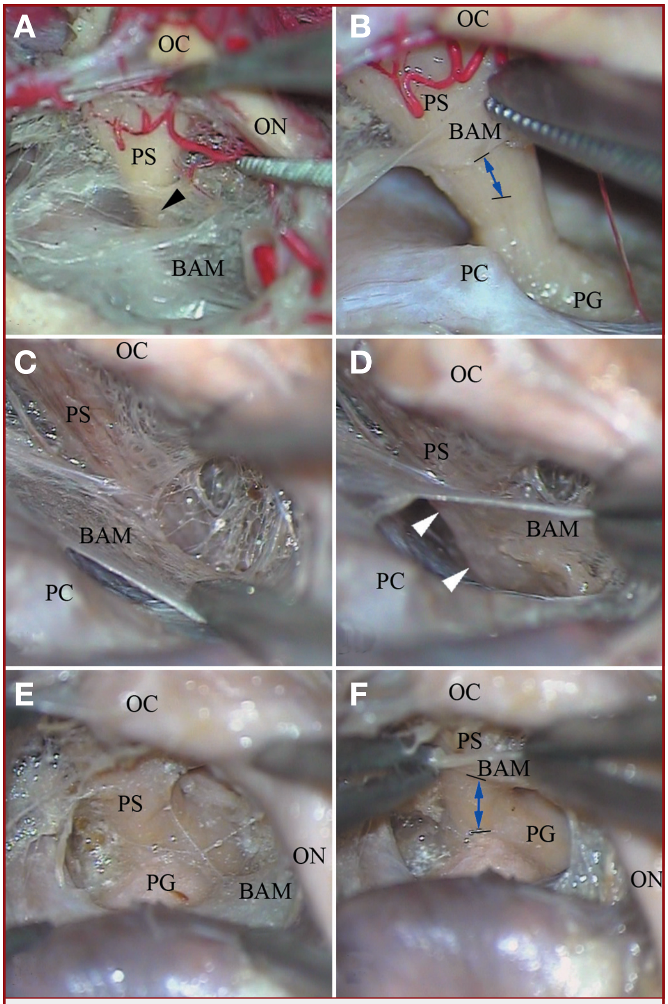

FIGURE 1. *Cadaveric dissection showing the relationship between the basal arachnoid membrane (BAM) and the pituitary stalk (PS).* **A**, *through the translucent BAM, the lower part of the PS (black arrowhead) could be seen in the extraarachnoid space between the BAM and diaphragma sellae (type IA).* **B**, *the upward extension of BAM (the arachnoid sleeve enveloping the PS) could only be stripped upward for a short distance (blue 2-head arrow) to the middle part of the PS.* **C** *and* **D**, *the BAM intersected with the PS at an oblique angle, which resulted in a longer distance (white arrowhead) between the lower end of the arachnoid sleeve enveloping the PS and superior surface of the pituitary gland (PG) on the posterior surface of the PS (type IB).* **E**, *the BAM directly contacted the superior surface of the PG (type II).* **F**, *the arachnoid sleeve enveloping the pituitary stalk could also be stripped upward to the middle part of PS (blue 2-head arrow). OC indicates optic chiasma; ON, optic nerve; PC, posterior clinoid process.*

Liliequist Membrane

The Liliequist membrane was situated posterior to the pituitary stalk. It arose from the basal arachnoid membrane covering

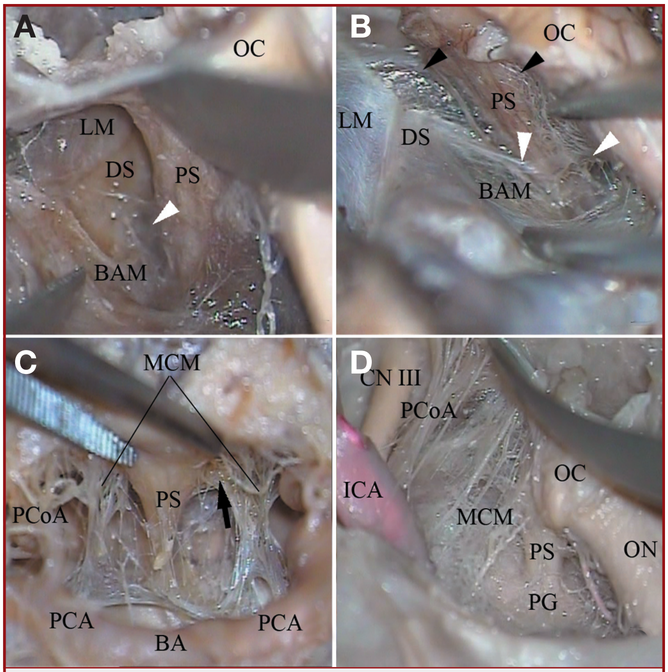

FIGURE 2. *Cadaveric dissection showing the relationship between the pituitary stalk (PS) and the arachnoid trabeculae originating from the Liliequist membrane (LM), basal arachnoid membrane (BAM), and medial carotid membrane (MCM).* **A**, *in this specimen, only the BAM sent out arachnoid trabeculae (white arrowhead) to attach to the posterior and lateral surfaces of the lower and middle parts of the arachnoid sleeve enveloping the PS.* **B**, *in this specimen, arachnoid trabeculae originating both from the LM (black arrowhead) and the BAM (white arrowhead) could be seen attaching to the posterior, lateral, anterior surface of the arachnoid sleeve enveloping the PS along its entire course, and to the inferior lateral surface of the optic chiasma (OC).* **C**, *anterior view of the attachments of MCM.* **D**, *posterior view of the attachments of the MCM (black arrow). BA indicates basilar apex; CN III, cranial nerve III (oculomotor nerve); DS, dorsum sellae; ICA, internal carotid artery; ON, optic nerve; PCA, posterior cerebral artery; PCoA, posterior communicating artery; PG, pituitary gland.*

the dorsum sellae, posterior clinoid processes, and adjacent anterior tentorial edge.

In 12 specimens, the well-developed diencephalic leaf of the Liliequist membrane extended upward, attached to the anterior edge of the mamillary bodies. It separated the chiasmatic cistern and interpeduncular cistern. In 11 specimens, a few arachnoid trabeculae originated from the superior surface of the Liliequist membrane and attached to the posterior, lateral surface of the upper and middle parts of the pituitary stalk, and to the inferolateral surface of the optic chiasma, which made the middle and upper part of the ASPS thicker (Figure 2B). In 1 specimen, there were no arachnoid trabeculae interconnecting the Liliequist membrane and the pituitary stalk (Figure 2A).

In 4 specimens, the diencephalic leaf of the Liliequist membrane was absent, and the chiasmatic cistern communicated freely with the interpeduncular cistern. Under these circumstances, there were no pituitary stalk related arachnoid trabeculae from the Liliequist membrane (Figure 2C).

Medial Carotid Membrane

The sagittally oriented medial carotid membrane, separating the chiasmatic cistern and the carotid cistern, varied greatly among different specimens and from side to side in individual specimens. In 5 specimens, the medial carotid membrane was absent unilaterally. The appearance of the medial carotid membrane varied from porous trabeculated wall to relatively intact membrane with small openings. The medial carotid membrane was originated from the basal arachnoid membrane inferomedial to the supraclinoid internal carotid artery and posterior communicating artery, and paramedian superior surface of the Liliequist membrane. Extending superiorly and medially, the medial carotid membrane attached to the pia mater covering the inferior surface of the optic chiasm, adjacent optic nerve, lateral surface of the infundibulum, and upper part of ASPS (Figure 2, C and D). Perforating arteries originating from the internal carotid artery and posterior communicating artery traveled on or through the medial carotid membrane to supply the pituitary stalk, optic chiasma, optic tract, and hypothalamus.

Histologic Findings

The ASPS was seen in all histologic specimens. Above the site where the pituitary stalk penetrated the basal arachnoid membrane to reach the pituitary gland, the upward extension of basal arachnoid membrane ran along the pituitary stalk (Figure 3, A-E). Therefore, the ASPS was arachnoid mater membrane, but thinner than the basal arachnoid membrane.

At the upper part of the pituitary stalk, the ASPS was seen attached to the top of the pars tuberalis. But whether it ended at the top of the pars tuberalis or turned downward between the pars tuberalis and the infundibulum stalk could not be determined in the Masson trichrome stain (Figure 3C). In histologic sections with immunohistochemical labeling for vimentin, it was clear the arachnoid sleeve ended at the top of the pars tuberalis and near the base of the infundibulum (Figure 3, D and E).

DISCUSSION

Architecture of Arachnoid Membrane Surrounding the Pituitary Stalk

Although a thorough knowledge of the microanatomy of the pituitary stalk is important in sellar and suprasellar surgery, the relevant literature provided little and incomplete information on the detailed topographic relationship between the pituitary stalk and surrounding arachnoid membrane.[2,4,6-13] According to Yaşargil[4] in his masterpiece, *Microneurosurgery*, the Liliequist membrane "is fused with the chiasmatic cistern around the infundibulum and pituitary stalk." Fox[2] proposed the existence of a "hypophyseal cistern" and made the following statement: "in many cases the membrane of Liliequist, which lies caudal to the pituitary stalk, sends an anterior reflection of arachnoid membrane in front of the pitu-

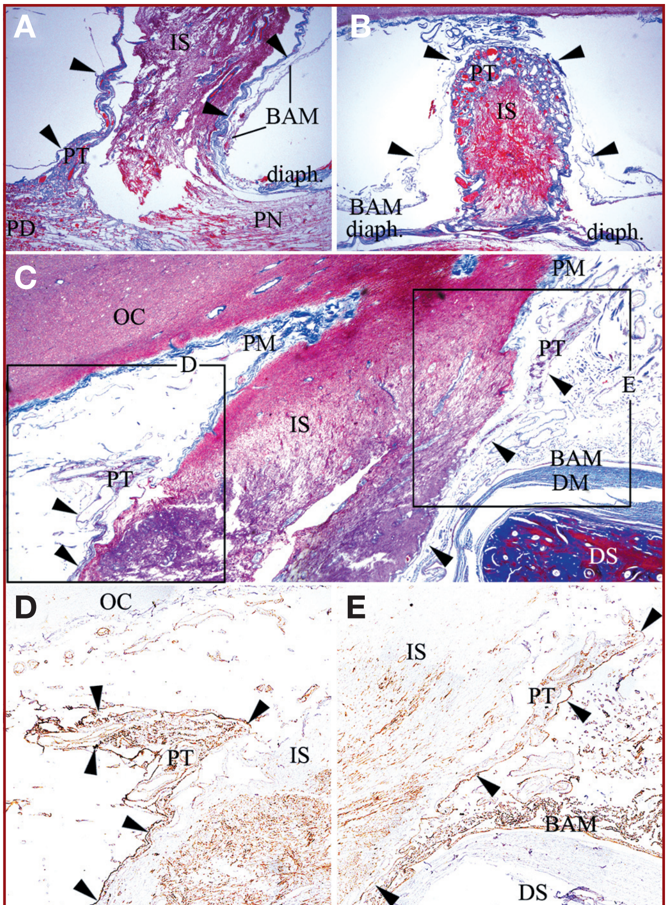

FIGURE 3. *Sagittal (***A***,* **C***,* **D***, and* **E***) and coronal (***B***) histologic sections through the pituitary stalk (Masson trichrome stain in* **A***,* **B***, and* **C** *with original magnification ×40, and vimentin labeling in* **D** *and* **E** *with original magnification ×100).* **A***, in this specimen, the arachnoid sleeve enveloping the pituitary stalk (ASPS) (arrowheads) started near the lower end of the pituitary stalk at the opening of diaphragma sellae, and the distance between the lower end of ASPS and the superior surface of pituitary gland was nearly equal on the anterior and posterior surfaces of the pituitary stalk.* **B***, in this specimen, there was a definite interval between the basal arachnoid membrane and the diaphragma sellae and superior surface of pituitary gland, and the ASPS (arrowheads) loosely covered the inferior part of the pituitary stalk and adhered to the middle part of the pituitary stalk more tightly.* **C***, the upper end of the ASPS cannot be determined in the upper part of the pituitary stalk because of tight adhesion in the Masson trichrome stain.* **D** *and* **E***, in the vimentin labeling specimen, it was demonstrated that the ASPS (arrowheads) ended at the upmost portion of the pars tuberalis (PT) on both the anterior (***D***) and posterior (***E***) surfaces of the upper part of pituitary stalk. BAM indicates basal arachnoid membrane; diaph., diaphragma sellae; DM, dural mater; DS, dorsum sellae; IS, infundibular stalk; OC, optic chiasma; PD, pars distalis; PM, pia mater; PN, pars nervosa.*

itary stalk. This situation puts the pituitary stalk inside its own hypophyseal cistern." Brasil and Schneider[6] stated that "Direct contact between MEM (Liliequist's membrane) and the posterior surface of the infundibulum was occasionally observed. The infundibulum was not surrounded by MEM in any specimen." In a more recent description, given by Lü and Zhu,[8] the mesencephalic leaf of the Liliequist membrane "attached to the posterior surface of the infundibulum and pituitary stalk" and "fused with the basal leaf of the chiasmatic membrane around the pituitary stalk."

As seen in our anatomical dissection and histologic sections, the pituitary stalk is enveloped by the arachnoid mater, namely, the "arachnoid sleeve enveloping the pituitary stalk" or ASPS, which is the direct upward extension of basal arachnoid membrane covering the diaphragma sellae. The ASPS encloses the pituitary stalk of variable length in all specimens. Its lower end can be infradiaphragmatic or supradiaphragmatic, and its upper end terminates at the top of the pars tuberalis and near the base of the infundibulum. In the majority of specimens, the arachnoid trabeculae originating from the Liliequist membrane, basal arachnoid membrane, and medial carotid membrane may reinforce the ASPS, as described in previous studies. In comparison with the previous results that the extension of arachnoid sleeves on the cranial nerves is extracranial in direction,[9,14,15] the ASPS on the pituitary stalk is a reverse type.

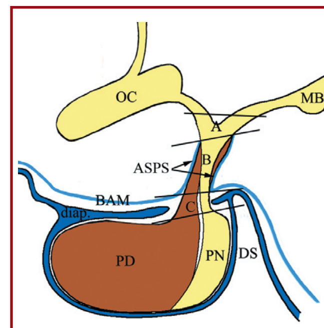

FIGURE 4. *The pituitary stalk can be divided into 3 parts according to their relationship with the arachnoid sleeve enveloping the pituitary stalk (ASPS).* **A***, subarachnoid part.* **B***, intraarachnoid part.* **C***, extraarachnoid part. BAM indicates basal arachnoid membrane; diap., diaphragma sellae; DS, dorsum sellae; MB, mamillary body; OC, optic chiasma; PD, pars distalis; PN, pars nervosa.*

From an anatomical point of view, the pituitary stalk can be divided into 3 parts according to their relationship with the ASPS (Figure 4). The first part, the subarachnoid part, is above the upper end of the ASPS, which comprises the base of the infundibulum. The second part, the intraarachnoid part, refers to the segment of pituitary stalk enveloped in the ASPS, which is the longest among the 3 parts. The third part, the extraarachnoid part, presents when there is an interval between the basal arachnoid membrane and the superior surface of the pituitary gland.

Based on the results from this study, the "hypophyseal cistern," initially described by Fox,[2] does exist in the majority of cases, and contains variable length of the pituitary stalk. The inner wall of this cistern is the ASPS, and the outer wall comprises arachnoid trabeculae originating from the basal arachnoid membrane, the Liliequist membrane, and the medial carotid membrane. Its completeness and length depend on the contribution by these 3 arachnoid membranes.

Embryogenesis of the Arachnoid Membrane Surrounding the Pituitary Stalk

Embryologically, the neural tube is enveloped by a mesenchymal layer, from which the meninges subsequently differentiate.[16]

Histologically, the hypophysis comprises a neural component (infundibular stalk and pars nervosa) derived from the embryonic diencephalon and an epithelial component (pars distalis, pars intermedia, and pars tuberalis) derived from the Rathke pouch. Chi and Lee[17] demonstrated that the mesenchymal cells around the Rathke pouch eventually form the pituitary capsule enveloping the whole pituitary gland, which is distinct from the pia arachnoid membrane. However, in cases of congenital absence of the adenohypophysis, the remaining neurohypophysis in the pituitary fossa was entirely surrounded by the pia arachnoid membrane, as reported by Brewer.[18] These facts suggest that the embryogenesis of the adenohypophysis may regulate the mesenchymal differentiation; that is, in normal development of the hypophysis, the mesenchymal cells differentiate into the pia arachnoid membrane in the suprasellar region covering the pituitary stalk and into the fibrous capsule in the pituitary fossa enveloping the pituitary gland.

In a detailed study of hypophyseal organogenesis in humans by Solov'ev et al,[19] the Rathke pouch is initially separated from the wall of the diencephalon by a layer of mesenchyme. They noticed that when the infundibulum contacts the Rathke pouch, newly formed connective tissue appears around the epithelial elements, this being absent only at the point of contact of the epithelial and neural elements of the organ. Because the pars tuberalis (originating from these epithelial elements) eventually wraps around the infundibular stalk (originating from the neural elements), it explains the results of our study: the ASPS only envelops the outer surface of the pituitary stalk and does not extend downward from the top of the pars tuberalis into the pituitary stalk between the infundibular stalk and the pars tuberalis.

Clinical Significance of the Arachnoid Membrane Surrounding the Pituitary Stalk

Although it is much thinner, the intact ASPS is physically much more flexible and durable than the neural tissue. In suprasellar meningiomas, though the pituitary stalk could be severely compressed, the ASPS may protect the pituitary stalk and provide a plane of cleavage for tumor dissection, which may partially explain why the incidence of postoperative hypophyseal dysfunction is fewer in patients with suprasellar meningiomas than that in patients with other intra-axial suprasellar tumors.[3,5,20,21]

The arachnoid membrane surrounding the suprasellar tumor can also act as a true barrier to limit its cisternal extension. In craniopharyngiomas, the tumor may arise from anywhere along the pituitary stalk, extending from the pituitary gland to the tuber cinereum, which means the tumor may arise in the extraarachnoid, intraarachnoid, or subarachnoid space. The varying patterns of ASPS and the location of the tumor origin relative to the ASPS may be relevant in determining growth patterns and the adhesion between the tumor and the suprasellar neurovascular structures.

CONCLUSION

Using the combination of anatomical dissection and histologic study, this study reveals that (1) the pituitary stalk is enveloped by arachnoid mater sleeve (the ASPS) in variable length, which is the direct upward extension of the basal arachnoid membrane; (2) the ASPS begins where the pituitary stalk penetrates the basal arachnoid membrane and ends at the top of the pars tuberalis; (3) the ASPS constantly presents and is reinforced by the arachnoid trabeculea originating from the basal arachnoid membrane, the Liliequist membrane, and the medial carotid membrane in the majority of specimens; and (4) the pituitary stalk can be divided into 3 parts according to their relationship with the ASPS: the subarachnoid, intraarachnoid, and extraarachnoid.

REFERENCES

1. Carmel PW. Craniopharyngioma: transcranial approaches. In: Apuzzo MLJ, ed. *Brain Surgery: Complication Avoidance and Management*. Vol 1. New York, NY: Churchill Livingstone; 1993:339-357.
2. Fox JL. *Atlas of Neurosurgical Anatomy: The Pterional Perspective*. New York, NY: Springer-Verlag; 1989:123-164.
3. Symon L, Rosenstein J. Surgical management of suprasellar meningiomas, part I: the influence of tumor size, duration of symptoms, and microsurgery on surgical outcome in 101 consecutive cases. *J Neurosurg*. 1984;61(4):633-641.
4. Yaşargil MG. *Microneurosurgery*. Vol 1. Stuttgart, Germany: Georg Thieme; 1984:5-54.
5. Yaşargil MG, Curcic M, Kis M, Siegenthaler G, Teddy PJ, Roth P. Total removal of craniopharyngiomas: approaches and long-term results in 144 patients. *J Neurosurg*. 1990;73(1):3-11.
6. Brasil AV, Schneider FL. Anatomy of Liliequist's membrane. *Neurosurgery*. 1993;32(6):956-961.
7. Inoue K, Seker A, Osawa S, Alencastro LF, Matsushima T, Rhoton AL Jr. Microsurgical and endoscopic anatomy of the supratentorial arachnoidal membranes and cisterns. *Neurosurgery*. 2009;65(4):644-665.
8. Lü J, Zhu XL. Microsurgical anatomy of Liliequist's membrane. *Minim Invas Neurosurg*. 2003;46(3):149-154.
9. Lü J, Zhu XL. Characteristics of distribution and configuration of intracranial arachnoid membranes. *Surg Radiol Anat*. 2005;27(6):472-481.
10. Rhoton AL Jr. The sellar region. *Neurosurgery*. 2002;51(4)(suppl):S335-S374.
11. Seeger W. *Endoscopic and Microsurgical Anatomy of the Upper Basal cisterns*. New York, NY: Springer-Verlag; 2008:69.
12. Vinas FC, Fandino R, Dujovny M, Chavez V. Microsurgical anatomy of the supratentorial arachnoidal trabecular membranes and cisterns. *Neurol Res*. 1994;16(6):417-424.
13. Yaşargil MG, Kasdaglis K, Jain KK, Weber HP. Anatomical observations of the subarachnoid cisterns during surgery. *J Neurosurg*. 1976;44(3):298-302.
14. Martins C, Yasuda A, Campero A, Rhoton AL Jr. Microsurgical anatomy of the oculomotor cistern. *Neurosurgery*. 2006;58(4)(suppl 2):ONS220-ONS228.
15. Ozveren MF, Uchida K, Tekdemir I, et al. Dural and arachnoid membraneous protection of the abducens nerve at the petroclival region. *Skull Base*. 2002;12(4):181-188.
16. O'Rahilly R, Müller F. The meninges in human development. *J Neuropathol Exp Neurol*. 1986;45(5):588-608.
17. Chi JG, Lee MH. Anatomical observations of the development of the pituitary capsule. *J Neurosurg*. 1980;52(5):667-670.
18. Brewer DB. Congenital absence of the pituitary gland and its consequences. *J Pathol Bacteriol*. 1957;73:59-67.
19. Solov'ev GS, Bogdanov AV, Panteleev SM, Yanin VL. Embryonic morphogenesis of the human pituitary. *Neurosci Behav Physiol*. 2008;38(8):829-833.
20. Fahlbusch R, Schott W. Pterional surgery of meningiomas of the tuberculum sellae and planum sphenoidale: surgical results with special consideration of ophthalmological and endocrinological outcomes. *J Neurosurg*. 2002;96(2):235-243.
21. Jung TY, Jung S, Choi JE, Moon KS, Kim IY, Kang SS. Adult craniopharyngiomas: surgical results with a special focus on endocrinological outcomes and recurrence according to pituitary stalk preservation. *J Neurosurg*. 2009;111(3):572-577.

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