A membranous structure separating the adenohypophysis and neurohypophysis: an anatomical study and its clinical application for craniopharyngioma

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OBJECT This study aimed to identify the membranous septation between the adeno- and neurohypophysis. The clinical impact of this septation in the surgical removal of infradiaphragmatic craniopharyngioma (Id-CP) is also clarified.

METHODS The sellar regions from 8 fetal and 6 adult cadavers were dissected. After staining first with H & E and then with picro-Sirius red, the membranous structures were observed and measured under normal light and polarization microscopy. The pre- and postsurgical images and intraoperative procedures in 28 cases of childhood Id-CP were reviewed and analyzed.

RESULTS There is a significant membranous septation (termed the adenoneurohypophysis septation [ANHS]) lying behind the intermediate lobe to separate the adeno- and neurohypophysis. The average thicknesses are $21.9 \pm 16.9 \mu\text{m}$ and $79.1 \pm 43.2 \mu\text{m}$ in fetal and adult heads, respectively. The median segment of the septation is significantly thicker than the upper and lower segments. The ANHS extends from the suprasellar pars tuberalis to the sellar floor, where it is fused with the pituitary capsule. During Id-CP surgery performed via a transcranial approach, the ANHS can be identified to reserve the neurohypophysis. Moreover, by understanding the anatomy of this membrane, the pituitary stalk was preserved in 3 patients (10.7%).

CONCLUSIONS There is a significant membrane separating the anterior and posterior lobes of the pituitary gland, which lies behind the intermediate lobe. Understanding the anatomy of this septation is important for identifying and preserving the neurohypophysis and pituitary stalk during Id-CP surgery.

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KEY WORDS craniopharyngioma; hypophysis; infradiaphragmatic; membrane; pituitary gland; septation; pituitary surgery

As the most important neuro-endocrinological organs in the human body, the hypothalamus-hypophysis axis and pituitary gland are in a dangerous zone for neurosurgery. Craniopharyngioma (CP) is a histologically benign tumor with malignant behavior that originates from the residual tissue of Rathke's pouch located along the intermediate lobe as far as the third ventricle floor.⁶ The location of this lesion makes resection difficult because neuro-endocrinological function can be compromised.

Many classifications based on the relationships of CP with the optic chiasm, ventricle, and pituitary stalk have been proposed, such as pre- or postchiasmatic, extra- or intraventricular, and transfundibular or infundibulotuberal

tumors.^{6,11} We recently proposed a topographic classification based on the tumor-membrane relationship,^{12,13} where the CP was divided into infradiaphragmatic, extraarachnoidal, intrarachnoidal, and subarachnoidal types. After analyzing 195 CP cases, we determined that the membranes can be used as a guide to safely remove tumors and protect the normal tissues. In addition, we proposed the true stratification structures of "intraventricular CP" and "infundibulotuberal CP," which also showed the close relationship of tumors to the arachnoid and pia mater.¹⁰ However, there are no publications describing infradiaphragmatic CP (Id-CP) and the related intrasellar membranes.

In 2009, we described the membranous layers covering

ABBREVIATIONS ANHS = adenoneurohypophysis septation; CP = craniopharyngioma; Id-CP = infradiaphragmatic CP.

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the surface of the pituitary gland,¹⁴ which led to the conclusion that the inner (lamina propria) and outer (pituitary capsule) layers cover the entire gland. After further observation, a membranous septation was observed between the anterior and posterior lobes. In the present study, the morphological features of this septation are depicted, and its importance for Id-CP surgery is also discussed.

Methods

Histological Sections and Staining

Heads from 8 fetal (ages 25–30 weeks) and 6 adult (4 men and 2 women) cadavers were embalmed with 10% formalin solution administered through the internal carotid arteries of the adults and the umbilical arteries of the fetuses. The pituitary gland, pituitary stalk, hypothalamus, and correlated parasellar structures were harvested. After decalcifying, dehydrating, and embedding the specimens in paraffin, serial sections were obtained in the coronal and sagittal direction at 8- μ m intervals. Four of every 10 sections with the required anatomical structures were selected for staining with H & E and then picro-Sirius red. An inverted optical polarization microscope (Olympus DP70; Olympus Corp.) and a software package (Olysia Bioreport, Olympus Corp.) were used to observe and measure the corresponding membranes.

Clinical Analysis

We collected the clinical data of 28 pediatric patients (age < 18 years) with Id-CP who presented in our department between January 2005 and September 2011. They had not undergone any previous surgical treatments or irradiation prior to transcranial tumor removal. Preoperative and postoperative MR images were selected and analyzed. The intraoperative procedures were imaged and recorded.

All measurement results were statistically analyzed with 1-way ANOVA and Student-Newman-Keuls tests. Differences were considered significant at $p < 0.05$.

Results

The Septation Between the Adeno- and Neurohypophysis

The significant membranous septation between the adeno- and neurohypophysis is depicted as a line in the sagittal sections (*yellow arrows* in Fig. 1). The septation was located behind the intermediate lobe and separated the neurohypophysis. However, there was no such membrane between the intermediate and anterior lobes. The septation extended from the suprasellar pars tuberalis into the intrasellar region and is termed the adenoneurohypophysis septation (ANHS). The other membranous structures in the sellar region, such as the pituitary capsule, diaphragm, and sellar floor dura, were also observed in the sections.

In some cases the pars tuberalis enveloped the pituitary stalk, as demonstrated by the adenocyte component behind the neurohypophysis. The membranous septation was also observed (*white arrows* in Fig. 2C). At the sellar floor, the septation appeared to fuse with the pituitary capsule (Fig. 2B). In the coronal sections, the membranous structure surrounded the neurohypophysis and pituitary stalk (Fig. 3), and the septation clearly separated

FIG. 1. Sagittal sections of a 30-week-old fetus. **A:** Gross view of the sellar region. A membranous structure (*yellow arrows*) separated the adeno- and neurohypophysis, which extended along the full length of the pituitary stalk from the suprasellar pars tuberalis to the intrasellar fossa. The corresponding areas of interest (*red squares*) are magnified in panels B and C. **B:** The septation extended upward to the pars tuberalis, where it appeared to fuse with the arachnoid trabeculus under the ventral side of the optic chiasm. **C:** The septation (*yellow arrows*) was located behind the intermediate lobe and Rathke's pouch. However, there was no significant membranous septation between the adenohypophysis and intermediate lobe. Picro-Sirius red stain. Adeno. = adenohypophysis; Infundi. = infundibulum; Inter. = intermediate lobe; Neuro. = neurohypophysis; Pars tuber. = pars tuberalis; Pit. capsu. = pituitary capsule; Rath. pouch = Rathke's pouch; S.F. dura = sellar floor dura; Trabe. = trabecula; Ventri. = ventricle.

the intermediate lobe and neurohypophysis. The same anatomical features were found in all adult sections. In addition, the intermediate lobe regressed significantly, as demonstrated by several small areas of degenerated Rathke's pouch (Fig. 4).

The thickness of the septation was $21.9 \pm 16.9 \mu\text{m}$ in fetal specimens and $79.1 \pm 43.2 \mu\text{m}$ in adults ($p < 0.001$). Moreover, the median ANHS segment closest to the intermediate lobe was significantly thicker than the upper and lower segments both in children and adults ($p < 0.001$, Table 1). However, no such difference was found between the upper and lower segments in fetuses or adults ($p = 0.107$ and $p = 0.77$, respectively).

Clinical Analysis

The 28 pediatric patients (8.1 ± 4.3 years old) with Id-CP underwent transcranial surgery. The median tumor size was $4.8 \pm 1.4 \text{ cm}$ (2.8–8 cm). During surgery, the tumor was observed to be covered by the diaphragm and suprasellar arachnoid (basal membranous arachnoid) and separated from the surrounding structures. The diaphragm was dissected with a round incision to expose the true tumor capsule wall. Then, following the interface of ANHS, the intrasellar part of the tumor was freed from the neurohypophysis, which was preserved in all cases

FIG. 2. Sagittal sections of a 25-week-old fetus. **A:** Gross view of the sellar region. The septation (yellow arrows) was significantly displayed between the adeno- and neurohypophysis. The corresponding areas of interest (red squares) are magnified in panels B and C. **B:** The septation (yellow arrows) was located behind the intermediate lobe, and Rathke's pouch was also observed. At the sellar floor, the septation seemed to fuse with the pituitary capsule. **C:** The magnified image shows that the pars tuberalis can envelop the whole pituitary stalk. The posterior part of the septation (white arrows) is also located between these 2 structures. Picro-Sirius red stain. Dor. sellae = dorsum sellae.

(Fig. 5). Because of fusion of the septation with the pituitary capsule, after radical removal of tumor at the bilateral sides along the septation, the medial wall of the cavernous sinus (which was composed of the lateral part of the pituitary capsule; see also Fahlbusch et al.⁵) was sometimes opened.

Some presurgical MRIs showed that the pituitary stalk was pushed to one side (Case 1 in Fig. 6, red arrow). As a result, the continuity of the pituitary stalk might be preserved during the surgery if the ANHS cleavage plane is followed. In 3 of 28 cases (10.7%), the stalk was preserved. However, in the majority of cases, the stalk was identified but could not be preserved because of its umbrella-shaped inflation (Case 2 in Fig. 6).

Discussion

Embryonic Development of the Membranous Septation Inside the Pituitary Gland

After carefully reviewing the literature, we did not find any publications describing the membranous septation (ANHS) that we identified. However, some descriptions of the embryonic development of the pituitary gland and

related meninges hinted at the origin of the septation. As reported by Chi and Lee,² the endomeninx (pia arachnoid) is visible around the primitive cerebral vesicle in 6-week-old embryos. Furthermore, the primitive neural tube and Rathke's pouch are also surrounded by mesenchymal cells, as reported by Chi and Lee and O'Rahilly and Müller.^{2,9} With the upward elongation of Rathke's pouch and its contact with the ventral diverticulum of the diencephalon of the neuroectoderm, the mesenchymal layer thickens around the developing adenohypophysis. Notably, the authors reported that when Rathke's pouch and the neurohypophysis finally fuse together, there is a temporary covering like the pia arachnoid around the neurohypophysis, which develops into a thin membrane of connective tissue that is continuous with the pituitary capsule as pituitary development continues. In 7-week-old embryos, the neurohypophysis begins to be wrapped by the enlarged adenohypophysis and also by the outer mesenchymal cells, which start to form a capsular structure. At 12 weeks, embryonic mesenchymal cells around the pituitary are fully differentiated to form a prominent pituitary capsule that is common to the adeno- and neurohypophysis.

In cases of congenital absence of the adenohypophysis,

FIG. 3. Coronal sections of a 30-week-old fetus. **A:** Section cut through the pituitary stalk. The septation is magnified (red square) in panel B. **B:** The membranous structure (yellow arrows) also separated the pituitary stalk from the adenohypophysis. **C:** Section cut through the intermediate lobe. The corresponding area of interest (red square) is magnified in panel D. **D:** The magnified image shows that the septation (yellow arrows) enveloped the neurohypophysis, which separated it and the intermediate lobe. Picro-Sirius red stain. Pit. stalk = pituitary stalk.

the remaining neurohypophysis in the pituitary fossa was still entirely surrounded by the pia arachnoid membrane, as reported by Brewer.¹ In contrast, Chi and Lee² reported a case of anencephaly. In a somewhat shallow sella tur-

cica, a dark tan excrescence was surrounded by a fibrous capsule that consisted entirely of adenohypophysis without a neural component. This capsule was clearly distinguishable from the cerebral pia arachnoid. The published

FIG. 4. Sagittal sections of adult specimens. **A:** Gross view of the sellar region. The septation extended along the full length of the pituitary stalk from the suprasellar pars tuberalis to the intrasellar fossa, which was thicker and more significant than was observed in fetal sections. The corresponding areas of interest (red squares) are magnified in panels B, C, and D. **B:** The upper segment of the septation (yellow arrows) was close to the pars tuberalis and seemed to be fused with the arachnoid trabeculus (Arach.). **C:** The median segment of septation was located behind the intermediate lobe and separate from the neurohypophysis (yellow arrows). The intermediate lobe was composed of degenerated Rathke's pouch. No significant membranous septation was identified between the adenohypophysis and intermediate lobe. **D:** The lower segment of septation fused with the pituitary capsule at the sellar floor (yellow arrows). Picro-Sirius red stain. See scale bar in panel B; panels C and D have the same magnification.

TABLE 1. Thickness of the septation (μm) between the adeno- and neurohypophysis in cadaveric specimens*

Location	Fetal Specimens†			Adult Specimens‡		
	Mean $\pm$ SD	Range	No.	Mean $\pm$ SD	Range	No.
Upper segment	14.1 $\pm$ 3.8§	8.0–24.0	78	62.3 $\pm$ 20.5¶	25.4–123.5	65
Median segment	41.0 $\pm$ 17.7**	11.2–82.0	70	114.9 $\pm$ 51.5††	54.3–261.6	50
Lower segment	11.3 $\pm$ 2.9	5.6–17.8	70	60.3 $\pm$ 25.8	29.9–133.9	50
Average	21.9 $\pm$ 16.9‡‡	5.6–82.0	218	79.1 $\pm$ 43.2	25.4–261.6	165

* Values in the "No." columns represent the total sum of the selected measuring points of each membrane.

† Between the 3 segments of fetus, $F = 177.287$, $p < 0.001$.

‡ Between the 3 segments of adults, $F = 39.559$, $p < 0.001$.

§ In fetal specimens, $p = 0.107$ versus the lower segment.

¶ In adult specimens, $p = 0.77$ versus the lower segment.

** In fetal specimens, $p < 0.001$ versus the other 2 segments.

†† In adult specimens, $p < 0.001$ versus the other 2 segments.

‡‡ In fetal specimens, $F = 315.236$, $p < 0.001$ versus the adult specimens.

histological evidence supports the existence of a membranous septation, which might histologically develop from the endomeninx (primitive pia arachnoid) surrounding the ventral diverticulum of the diencephalon (neurohypophysis).

Growth Patterns and Surgery for Id-CP

According to the pioneering histological findings published by Erdheim,⁴ it was hypothesized that CPs arose from the epithelial cell nests of an incompletely involutioned hypophyseal-pharyngeal duct, which are mainly found in

2 areas: 1) at the anterior-superior surface of the junction of the pituitary gland and the stalk, and 2) at the interface between the pars tuberalis and the infundibulum. The development of CPs from these 2 nests would give rise to infrasellar/subdiaphragmatic CPs and infundibulotuberal CPs, respectively. This preferential location for subdiaphragmatic CPs could account for lesion expansion in the area where the stalk penetrates through the diaphragm and the gross distortion/infiltration of the stalk, making safe dissection of this structure impossible.¹¹

In our study, a septation (the ANHS) was identified be-

FIG. 5. Surgical procedures to resect an Id-CP. **A:** Pre-surgical MRI revealed an intra- and suprasellar Id-CP that occupied the anterior part of the third ventricle and caused minor hydrocephalus. **B:** Via the frontobasal interhemispheric approach, the tumor covered by the diaphragm was exposed, and a layer of basal membranous arachnoid (black arrow) covered the diaphragm. **C:** Both optic nerves were bluntly dissected from the tumor because of the diaphragm and arachnoid separation. **D:** At the top of the tumor, the pituitary stalk was inflated. The dotted lines with scissors indicate the round incision used to open the diaphragm. **E:** After exposure at the dorsum sellae, the tumor was found to be completely covered by the diaphragm. The Liliequist membrane could also be identified. **F:** After the round incision of the diaphragm (scissors and arrows) was made, the tumor was observed. It was not difficult to bluntly separate the tumor from the neurohypophysis with a microdissector. The surface of the neurohypophysis was smooth, and a membranous septation appeared to cover it. **G:** The incision (white arrows) also revealed the smooth neurohypophysis bilaterally. **H:** The neurohypophysis was preserved after tumor removal. The anterior sphenoidal planum and posterior Liliequist membrane were also observed. Dia. = diaphragm; Lilie. = Liliequist; O.N. = optic nerve; P.S. = pituitary stalk; S.P. = sphenoidal planum; T. = tumor.

FIG. 6. Intraoperative Id-CP findings. In Case 1, presurgical MRI studies (**A1 and 2**) revealed an intra- and suprasellar cystic lesion with enhanced cystic walls. On coronal MRI, the pituitary stalk was identified at the left side of the tumor as indicated by the *red arrow*. After tumor removal via a right frontal-temporal approach (**B**), the pituitary stalk and neurohypophysis were preserved and were covered by a smooth membranous structure. The postsurgical MRI studies (**C1 and 2**) confirmed that the tumor was totally removed. The *green arrow* indicates the preserved pituitary stalk. In Case 2, a cystic lesion with a significant “snowman” sign was located in the intra- and suprasellar region (**D1 and 2**), which indicated that part of the tumor was protruding through the enlarged Pacchionian foramen. No pituitary stalk could be identified in either the sagittal or coronal images. The frontobasal interhemispheric approach was selected. During intrasellar tumor removal (**E**), the septation was used as an interface to free the tumor from the neurohypophysis. Because the septation was fused to the pituitary capsule, the medial wall of the cavernous sinus was opened during tumor removal. Gelfoam (*black arrows*) was used to stop the venous bleeding. Postsurgical MRI studies (**F1 and 2**) confirmed that the tumor was totally removed. Dors. sell. = dorsum sellae; Plan. sphen. = planum sphenoidale.

hind the intermediate lobe and provides an interface cleavage between the adeno- and neurohypophysis. Moreover, the median segment of the ANHS close to the neurohypophysis was significantly thicker than the upper segment near the stalk ($p < 0.001$, Table 1). As indicated in Fig. 7, the extreme thinness of the upper segment might allow the greatest possibility for Id-CP intrusion into the distal part of stalk, causing umbrella-shaped inflation due to chronic pressure, which occurred in a majority of Id-CP cases (25 of 28 [89.3%]). Unfortunately, the pituitary stalk could be preserved in only 3 patients with Id-CP. The thinner upper portion of the ANHS might not act as an effective anatomical barrier to protect the stalk. Interestingly, the median part of the septation was always thicker, and this might explain why no Id-CPs were found protruding into the neurohypophysis in published clinical descriptions. During surgery for the present Id-CP cases, the neurohypophysis was preserved with a relatively smooth surface. In contrast, the adenohypophysis is difficult to preserve because there is no membranous septation between it and the intermediate lobe.

On the other hand, the different thicknesses between fetuses and adults may underlie the different occurrence

rates of Id-CP in children and adults. As we previously reported,¹² Id-CP is more common in pediatric patients (34 of 81 [42%]) than in adults (8 of 114 [7%]). However, the suprasellar extraventricular type of CP was much more frequent in adult (32 of 114 cases [28.1%]) than in pediatric cases (3 of 81 cases [3.7%]). The membranous septation might be an important reason for these differences. The average thickness of the ANHS in adults was almost 4 times thicker than in fetuses ($79.1 \pm 43.2 \mu\text{m}$ and $21.9 \pm 16.9 \mu\text{m}$, respectively). Because the origin of CP is at the anterior-superior surface of the junction of the pituitary gland and the stalk, the thicker and stronger septation might protect the stalk from being affected by CP in adults. The other possible reason is the inclination angle of the stalk. In our previous publication,⁸ MR images obtained in 157 children and 323 adults who had normal anatomical locations for the optic chiasm and pituitary stalk were analyzed. We found that children had a larger inclination angle than adults (65.87° vs 56.36° , $p = 0.012$), which indicated that the stalk in children is more vertically oriented than in adults. Moreover, adults had a greater percentage of postfixed stalks (in other words, pituitary stalks located close to the dorsum sellae; 43.7% vs 24.8%,

FIG. 7. Schematic drawings depicting the growth pattern of Id-CP. **A:** Normal sellar region and the ANHS structure. **B1–3:** The growth process of Id-CP in the sagittal position. The thinnest upper segment of the ANHS might minimize the protective function of this septation for the pituitary stalk. The stalk appears as an umbrella-shaped structure. **C:** In the coronal view, some Id-CP tumors (**C1**) displace the stalk laterally, which allows the continuity of the pituitary stalk and hypothalamic-pituitary gland to be preserved after tumor removal. However, in other cases (**C2**), the stalk cannot be preserved even after identification because of severe inflation and invasion. Copyright Yuntao Lu. Published with permission.

$p < 0.001$), whereas centered stalks (63.1% vs 48.3%, $p = 0.002$) were more prevalent in children. Additionally, the pituitary stalk is more frequently squeezed and deformed by the dorsum sellae in adults than in children (15.8% vs 7.6%, respectively; $p < 0.001$). For the 2 reasons mentioned above, tumors in adults might more easily grow toward the subarachnoid cavity instead of inflating and infiltrating the stalk.

With regard to pediatric Id-CP surgery, many publications^{3,5,7} agree that the recurrence of Id-CP would be mostly from residual intrasellar tumor. In this study, our techniques of gross-total resection of this part of the tumor are proposed based on the intrasellar membranous structures. The suprasellar tumor can be freed more easily because it is covered by a complete or incomplete diaphragm and basal membranous arachnoid.^{12,13} The diaphragm can be dissected using a round incision to expose the tumor capsule (Fig. 5). At the dorsum sellae, the septation interface needs to be carefully observed. By following the septation, the intrasellar tumor can be separated from the neurohypophysis.

Conclusions

A membranous septation (the ANHS) was identified between the adeno- and neurohypophysis, and behind the intermediate lobe and Rathke's pouch. The septation extends from the pars tuberalis to the sellar floor. It is likely that the septation develops from the endomeninx (primitive pia arachnoid) surrounding the ventral diverticulum of the diencephalon (neurohypophysis). This septation can

serve as a potential surgical cleavage target when removing the intrasellar portion of an Id-CP. Understanding the morphological features of this septation is important for optimizing total tumor resection and identifying and protecting the neurohypophysis and pituitary stalk.

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Conception and design: Qi. Acquisition of data: Lu, Xu, Pan. Analysis and interpretation of data: Lu. Drafting the article: Lu. Critically revising the article: Lu. Reviewed submitted version of manuscript: Lu, Pan. Statistical analysis: Lu. Administrative/technical/material support: Lu, Xu, Shi. Study supervision: Lu, Pan.

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