Investigation of the Presence of Arachnoid Granulation in Fetuses and Early Infancy

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Abstract

The aim of our study is to investigate the importance of arachnoid granulation in cerebrospinal fluid physiology in fetuses and early infancy. Using the random sampling method, postmortem fetuses more than 26 weeks of gestation age and the children under the age of 1 year were chosen from the autopsy materials. Two male and two female intrauterine dead fetus; three male and three female, totally six children under the age of 1 year and one 3-year-old male were included in this study. In cases of intrauterine fetuses more than 26 weeks of gestation and children under the age of 1 year, complete invagination of arachnoid villi into the superior sagittal sinus was examined histologically. In the intrauterine period and in the first 6 months of life, arachnoid villi structures were not found in histologic preparations although in preparations taken after the 6 months of life samples showed similarities to arachnoid granulations. These structures were considered as arachnoid villi drafts after immunohistochemical analysis. In the control case who were 3 years old, maturation of arachnoid villi was complete and the arachnoid villi were invaginated into the superior sagittal sinus as fingerlike extensions. In our study, we think that the failure after E3V intervention in the treatment of hydrocephalus in cases under the age of 1 years may be related to the completion of arachnoid granulation development after the 18th month of life and the immature resorption capacity in this period.

Keywords

- cerebrospinal fluid
- arachnoid granulation
- dural wall
- anlage

Introduction

Approximately, 60% of the human body is water.[1] Water is found in intracellular, interstitial, extracellular, or intravascular compartments and as cerebral spinal fluid (CSF) in the brain. It wraps around the cerebrum and the spinal cord spreads in it and flows around it. CSF is the major component of this system.

The cerebral fluid system exhibits a dynamic structure, and intracranial fluids are preserved in a very delicate balance in separate compartments. This delicate balance is of great importance for the continuation of optimal neuronal function. Understanding the dynamics related to the production, circulation, and absorption of CSF gives an important insight into the pathophysiology of CSF-related diseases.[2] It is an important preliminary step to plan and implement treatment strategies for related diseases. In recent years, arachnoid granulations, which were classically known to play a critical role in CSF absorption, are under the spotlight regarding the importance of cerebral absorption of lymphatic drainage.[3] In the present study, we aimed to investigate the histopathological presence of arachnoid granulations, which are the major components of CSF absorption, in fetuses and cases under 1 year of age.

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Table 1 Cases general characteristics

Case	Gender	Age	Cause of death
1	M	38-mo old child	Car accident
2	F	32-wk fetus	Ablation placenta
3	F	28-wk fetus	Ablation placenta
4	M	34-wk fetus	IUGR
5	M	5-mo old child	Sudden infant death syndrome
6	M	36-wk fetus	IUGR
7	M	8-mo old child	Sudden infant death syndrome
8	F	1-mo old child	Respiratory system disease
9	F	10-mo old child	Respiratory system disease
10	F	7-mo old child	Respiratory system disease
11	M	4-mo old child	Dehydration

Abbreviations: IUGR, intrauterine growth retardation.

Materials and Methods

The present study was discussed and approved at the meeting between the Ege University Faculty of Medicine Ethics Committee for Clinical Research and T.C. Ministry of Justice Forensic Medicine Institute.

The cases were randomly sampled from autopsy materials of fetuses over 26 weeks and children under 1 year of age brought to the Macroscopic Laboratory of Ege University Faculty of Medicine, Department of Pathology, and the Autopsy Hall of Morgue Department of Forensic Medicine Council in Izmir for autopsy purposes. Autopsies were performed within 24 hours of death.

The study included: four intrauterine fetal death cases over 26 weeks (two male and two female), six autopsy cases under 1 year of age (three boys and three girls), and one boy at 3 years of age for the control case (►**Table 1**). Autopsy cases with a known pathology of the central nervous system were excluded from the study. Autopsies were performed within the first 24 hours of death. While the samples were taken for the study, an interauricular line was determined to pass through the vertex in the frontal direction. Calvarium was exposed through an interauricular incision line until the supraorbital region in the anterior and the inion in the posterior. A vibrating dissection saw (Electronic Power Gipssage, Germany) was used in cases younger than 1 year of age, and surgical scissors (Metzenbaum, Medikon, England) were used in postmortem fetuses >24 weeks. Since the calvarium and dura are agglutinated especially in fetuses, the vertex dura was removed together with the bone up to the confluens sinuum in connection with the superior sagittal sinus, following the observation of the calvarium from the anterior of the superior sagittal sinus on the midline. Samples were placed and preserved in a 10% formalin solution. The materials, which were kept in formalin for an average of 30 days, were placed on the cassettes in serial sections from the frontal toward the caudal at 1-mm intervals. In nine samples, the dura was preserved in a 4% acid solution before tissue monitoring since it could not be separated from the bone. After 3 days of acid treatment, the materials were monitored on a routine tissue tracking device in serial sections. After 16 hours, the samples were blocked by embedding in paraffin. The blocks were cut at a 4-µm thickness on a microtome and stained with hematoxylin and eosin (►**Fig. 1**).

In histological examination, all sections were evaluated for the presence of arachnoid granulation under the microscope. Epithelial membrane antigen (EMA) was applied immunohistochemically to three cases with suspicious structures, which were considered to be the outlines of arachnoid granulation, as well as to the control. Five-micron thick sections obtained from a paraffin block with EMA-suspicious structures were placed on electrostatically charged slides (X-traTM, Surgipath Medical Industries, Richmond, IL, United States) and dried at 50°C for 2 hours. Primary antibody Anti-

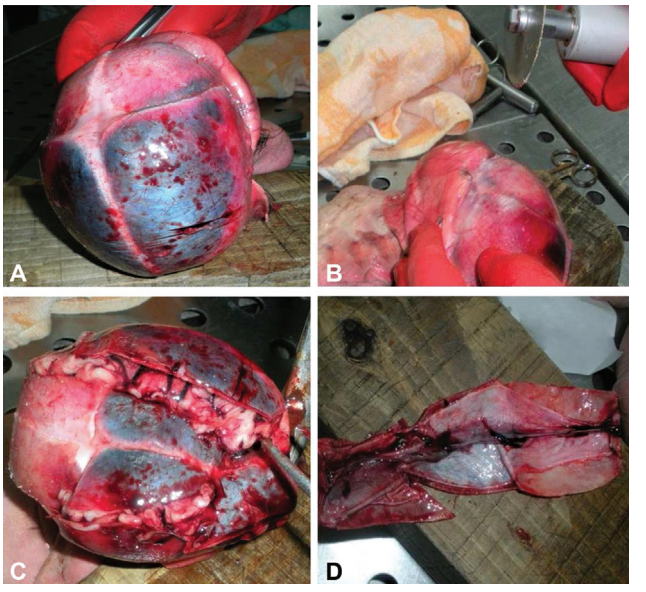

Fig. 1 (**A**) Calvarium view after scalp detachment in a patient who was taken to the autopsy table. (**B**) Removal of the calvarium with a dermatome. (**C-D**) Removal of the superior sagittal sinus with dura and calvarium.

Table 2 Immunohistochemistry and AG presence table

Case number	Gender	Age	EMA	AG
1	M	38-mo old child	Implemented	(+)
2	F	32-wk fetus	Not implemented	None
3	F	28-wk fetus	Not implemented	None
4	M	34-wk fetus	Not implemented	None
5	M	5-mo old child	Not implemented	None
6	M	36-wk fetus	Not implemented	None
7	M	8-mo old child	Implemented	Outline of AG
8	F	1-mo old child	Not implemented	None
9	F	10-mo old child	Implemented	Outline of AG
10	F	7-mo old child	Implemented	Outline of AG
11	M	4-mo old child	Not implemented	None

Abbreviations: AG, arachnoid granulation; EMA, epithelial membrane antigen.

EMA (clone EMA, Dako Cytomation, 1:100 dilution, catalog no: M3619) was dripped manually. Following incubation for 32 minutes at 37°C, the sections were manually cross-stained with hematoxylin and eosin and bluing solution, dehydrated, cleared in xylene, and placed on the slices.

Results

Autopsy materials were taken from cadavers, six of which were boys and five girls. The mean fetus age was 32 weeks, while the mean age in cases under 1 year of age was 5.8 months. One 3-year-old case was used as a control case for histological imaging. The causes of death were as follows: ablatio placenta in two cases, intrauterine developmental retardation in two cases, sudden infant death syndrome in two cases, respiratory disease in three cases, secondary dehydration in one case, and car accident in one case.

In histological examination, we observed arachnoid granulations forming papillary protrusions into the sinus, increased cellularity, and immunohistochemical EMA positivity only in control case 1. In cases numbered 2, 3, 4, 5, 6, 8, and 11, and infantile cases under 6 months, no structure was found to be stained in the light microscope, consistent with the histology of arachnoid granulation (►**Table 2**). In cases older than 6 months with numbers 7, 9, and 10, small papillary structures were observed with an immature appearance, which we thought was an anlage of arachnoid granulation. However, in the immune histochemical examination, these immature structures demonstrated negative results for EMA staining (►**Fig. 2**). Consistent with the literature, no male–female difference was observed among the cases.

The presence of EMA negativity in these structures, which were considered arachnoid granulation outlines due to their morphological similarity, was associated with incomplete maturation. Although these outlines were morphologically similar to the structures of arachnoid granulation, no definitive assessment of whether they function was made.

Discussion

Since the 16th century, studies have been performed by various researchers in different science institutions around the world, from clinicians such as Cushing and Dandy to physiologists such as Weed, to understand the physiology of CSF circulation and absorption.[4] Understanding and resolving this delicate balance is vital for establishing pathophysiology and treatment models of CSF-related diseases.

Arachnoid granulation is classically thought of as the main component of CSF reabsorption. Vesalius and Willis are known for their studies conducted in the 16th and 17th centuries to establish the structure of arachnoid granulations. However, in 1721, Pacchioni was the first to describe his views on the sectarian roles of lacuna around the superior

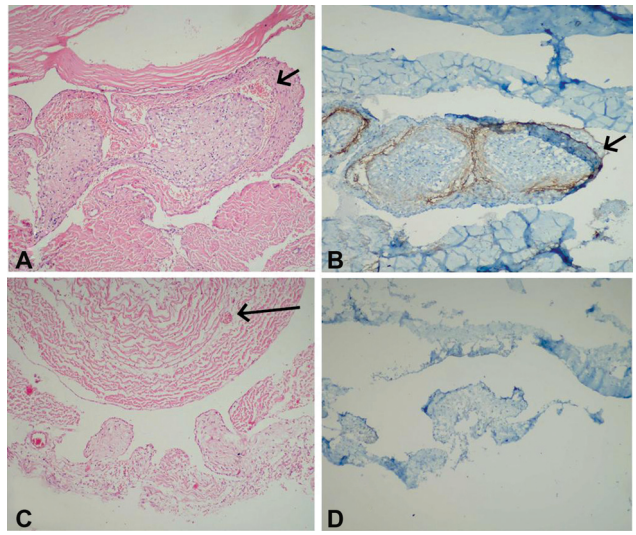

Fig. 2 (**A**) Positive control HxE, x100 staining of case 1 (*arrow indicates arachnoid granulation*). (**B**) EMA positive picture of case 1 EMAx100 (*arrow indicates arachnoid granulation*). (**C**) Arachnoid granulation anlage HEx100 staining of case 9 (*arrow pointing to the outline*). (**D**) EMA negative picture of case 9, EMAx100 staining. EMA, epithelial membrane antigen.

sagittal sinus in dissection studies. In the late 19th century, Luschka mentioned that the arachnoid structures were invaginated with the superior sagittal sinus.[5] Quincke injected sulfide (red mercury) into the CSF in animal models and showed that the material distributed around the superior sagittal sinus under the microscope, mentioning that the villi herein play a role in CSF circulation. In 1875, Key and Retzius demonstrated the role of choroid plexus in CSF drainage and investigated the infusion of gelatin in human cadaver samples in which they showed that this substance entered villous structures and then switched to lateral lacunae, passing through venous sinuses and CSF circulating back into the blood. They also found stained substance in cervical lymph nodes.[6] In 1901, Cushing mentioned one-way absorption of arachnoid projections of CSF, and then, in 1914, Weed conducted further studies on this subject, reporting that these structures represented a semipermeable blind diverticulum interspersed between the venous blood in the cerebral sinuses and the CSF in the subarachnoid space.[7,8]

The revision of the old information about CSF flow was first started with the studies by Welch et al. Welch and Friedman first focused on Cushing's work on arachnoid villi and ultimately found that the statements were valid. Perfusion studies on cranial arachnoid villus in the green monkey form the basis of these studies. Their experiments demonstrated that a kind of balance allows the passage of cerebrospinal fluid as well as some proteins and particles from the arachnoid villi to the cranial sinuses.[9] In the classical imaging methods, arachnoid granulation is usually seen in humans after the first 12 months of life, while it is not observed in earlier infants and animal models.[7,10] Similar to our histological study, Gomez et al evaluated 27 human fetuses and newborns by removing the superior sagittal sinus and confluens sinuum in the postmenstrual 26 to 54 weeks period. However, unlike our study, they evaluated electron microscopy images, not light microscopy. In this study, they observed oval depressed areas on the sinus wall in 26-week fetuses and reported that arachnoid tissue was histologically clustered on the dural wall. They reported that the recesses in the wall became more irregular at week 30 and that arachnoid granulations were observed under the electron microscope at week 39.[11]

In our study, papillary protrusions into the sinuses, increased cellularity, and arachnoid granulation structures showing EMA positivity were monitored immunohistochemically in only one control case. No structure was found to be stained in the light microscope compatible with the histology of arachnoid granulation in intrauterine and infantile cases under 6 months. Small papillary structures with an immature appearance, which were thought to be arachnoid granulation outlines, were observed in cases older than 6 months. However, in the immune histochemical examination, these immature structures demonstrated negative results for EMA staining. The presence of EMA negativity in these structures, which were considered arachnoid granulation outlines due to their morphological similarity, was associated with incomplete maturation.

In the literature, there are a limited number of studies mentioning the morphology of human arachnoid villi and granulations. In the studies conducted by Le Gros, Ferner, and Thomas at different times, as in our study, it was mentioned that arachnoid cell clusters that can be observed in the early fetus will be the precursors of arachnoid villi and granulations that will form in the later stages of life. In our study, no male–female difference was found between the cases, which was consistent with the literature. Available studies on this subject are generally related to the size or location of arachnoid granulations. Our study aimed to evaluate arachnoid villi, which are of great importance for CSF absorption and are considered to have completed their morphological maturation. Arachnoid villi were observed to be structures completing maturation and forming finger-like projections into the superior sagittal sinus in our 36-month postnatal control case. Available images of arachnoid villi and granulation during fetal and early infancy in the literature may show parallelism with the structures that we considered to be arachnoid granulation patterns. However, we think that granulation with the function of functional absorption within the first postnatal year is out of the question.

In the treatment of hydrocephalus, ventriculoperitoneal (VP) shunt insertion is associated with a high failure rate and many complications in early infancy and premature infants, as in every period of life.[12,13] In the light of studies on hydrocephalus, E3V (endoscopic third ventriculostomy) is a good alternative in the treatment of obstructive, noncommunicative hydrocephalus, especially in cases of aqueduct stenosis. However, E3V is still a controversial treatment option in young children, especially infants younger than 6 months. In their study with 23 patients in 2009, Ogiwara et al mentioned that age is an important prognostic factor for E3V, but hydrocephalus etiology bears more significance when planning surgery.[14] In 2006, DiRocco compared shunt surgery and E3V, reporting that there was no difference between these two surgical procedures in terms of complications.[15]

In 2004, Koch and Wagner mentioned that CSF reabsorption disorders are one of the minor effective causes of E3V failure.[16] Available studies report varying rates of success in hydrocephalus treatment in patients under 1 year of age. Although these varying rates are mostly associated with the etiology of hydrocephalus, CSF resorption disorder is mentioned in a limited number of studies. Publications are mentioning that the success of E3V is low following diseases that impair CSF absorption in adult cases, such as a former history of meningitis or hemorrhage.[17] After the evaluation of our study, we think that this failure in E3V treatment may be due to the incomplete maturation of arachnoid granulations.

Conclusion

In line with the results of our study, we think that the failure after E3V intervention in the treatment of hydrocephalus in cases under the age of 1 years may be related to the

completion of arachnoid granulation development after the 18th month of life and the immature resorption capacity in this period. We would like to emphasize that, in prospective studies with larger series, it may be useful to evaluate the age component as an important parameter as well as the etiology of the disease in the treatment planning for hydrocephalus.

Conflict of Interest

None declared.

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