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Original Article

Aquaporin-9 as a biomarker for hydrocephalus: Insights from experimental rat models

Catur Kusumo[1], Muhammad Arifin Parenrengi[2], Wihasto Suryaningtyas[1], Achmad Fahmi[3], Budi Utomo[4], I Ketut Sudiana[5]

[1]Department of Neurosurgery, Airlangga University/Dr. Soetomo General Academic Hospital, [2]Department of Neurosurgery, Dr. Soetomo General Academic Hospital, Departments of [3]Neurosurgery, [4]Public Health, [5]Anatomical Pathology, Faculty of Medicine, Universitas Airlangga-Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

E-mail: *Catur Kusumo - kusumocatur49@gmail.com; Muhammad Arifin Parenrengi - muhammad.arifin@fk.unair.ac.id; Wihasto Suryaningtyas - wihasto-s@fk.unair.ac.id; Achmad Fahmi - achmadfahmibaabud@yahoo.com; Budi Utomo - budiutomo@fk.unair.ac.id; I Ketut Sudiana - ik.sudiana@yahoo.com

***Corresponding author:**
Catur Kusumo,
Department of Neurosurgery,
Airlangga University/
Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

kusumocatur49@gmail.com

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ABSTRACT

Background: Hydrocephalus, characterized by ventricular enlargement often associated with elevated intracranial pressure, necessitates reliable biomarkers for accurate diagnosis. Aquaporin-9 (AQP-9), localized at the interface of cerebrospinal fluid (CSF) spaces and blood vessels, plays a critical role in brain water homeostasis but remains underexplored in the context of hydrocephalus. Further investigation into AQP-9 expression in CSF is essential to elucidate its potential as a diagnostic biomarker and its role in hydrocephalus pathophysiology.

Methods: This experimental study utilized 10–12-week-old Sprague–Dawley rats (*Rattus norvegicus*) weighing 200–250 g, randomly assigned to three groups. CSF served as the primary unit of analysis. AQP-9 levels were quantified using the enzyme-linked immunosorbent assay Sandwich method, with CSF sampling conducted at 7-day intervals over 21 days.

Results: AQP-9 levels were significantly elevated in hydrocephalic mice compared to controls, with the highest levels on day 21 (887.62 ± 88.72). CSF drainage resulted in a notable reduction in AQP-9 levels at all time points. Statistical analysis confirmed significant differences across groups ($P < 0.05$), with *post hoc* tests showing meaningful reductions in AQP-9 levels after drainage compared to hydrocephalic states. These findings suggest AQP-9 as a potential biomarker for hydrocephalus diagnosis and monitoring therapeutic response.

Conclusion: AQP-9 shows promise as a biomarker for hydrocephalus, with levels reflecting disease progression and decreasing after CSF drainage. This highlights its potential for diagnosis and therapeutic monitoring, warranting further validation.

Keywords: Aquaporin-9, Biomarker, Cerebrospinal fluid drainage, Hydrocephalus, Ventricular cerebrospinal fluid

INTRODUCTION

Hydrocephalus is characterized by ventricular enlargement, often accompanied by elevated intracranial pressure (ICP) due to disruptions in cerebrospinal fluid (CSF) formation, flow, or absorption. Key sites for CSF flow obstruction include the foramen of Monro, the aqueduct of Sylvius, the fourth ventricle, and the arachnoid villi. CSF plays a crucial role in maintaining brain homeostasis, acting as a protective barrier, distributing nutrients, supporting inter-neuronal connections, and removing metabolic waste.[7,8,9]

Aquaporins (AQPs), particularly AQP-9, have emerged as potential biomarkers for hydrocephalus diagnosis and management. While AQPs have been studied in conditions such as idiopathic normal pressure hydrocephalus and shunt malfunctions, the specific role of AQP-9, a water channel protein involved in cerebral fluid regulation, remains underexplored.[3,5,6,13]

This study investigates AQP-9 expression in a Sprague–Dawley rat model of kaolin-induced hydrocephalus, comparing levels in ventricular CSF before and after CSF drainage. By elucidating AQP-9's role in hydrocephalus pathophysiology, this research aims to contribute to the development of targeted therapeutic strategies for improved patient outcomes.

MATERIALS AND METHODS

This experimental study was conducted at the Biosciences and Biomolecular Laboratory, Brawijaya University, Malang, and Veterinary Department Airlangga University in 2021. The study utilized 10–12-week-old Sprague–Dawley rats (*Rattus norvegicus*) weighing 200–250 g, with no prior interventions. The independent variables were hydrocephalus status and observation timeline, while AQP-9 levels in CSF served as the dependent variable.

Three groups were established through simple randomization, each comprising 12 rats. Measurement of rat CSF levels was performed intermittently. K0 (control) – normal rats with measurements on day 0; K1 (hydrocephalus-induced) – measurements on days 7, 14, and 21; and K2 (hydrocephalus-induced with CSF drainage) – measurements on the same intervals. The rats were acclimatized for 7 days before procedures under standardized conditions.

Hydrocephalus was induced by injecting 20–30 µL of 20% sterile kaolin suspension into the cisterna magna under anesthesia. CSF drainage was performed via a 5Fr NGT catheter implanted at the anterior bregma and secured for drainage access. AQP-9 levels were quantified using enzyme-linked immunosorbent assay (ELISA) with the Sandwich method. This research reagent uses Cloud-clone SEA578Hu ELISA Kit for AQP-9.

Statistical analyses included descriptive and hypothesis testing using the Statistical Package for the Social Sciences software. Normality was assessed with the Kolmogorov–Smirnov test. Group comparisons were conducted using one-way analysis of variance (ANOVA), and *post hoc* analyses employed the least significant difference test. Results with $P < 0.05$ were considered statistically significant.

RESULTS

The highest AQP-9 levels were observed in hydrocephalic mice on day 21 (887.62 ± 88.72), while the lowest levels were in the control group (92.36 ± 13.19) [Table 1]. ANOVA analysis demonstrated a significant difference in AQP-9 levels across groups ($P < 0.05$). *Post hoc* analysis revealed a significant increase in AQP-9 levels in hydrocephalic mice compared to controls on days 7 ($P = 0.004$), 14 ($P = 0.000$), and 21 ($P = 0.000$) [Table 2].

After CSF drainage, AQP-9 levels significantly decreased compared to hydrocephalic mice. ANOVA analysis confirmed significant differences across groups ($P < 0.05$), as shown in Table 3. *Post hoc* analysis, as presented in Table 4, revealed significant reductions in AQP-9 levels in the 7-day ($P = 0.000$), 14-day ($P = 0.000$), and 21-day ($P = 0.000$) drainage groups compared to the hydrocephalic groups. These results emphasize the dynamic modulation of AQP-9 expression in hydrocephalus and its marked decline following CSF drainage, reinforcing its potential utility as a

Table 1: AQP-9 expression levels in the CSF of control and comparison groups, hydrocephalic rats, and rats after CSF drainage.

No.	Research groups	Mean±Standard Deviation
1	Control	92.36±13.19
2	Hydrocephalus D-7	252.87±38.14
3	Hydrocephalus D-14	633.87±100.96
4	Hydrocephalus D-21	887.62±88.72
5	Drainage D-7	160.36±35.36
6	Drainage D-14	176.63±25.00
7	Drainage D-21	220.60±282.87
	Average Total	346.33±282.87

AQP-9: Aquaporin-9, CSF: Cerebrospinal fluid

Table 2: Comparison of AQP-9 levels in the control group versus the hydrocephalus group.

Group	Comparison	Mean difference	Standard error	*P*-value
Control	H-7	−160,515	40.513	0.004*
Control	H-14	−541,515	40.513	0.000*
Control	H-21	−795,258	40.513	0.000*

AQP-9: Aquaporin-9, *: Indicates the significance of the p value

Table 3: Comparison of AQP-9 levels in the control group versus post-CSF drainage.

Group	Comparison	Mean difference	Standard error	*P*-value
Control	Drainage D-7	−68.000	15.632	0.023
Control	Drainage D-14	−84.273	15.632	0.000
Control	Drainage D-21	−1287.242	15.632	0.000

AQP-9: Aquaporin-9, CSF: Cerebrospinal fluid

Table 4: Comparison of AQP-9 levels in the hydrocephalus group versus post-CSF drainage.

Group	Comparison	Mean difference	Standard error	*P*-value
H-7	Drainage D-7	92.515	69.801	0.023*
H-14	Drainage D-14	457.242	102.014	0.000*
H-21	Drainage D-21	667.015	105.410	0.000*

AQP-9: Aquaporin-9, CSF: Cerebrospinal fluid, *: Indicates the significance of the p value

biomarker for tracking disease progression and evaluating therapeutic interventions.

DISCUSSION

AQP-9, hydrocephalus, and animal models

AQP-9, a member of the AQP family, plays a critical role in the transport of water and small molecules such as glycerol, urea, and lactate across cell membranes. Its unique ability to facilitate the movement of these metabolites is integral to energy metabolism and fluid homeostasis.[10,11] While the involvement of AQPs such as AQP-1 and AQP-4 in hydrocephalus has been well-documented,[14] evidence regarding AQP-9's role remains limited. A 2020 systematic review identified only one experimental study assessing AQP-9 expression in hydrocephalus models,[3] underscoring the need for further research in this area.

Rodent models, such as those using Sprague–Dawley rats, are frequently employed to investigate hydrocephalus pathophysiology due to their anatomical similarity to humans, particularly regarding ventricular structures and CSF pathways.[4] Techniques such as kaolin injection into the cisterna magna effectively induce hydrocephalus in these models, enabling analysis of AQP expression changes. Studies have demonstrated compensatory upregulation of AQPs like AQP-4 in the hippocampus and parietal cortex under these conditions, suggesting a role in mitigating fluid accumulation while potentially contributing to hydrocephalus progression.[1,12]

Expression levels of AQP-9 in hydrocephalus

This study revealed a significant increase in AQP-9 expression in the CSF of hydrocephalus-induced mice compared to controls, with the highest levels observed in mice with 21 days of hydrocephalus (mean: 887.62 ± 88.72 vs. 92.36 ± 13.19 in controls). *Post hoc* analysis confirmed significant differences in expression between days 7, 14, and 21 ($P = 0.000$), indicating a progressive increase with prolonged hydrocephalus. Elevated AQP-9 likely reflects cellular adaptations to ICP and fluid accumulation. AQP-9's ability to transport metabolites such as lactate and glycerol may also contribute to metabolic stress during hydrocephalus, particularly under hypoxic conditions.[2]

Effects of CSF drainage on AQP-9 expression

CSF drainage effectively reduced AQP-9 expression, particularly in mice with 21-day hydrocephalus, though levels remained elevated compared to controls. This suggests that drainage mitigates fluid accumulation but does not immediately restore AQP-9 expression to baseline. *Post hoc* analysis demonstrated statistically significant reductions across all time points, highlighting the protective effects of drainage in alleviating fluid overload and improving physiological conditions. These findings emphasize the role of AQP-9 in hydrocephalus and its response to therapeutic interventions.[1]

Implications and limitations

The relatively low mean value in the 1st week suggests that the CSF drainage system may not yet be working optimally. This stage can be considered the initial phase of adaptation, in which the brain system is just beginning to adapt to increased ICP. Most likely, the compensatory system in hydrocephalus patients is still in the early activation stage, so drainage efficiency has not been fully achieved.

The difference in mean CSF drainage increased significantly in the 2nd week, indicating that there was a marked increase in CSF drainage capacity. This phase indicates an increase in adaptation or perhaps a clinical intervention that plays a role in improving CSF flow efficiency. Physiologically, the 2nd week may be a transitional phase, where the expression of water channel proteins such as AQP-9 may change part of the body's compensatory mechanism in the face of increased CSF volume.

A greater increase in the mean difference in CSF drainage occurred in the 3rd week, which not only showed a significant improvement from the previous period but also signaled that the CSF drainage system was in a more stable and efficient state. This condition describes the stage of optimal adaptation or clinical improvement in hydrocephalus patients. By the 3rd week, the CSF drainage system can be assumed to have reached stability, where the level of ICP has decreased significantly along with the increase in drainage ability.

Overall, the analysis showed a significant upward trend in the difference in mean CSF drainage from week 1 to week 3. This increase in CSF drainage efficiency was associated with a decrease in ICP and potential improvement in the degree of hydrocephalus. Significant "*P*" values at each period indicated that the changes occurring at each week were statistically significant as

well as indicating potential physiologic adaptation or improvement in the patient.

These results clinically underscore the importance of regular monitoring of CSF drainage to evaluate the progression of the hydrocephalus condition in patients, as well as determining appropriate interventions at each phase to optimize treatment outcomes. The improvement at week 3 suggests that the CSF drainage system has undergone better adaptation, making an important contribution to the clinical improvement of hydrocephalus patients.

The study underscores AQP-9's potential as a biomarker for hydrocephalus progression and response to CSF drainage therapy. However, limitations exist. The study did not investigate the long-term effects of CSF drainage on AQP-9 expression or elucidate the molecular mechanisms regulating AQP-9 in hydrocephalus. In addition, reliance on animal models limits direct extrapolation to human pathology, necessitating further studies to explore AQP-9's role in fluid dynamics and optimize therapeutic strategies for hydrocephalus patients.

CONCLUSION

This study identifies AQP-9 as a potential biomarker for hydrocephalus, showing significantly elevated expression in ventricular CSF of hydrocephalus-induced Sprague–Dawley rats that increase with disease duration. CSF drainage effectively reduces AQP-9 levels, though not to baseline, indicating ongoing compensatory mechanisms. The findings highlight AQP-9's diagnostic and therapeutic relevance, with further research needed to explore its molecular regulation, long-term dynamics, and clinical applicability for improved hydrocephalus management.

Ethical approval: The Institutional Review Board approved the research/study at the Animal Care and Use Committee (ACUC), Airlangga University, number 2.KE.091.07.2021, dated July 15, 2021.

Declaration of patient consent: Patient's consent is not required as there are no patients in this study.

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