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Intracranial Pressure Measurement of and Investigation of the Effect of Aquaporin on Hydrocephalus Induced by Experimental Kaolin and Autologous Blood Injection: An Animal Study

Deneysel Kaolin ve Otolog Kan Enjeksiyonu ile İndüklenen Hidrosefali Üzerine Aquaporin Etkisinin İntrakraniyal Basınç Ölçümü ve Araştırılması: Hayvan Deneyi

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ABSTRACT Objective: Hydrocephalus is a condition in which brain tissue is damaged due to ventricular enlargement. In experimental models, hydrocephalus was induced by injecting various substances into the cerebrospinal fluid pathway or creating a subarachnoid hemorrhage model. **Material and Methods:** Five experimental groups were formed. The stereotaxic frame was placed in accordance with the coordinates calculated for the cisterna magna. In Group 1, only a spinal puncture was performed. In Group 2, a hydrocephalus model was created by injecting kaolin (Group 2A) and autologous blood (Group 2B). A hydrocephalus model was created with kaolin in Group 3, autologous blood in Group 4, and acetazolamide treatment was applied to both groups post-injection. Autologous blood was taken from the experimental groups before decapitation, and the levels of tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-1 were measured by the ELISA method. After histological staining, the lateral ventricle size was measured. Intracranial pressure (ICP) measurements were taken on days 0 and 7 in all groups. **Results:** There was a significant increase in ICP in Groups 2A and 2B. TNF-α and IL-1 values increased more significantly in the groups that did not receive acetazolamide treatment compared to the group that received treatment. **Conclusion:** There was an increase in ventricle dimensions and ICP as well as TNF-α and IL-1 levels in both hydrocephalus models. Acetazolamide treatment was seen to be significantly more effective in kaolin group. This study is important because it is the first in the literature to perform biochemical and histopathological examination and ICP measurements all in the same hydrocephalus model.

Keywords: Hydrocephalus; interleukin 1; tumor necrosis factor-alpha; acetazolamide; intracranial pressure

ÖZET Amaç: Hidrosefali, ventriküler genişleme nedeniyle beyin dokusunun hasar gördüğü bir durumdur. Deneysel modellerde, beyin omurilik sıvısı yollarına çeşitli maddeler enjekte edilerek veya bir subaraknoid kanama modeli oluşturularak hidrosefali meydana getirildi. **Gereç ve Yöntemler:** Toplam 5 deney grubu oluşturuldu. Stereotaktik frame sisterna magna konumunun tespiti için kullanıldı. Grup 1'de sadece spinal ponksiyon yapıldı. Grup 2'de kaolin (Grup 2A) ve otolog kan (Grup 2B) enjekte edilerek hidrosefali modeli oluşturuldu. Grup 3'te kaolin enjeksiyonu, Grup 4'te otolog kan enjeksiyonu ile hidrosefali modeli oluşturuldu ve enjeksiyon sonrası her iki gruba da asetazolamid tedavisi uygulandı. Dekapitasyon öncesi deney gruplarından otolog arter kanı alındı ve tümör nekrozis faktör-alfa (TNF-α) ve interlökin (IL)-1 seviyeleri ELISA yöntemi ile ölçüldü. Histolojik boyamadan sonra da lateral ventrikül boyutu ölçüldü ve grup içi ortalamalar hesaplandı. Tüm gruplarda 0 ve 7. günlerde intrakraniyal basınç [intracranial pressure (ICP)] ölçümleri alındı. **Bulgular:** 2A ve 2B gruplarında ICP'de anlamlı bir artış oldu. Asetazolamid tedavisi almayan gruplarda, tedavi gören gruba göre TNF-α ve IL-1 değerleri anlamlı olarak daha arttı. **Sonuç:** Çalışmamızda oluşturulan 2 ayrı hidrosefali modelinde, TNF-α, IL-1, ICP ve ventrikül boyutlarında artış saptandı, Asetazolamid tedavisinin daha çok kaolin grubunda etkili olduğu anlaşıldı. Bu çalışma, literatürde biyokimyasal ve histopatolojik inceleme ile ICP ölçümlerini aynı hidrosefali modelinde gerçekleştiren ilk çalışma olması nedeniyle önemlidir.

Anahtar Kelimeler: Hidrosefali; interlökin-1; tümör nekrozis faktör-alfa; asetazolamid; intrakraniyal basınç

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Hydrocephalus is a condition in which brain tissue is damaged due to enlargement of the ventricles, which in turn causes neurological losses.[1] The balance between cerebrospinal fluid (CSF) formation and absorption deteriorates, and hydrocephalus develops due to pathological causes such as trauma, infection, bleeding (subarachnoid hemorrhage), tumor, and ischemia.[2] In one subarachnoid hemorrhage (SAH) model, the autologous arterial blood of the animal is injected into the cisterna magna with the help of a needle in the prone position.[3] Hydrocephalus exacerbates neurological deficits after SAH and manifests in various neurological conditions ranging from simple cognitive dysfunction to severe gait disturbance. SAH is a common cause of chronic communicating hydrocephalus encountered in the clinic and yields clinical findings associated with hydrocephalus in 20% of patients.[1] Another hydrocephalus model is the kaolin injection that Feng et al.[4]

MATERIAL AND METHODS

The study was initiated after obtaining approval from Bursa Uludağ University Animal Research Ethics Committee (HADYEK), Bursa, Türkiye (date: January 08, 2021, no: 2021-01/08). All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Our study was carried out in accordance with the principles of the Declaration of Helsinki.

In the experimental setup, 4 groups (n=7) were formed of 28 female rats of Sprague-Dawley strain weighing between 250-300 g (Table 1). General anesthesia was administered with 100% sevoflurane (250 mL solution) as part of the surgical procedures. All surgical procedures were performed by the same surgeon, and a 3.5x magnification loupe was used during the procedure.

TABLE 1: Experimental groups and study design.

Groups	
Group 1	Puncture+ICP measurement (day 0 and 7) (4 animals)
Group 2A	Kaolin injection+ICP measurement (days 0 and 7) (6 animals)
Group 2B	Autologous blood injection+ICP measurement (days 0 and 7) (6 animals)
Group 3	Kaolin injection+acetazolamide treatment+ICP measurement (day 0 and 7) (6 animals)
Group 4	Autologous blood injection+acetazolamide treatment+ICP measurement (day 0 and 7) (6 animals)

ICP: Intracranial pressure.

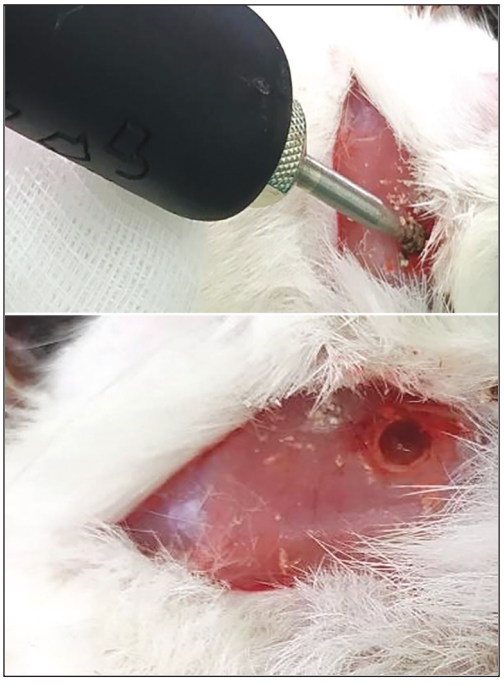

FIGURE 1: A burr hole was created on the right Kocher's point with the help of a perforator.

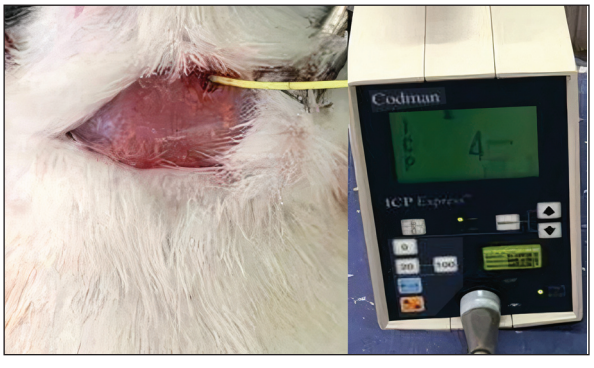

FIGURE 2: Intraparenchymal ICP monitor was connected and ICP was measured. ICP: Intracranial pressure.

Experimental Hydrocephalus Model 1 (Kaolin Injection): After general anesthesia, rats were placed supine on the surgical table, and a burr hole was opened on the right Kocher's point with a perforator (Figure 1). An intraparenchymal intracranial pressure (ICP) monitor was connected, and an ICP measurement was taken (Figure 2). Then, the ICP monitor was removed, and the wound was sutured. In order to induce hydrocephalus, the rat was

placed in a prone position. To access the fourth ventricle, the rat's skull was placed on the stereotaxy device by the external auditory canal. The scalp was cut from eye to the nape and the periosteum on it was peeled off. To secure the cannula to be inserted, the skull was drilled at three more points with the dentist's tour, and screws were driven into the skull (approximately 1 cm away from the drilled point). Coordinates were determined by consulting the stereotaxy atlas.[5] The fourth ventricle was located, and a 28-gauge cannula was inserted. CSF flow was sighted from the cannula. No additional procedure was performed in Group 1. Group 2A was then injected with 0.05 mL of kaolin suspension prepared with 250 mg/mL 0.9% NaCl. Group-3 rats received 20 mg/kg acetazolamide intraperitoneally 24 hours after the procedure. After the 7-day follow-up period, an ICP measurement was taken by entering the same burr hole again. The left side of the xiphoid process was taken as the reference point in advancing into the chest cavity at an angle of 30 degrees, autologous arterial blood was withdrawn from the heart for biochemical examination, and rats were decapitated. Subsequently, the cerebrum was completely removed for the histopathological examination of ventricular dilation (Figure 3). The ventricular dimensions were examined by taking pathological sections.

Experimental Hydrocephalus Model 2 (Autologous Blood Injection): The same surgical procedure was performed in model 2 rats as in model 1. In Group 2B, 0.05 mL of non-heparinized autologous arterial blood was sampled from the heart by advancing at an angle of 30 degrees in the chest cavity with the left side of the xiphoid process as the reference point and was injected into the percutaneous subarachnoid space using the stereotaxic frame under sterile conditions. Group 4 rats were treated with 20 mg/kg Acetazolamide intraperitoneally 24 hours after the procedure. After the 7-day follow-up period, ICP was measured by entering the same burr hole again. Autologous arterial blood sampled from the heart was sent for biochemical examination, and the rats were decapitated. Subsequently, the cerebrum was completely removed for histopathological examination and measurement of the ventricular dimensions (Figure 3).

BIOCHEMICAL EXAMINATION

Biochemical analysis was performed with the Biovision rat tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-1 beta ELISA kit (BioVision, Inc. Headquarters 155 South Milpitas Blvd. Milpitas, California 95035).

In order to determine the TNF-α and IL1 beta values of all groups, 5 mL of blood samples were transferred into ethylene diamine tetra acetic acid tubes. All the blood serums were separated by centrifugation at 1,000 g for 15 minutes. The serum samples were stored at -20°C until ELISA experiments were conducted. Standard dilutions were prepared. In standard dilution series, 100 µL of each sample was added into the wells, which were then sealed with a plate coater and incubated at 37°C for 1 hour. After incubation, the liquid in each well was discarded. 100 µL of Detection Reagent A working solution was added to each well, and the wells were covered with

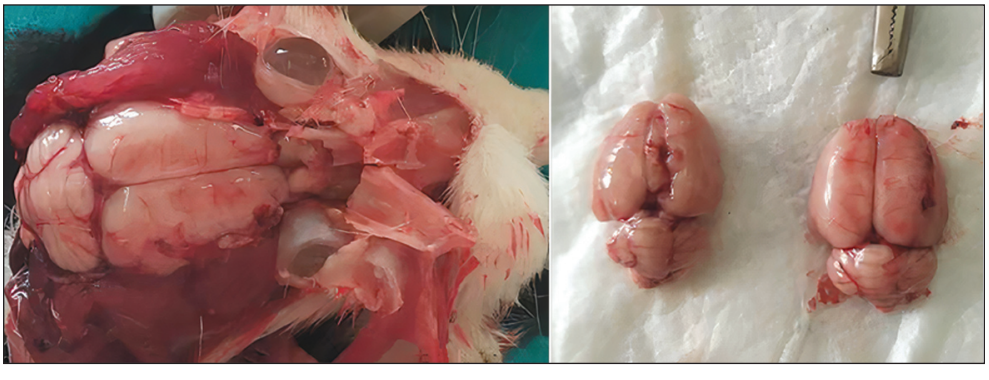

FIGURE 3: Cerebrum was totally removed. Sulcal effacement and swollen brain tissue were observed macroscopically (as seen in the brain tissue marked with the piece of equipment) in the groups with hydrocephalus.

a plate sealer and incubated at 37°C for 1 hour. 100 µL of Detection Reagent B working solution was added to each well, and the wells were covered with a plate sealer and incubated at 37°C for 30 minutes. After incubation, each well was washed 5 times. 90 µL of substrate solution was added to each well, and the wells were covered with a plate sealer and incubated at 37°C for 10-20 minutes. Samples were measured at 450 nm in a ChroMate microplate reader, and the obtained data were listed for statistical analysis.

HISTOPATHOLOGICAL EXAMINATION

After the animals were euthanized, their skulls were opened and their brains were taken out. The brain tissue was sliced horizontally on the coronal plane into 6 equal parts at right angles to cover both hemispheres. Sections were taken from the rostral diencephalon region (including the 3rd and lateral ventricles) and fixed in 10% formaldehyde buffered for 48 hours, and then the tissues were driven through graded alcohols and xylol series and embedded in paraffin. Tissues were cut with a microtome at 4µ thickness and stained with Hematoxylin & Eosin (Figure 4a, Figure 4b, Figure 4c, Figure 5a, Figure 5b). The histopathological examination was modified from a previous study, and the flattening of the ependymal cells in the ventricular cavities from the cubic structure was determined by scoring from one to three (+: cubic structure and ciliary formation, ++: flat structure and ciliary structure and +++: flat structure and ciliary structure loss) (Figure 4a, Figure 4b, Figure 4c).[6] The lateral ventricular perimeter was measured under the microscope at 40x magnification and recorded with the analySIS Five LS Research Soft Imaging System (Olympus corporation, Japan) program, and the mean lateral ventricular perimeter was calculated (Figure 6a, Figure 6b).

STATISTICAL ANALYSIS

Within the scope of the project, measurements were made in 4 different parameters (Ventricle sizes, ICP values, TNF-α, IL-1β) in 5 animal groups (Group 1, Group 2A, Group 2B, Group 3, Group 4). The quantitative data obtained were first subjected to the normality test. One-way ANOVA test was applied to the normally distributed data. Groups were compared using the Tukey test. A 95% confidence interval was chosen as a measure of reliability, and statistical significance was accepted as $p<0.05$.

RESULTS

COMPARISON OF ICP VALUES

The ICP measurements graphical view of the animals on days 0 and 7 are shown in Figure 7. The mean ICP values of Group 1 (puncture ICP measurement) on days 0 and 7 were 5.75 mmHg and 6.5 mmHg, respectively ($p>0.05$) (Table 2). This indicates that there is no pathology to increase the ICP pressure due to the procedures the subject is exposed to during CSF puncture and ICP measurement. The mean ICP values of Group 2A (kaolin injection+ICP measurement) mean ICP values on days 0 and 7 were 9 mmHg and 27.1 mmHg, respectively ($p<0.0001$). The mean ICP values of Group 2B (autologous blood injection+ICP measurement) on days 0 and 7 were 9 mmHg and 19.66 mmHg, respectively ($p=0.0055$).

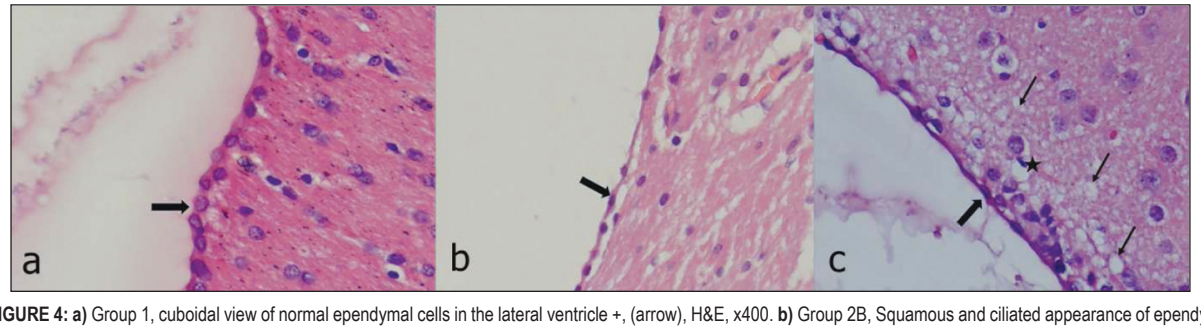

FIGURE 4: a) Group 1, cuboidal view of normal ependymal cells in the lateral ventricle +, (arrow), H&E, x400. **b)** Group 2B, Squamous and ciliated appearance of ependymal cells in the lateral ventricle ++, (arrow), H&E, x400. **c)** Group 2A, Flat and non-ciliated appearance of ependymal cells in the lateral ventricle +++, (arrow) degeneration in oligodendrocytes (star), vacuolar degeneration in subependymal tissue. H&E, x400.

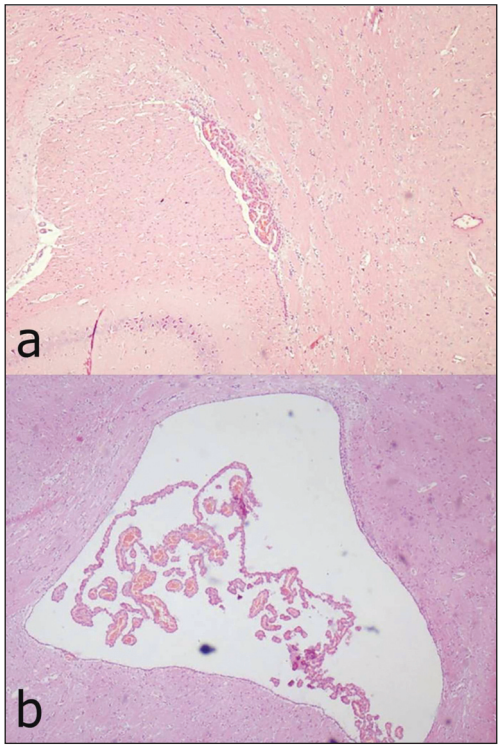

FIGURE 5: a) Group 2B, normal appearance of choroid plexuses (arrow) in the lateral ventricles, H&E, x40. **b)** Group 2A, hyperemic appearance due to enlargement of the lateral ventricles (arrow), H&E, x40.

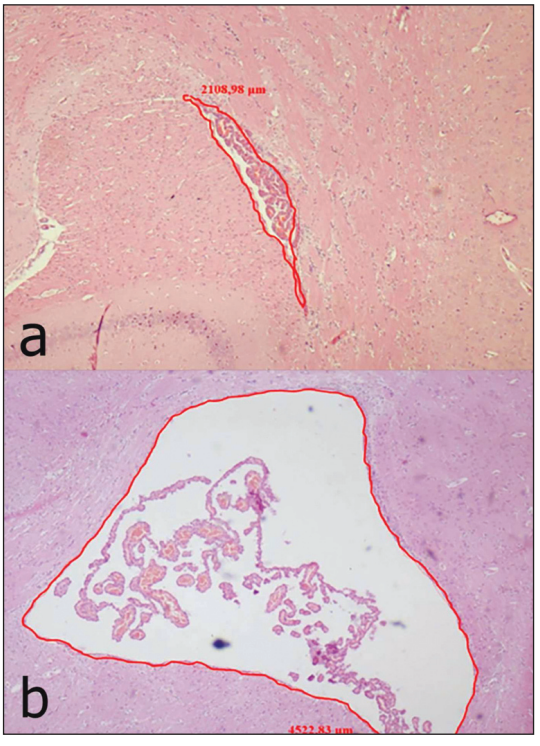

FIGURE 6: a) Group 1 (control). 2108.98 µm (measurement with the AnalySIS Five LS Research Soft Imaging System) H&E, x40. **b)** Group 3. 4522.82 µm (measurement with the AnalySIS Five LS Research Soft Imaging System) H&E, x40.

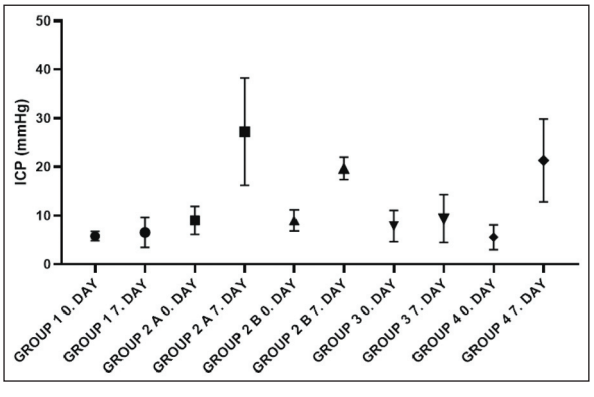

FIGURE 7: Graphical view of ICP measurements. ICP: Intracranial pressure.

The mean ICP values of Group 3 (kaolin injection+acetazolamide treatment+ICP measurement) were 7.83 mmHg and 9.33 mmHg, respectively ($p>0.05$). This indicates that acetazolamide treatment was effective in the kaolin-induced hydrocephalus model. The mean ICP values of Group 4 (autologous blood injection+acetazolamide treatment+ICP measurement) were 5.5 mmHg and 21.33 mmHg, respectively ($p<0.0001$), which shows that acetazolamide treatment was not effective in the autologous blood-induced hydrocephalus model.

TABLE 2: Statistical comparison of the ICP values of the groups on days 0 and 7.

PAIRED	Mean diff,	95.00% CI of diff,	Significant?	Summary	p value
Group 1 day 0 vs. day 7	-0.75	-10.81 to 9.309	No	ns	>0.9999
Group 2A day 0 vs. day 7	-18.17	-26.38 to -9.954	Yes	****	<0.0001
Group 2B day 0 vs. day 7	-10.67	-18.88 to -2.454	Yes	**	0.0055
Group 3 day 0 vs. day 7	-1.5	-9.713 to 6.713	No	ns	0.9928
Group 4 day 0 vs. day 7	-15.83	-24.05 to -7.620	Yes	****	<0.0001

ICP: Intracranial pressure; CI: Coffidence interval.

HISTOPATHOLOGICAL FINDINGS

The enlargements of the lateral ventricles in the macroscopic sections of the groups are shown in Figures 8 and Figure 9.[6] In the first group, hyperemia was not present in the lateral and 3rd ventricles, and ependymal cells preserved their shape (Figure 4a).[6] Group 2B had hyperemia (4/6) of the choroid plexus located in both the lateral and 3rd ventricles, and ependymal cells lost their cubic form and became flattened (3/6) (Figure 4b, Figure 5a). Vacuolization in subependymal tissues (4/6) and degeneration in oligodendrocytes (4/6) were noted. In Group 2A, bleeding increased in the 3rd and lateral ventricles (5/6), and the ependymal cells were flattened and lost their cilia (4/6) (Figure 4c, Figure 5b). Vacuolization in subependymal tissue and degeneration (5/6) in oligodendrocytes were observed.[7] In Group 4, bleeding and hyperemia were observed in the 3rd ventricles and laterals (3/6), while ependymal cells mainly were normal (4/6). However, vacuolization in subependymal tissue and degeneration in oligodendrocytes were noted in the whole group. Inflammatory cells were found in the parenchymal region in one animal. One animal had siderocytes in the lateral ventricles, parenchymal necrosis, and cell infiltrations. In Group 3, hyperemia was observed in all of the lateral ventricles (6/6) and the 3rd ventricle (4/6). It was noted that the ependymal cells were mostly flattened (5/6). Vacuolization and degeneration of oligodendrocytes were observed in all subependymal tissue (Figure 4c). Ependymal cells did not show any change in Group 1, and their cubic forms were preserved.[8] Besides, ependymal damage was observed to be the same in Groups 2A and 3 (1.83) and more than Groups 4 and 2B (1.33 and 1.5, respectively). Comparison between the groups showed group 4 to have the highest mean ventricle dimension (8283.452 µm) (Figure 10). The mean ventricular widths were

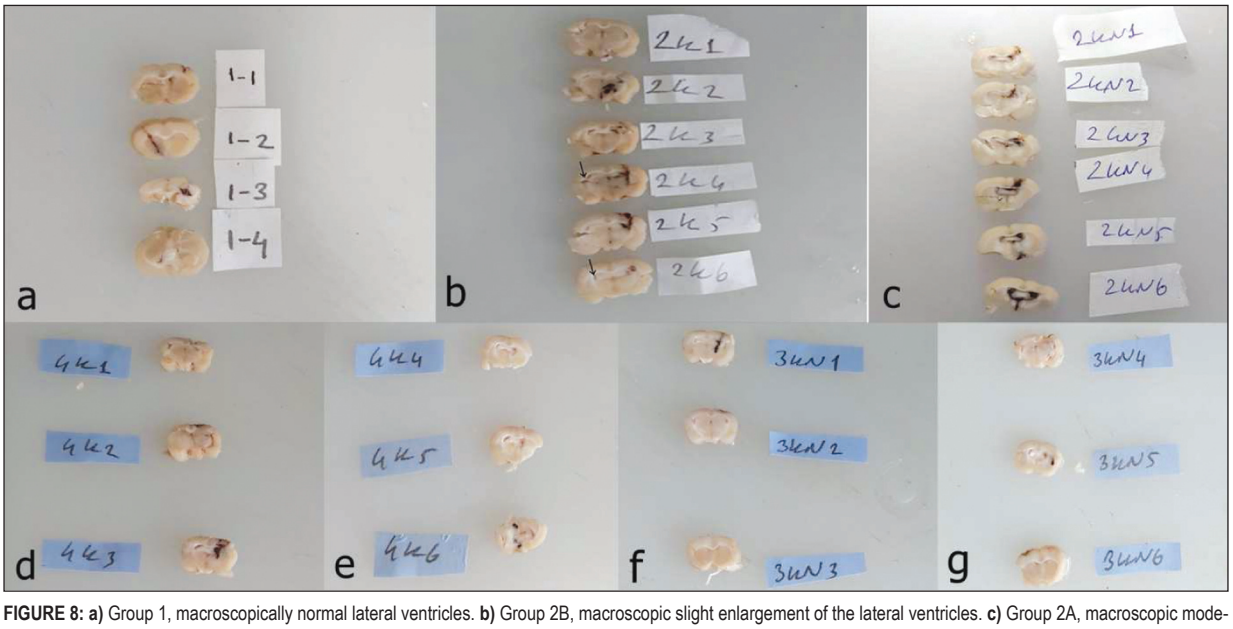

FIGURE 8: a) Group 1, macroscopically normal lateral ventricles. **b)** Group 2B, macroscopic slight enlargement of the lateral ventricles. **c)** Group 2A, macroscopic moderate enlargement of the lateral ventricles. **d, e)** Group 4, macroscopic moderate enlargement of the lateral ventricles. **f, g)** Group 3, macroscopic slight enlargement of the lateral ventricles.

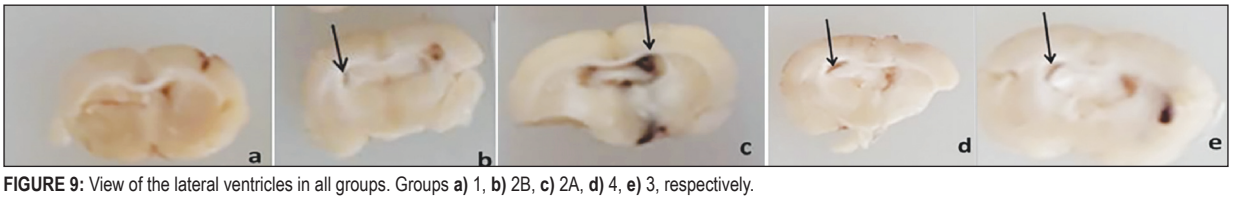

FIGURE 9: View of the lateral ventricles in all groups. Groups **a)** 1, **b)** 2B, **c)** 2A, **d)** 4, **e)** 3, respectively.

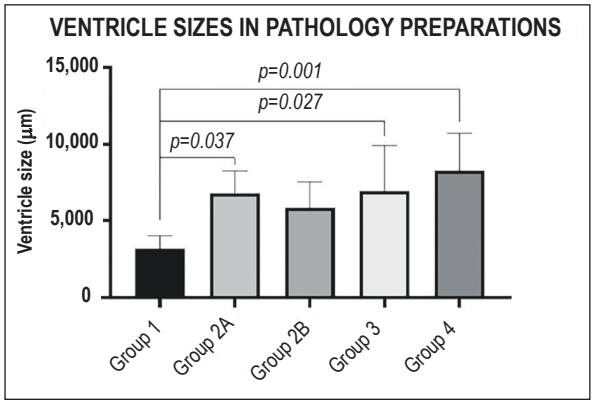

FIGURE 10: Graphical view of ventricle dimensions.

6941.667 µm and 6781.21 µm in Groups 3 and 2A, respectively. It was 5851.923 µm in Group 2B, and 3264.683 µm in Group 1 (Figure 6a, Figure 6b).[8]

Evaluation of the average ventricular sizes showed that the mean ventricular size in Group 1 (3264.68 µm) was considerably smaller than the other groups (Figure 10). The mean ventricular size in Group 2A was 6781.21 µm, and the mean ventricular size in Group 2B was 5851.92 µm. Ventricular dimensions of Group 2A (kaolin) were slightly larger than Group B (autologous blood). In Groups 3 and 4, which had been administered acetazolamide treatment, the mean ventricle sizes were 6941.83 µm and 8283.45 µm, respectively. It was observed that the ventricle sizes increased less in Group 3, which had been administered kaolin injection and acetazolamide treatment, compared to Group 4, which had been administered autologous blood injection and acetazolamide treatment. An increase in ventricle size was observed in our pathology preparations, and the comparison of acetazolamide-administered groups (kaolin vs. autologous blood injection) revealed smaller ventricle sizes in the kaolin injection group; however, the effect of acetazolamide treatment was not statistically significant.

Pairwise analysis of the ventricle sizes displayed a significant difference between Group 1 and Group 2A, Group 2B, Group 3, and Group 4. No significant difference was found between the other groups (Table 3).

Tukey's multiple comparisons test detected a significant increase in ventricle size in Group 2A, Group 3, and Group 4 compared to Group 1.

BIOCHEMICAL EXAMINATION

TNF-α and IL-1β values detected in the groups are shown in Table 4. TNF-α mean value were measured as 51 pg/mL in Group 1, 428.33 pg/mL in Group 2A, 437.5 pg/mL in Group 2B, 208.33 pg/mL in Group 3, and 191.66 pg/mL in Group 4. TNF-α values were significantly increased in other groups compared to Group 1. TNF-α values were found to be approximately half as low in Groups 3 and 4 which were administered acetazolamide treatment, compared to Groups 2A and Group 2B which did not receive treatment. Statistical analysis determined that TNF-α values were significantly increased in other groups compared to Group 1. However, while Group 2A and Group 2B had $p<0.0001$, p-values of Group 3 and Group 4 were $p=0.0007$ and $p=0.0025$, respectively. These figures indicate that TNF-α values increased more significantly in the groups which were not administered acetazolamide treatment compared to the group which were. Among groups receiving acetazolamide treatment, the increase in TNF-α was less in Group 4 with autologous blood injection, compared to Group 3 with kaolin injection, and accordingly, the efficacy of acetazolamide in reducing TNF-α was higher in the group that received autologous blood. However, there was no statistically significant difference between Group 2A and 2B, and between Group 3 and 4 (Table 5).

Group 1 IL-1β mean values were 3250 pg/mL in Group 1, 38458.33 pg/mL in Group 2A, 40416.66 pg/mL in Group 2B, 26283.33 pg/mL in Group 3,

TABLE 3: Pairwise analysis of ventricle dimensions in pathology preparations.

		p value	Significantly different (p<0.05)?
Group 2A	Group 1	0.0004	Yes
Group 2B	Group 1	0.006	Yes
Group 3	Group 1	0.014	Yes
Group 4	Group 1	0.0007	Yes
Group 2B	Group 2A	0.3346	No
Group 3	Group 2A	0.9085	No
Group 4	Group 2A	0.2279	No
Group 3	Group 2B	0.4545	No
Group 4	Group 2B	0.0733	No
Group 4	Group 3	0.4149	No
			Statistically significant

TABLE 4: TNF-α and IL-β values detected in the groups.

	TNF-α (pg/mL)	IL-1β (pg/mL)
Group 1	30	2,500
	45	5,700
	54	3,600
	75	1,200
Group 2A	450	40,000
	520	44,500
	465	30,000
	385	35,500
	350	39,250
	400	41,500
Group 2B	475	42,000
	525	37,500
	450	42,500
	350	37,500
	400	45,500
	425	37,500
Group 3	250	25,000
	200	19,800
	250	27,400
	150	23,250
	175	32,750
	225	29,500
Group 4	150	20,000
	250	18,500
	175	19,500
	125	22,500
	200	27,500
	250	15,000

TNF-α: Tumor necrosis factor-alpha; IL-β: Interleukin beta.

20500 pg/mL in Group 4. Compared to Group 1, IL-1β values were significantly increased in other groups. IL-1β values decreased by approximately half in Group 3 and Group 4 which received acetazolamide treatment, compared to Group 2A and Group 2B which did not. Statistical analysis determined that IL-1β values increased significantly in other groups compared to Group 1. A significant difference was also found between Group 2A and Group 2B, Group 4 and Group 3. These values mean that IL-1β values increased more significantly in the groups that did not receive acetazolamide treatment compared to the group that did. Among groups receiving acetazolamide treatment, the increase in IL1β values increased was less in Group 4 with autologous blood injection, compared to Group 3 with kaolin injection, and accordingly, the efficacy of acetazolamide in reducing IL-1β was higher in the group that received autologous blood injection. However, no statistically significant difference was found between Group 2A and Group 2B, and between Group 3 and Group 4 (Table 6).

DISCUSSION

CSF is secreted from the choroid plexus and ependymal cells situated in the wall of the lateral, 3rd, and 4th ventricles and is in a state of balance in the body.[5] CSF circulation between the subarachnoid distance, brain, and the ventricles depends on the nutritive and excretory balance in the interstitial fluid. CSF reabsorption occurs with the help of aquaporin (AQP) 4 from the capillary wall or arachnoid villi. Aquaporin channels assist fluid flow and ion passage between the ventricles enabling CSF circulation. Over time, the balance between CSF formation and absorption

TABLE 5: TNF-α Tukey's multiple comparisons test.

Tukey's multiple comparisons test	Mean diff,	95.00% CI of diff,	Significant?	Summary	Adjusted p value
Group 1 vs. Group 2A	-377.3	-475.0 to -279.7	Yes	****	<0.0001
Group 1 vs. Group 2B	-386.5	-484.1 to -288.9	Yes	****	<0.0001
Group 1 vs. Group 3	-157.3	-255.0 to -59.71	Yes	***	0.0007
Group 1 vs. Group 4	-140.7	-238.3 to -43.05	Yes	**	0.0025
Group 2A vs. Group 2B	-9.167	-96.48 to 78.15	No	ns	0.9978
Group 2A vs. Group 3	220	132.7 to 307.3	Yes	****	<0.0001
Group 2A vs. Group 4	236.7	149.4 to 324.0	Yes	****	<0.0001
Group 2B vs. Group 3	229.2	141.9 to 316.5	Yes	****	<0.0001
Group 2B vs. Group 4	245.8	158.5 to 333.1	Yes	****	<0.0001
Group 3 vs. Group 4	16.67	-70.65 to 104.0	No	ns	0.979

TNF-α: Tumor necrosis factor-alpha; CI: Coffidence interval.

TABLE 6: Evaluation of IL-1β values by Tukey's multiple comparisons test.

Tukey's multiple comparisons test	Mean diff,	95.00% CI of diff,	Significant?	Summary	p value
Group 1 vs. Group 2A	-35208	-43095 to -27321	Yes	****	<0.0001
Group 1 vs. Group 2B	-37167	-45054 to -29280	Yes	****	<0.0001
Group 1 vs. Group 3	-23033	-30920 to -15146	Yes	****	<0.0001
Group 1 vs. group 4	-17250	-25137 to -9363	Yes	****	<0.0001
Group 2A vs. Group 2B	-1958	-9013 to 5096	No	ns	0.9215
Group 2A vs. Group 3	12175	5121 to 19229	Yes	***	0.0003
Group 2A vs. Group 4	17958	10904 to 25013	Yes	****	<0.0001
Group 2B vs. Group 3	14133	7079 to 21188	Yes	****	<0.0001
Group 2B vs. Group 4	19917	12862 to 26971	Yes	****	<0.0001
Group 3 vs. Group 4	5783	-1271 to 12838	No	ns	0.1447

IL-β: Interleukin beta; CI: Coffidence interval.

deteriorates, and hydrocephalus develops due to pathological causes such as trauma, infection, bleeding, tumor, and ischemia.[2]

Acetazolamide, a carbonic anhydrase inhibitor, is a drug that regulates CSF in the treatment of hydrocephalus and has widespread clinical use.[2]

AQPs are transmembrane channel proteins that ease water movement across plasma membranes in secretory and absorptive cells and they have 13 subtypes. The most common ones found in the CNS are AQP-1, AQP-9, and especially AQP-4.[9]

In experimental models, hydrocephalus is induced by injecting various substances into the CSF tract or by developing a SAH model. It has been shown that the expression of AQP-1 does not change in response to hydrocephalus, especially in the kaolin injection-induced chronic hydrocephalus model. Therefore, pharmacological blockade of AQP-1 expression may provide a therapeutic benefit by reducing CSF production. Despite few studies reporting an inhibitory effect on AQP-1 and AQP-4, most studies found that acetazolamide, a carbonic anhydrase inhibitor, has no effect on AQPs.[10]

IL-1β is secreted by immune cells in case of infection, cell damage and inflammation. In addition, IL-1β is thought to cause hydrocephalus by causing excessive secretion of CSF from the choroid plexus.[11,12] Statistical analysis determined that IL-1β values increased significantly in other groups compared to Group 1. A significant difference was also found between Group 2A and Group 2B, Group 4 and Group 3. These values mean that IL-1β values increased more significantly in the groups that did not receive acetazolamide treatment compared to the group that did. Among groups receiving acetazolamide treatment, the increase in IL-1β values was less in Group 4 with autologous blood injection, compared to Group 3 with kaolin injection, and accordingly, the efficacy of acetazolamide in reducing IL-1β was higher in the group that received autologous blood injection. However, no statistically significant difference was found between Group 2A and Group 2B, and between Group 3 and Group 4.

Although clinical severity in patients with SAH is associated with other markers, it is also associated with high TNF alpha levels.[13] TNF-α values increased more significantly in the groups which were not administered acetazolamide treatment compared to the group which were. Among groups receiving acetazolamide treatment, the increase in TNF-α values was less in Group 4 with autologous blood injection, compared to Group 3 with kaolin injection, and accordingly, the efficacy of acetazolamide in reducing TNF-α value was higher in the group that received autologous blood injection. However, there was no statistically significant difference between Group 2A and Group 2B, and between Group 3 and Group 4

Our study created 2 hydrocephalus models with kaolin and autologous blood injection. We compared the ICP value, ventricle dimensions, and IL-1 and TNF-α values after acetazolamide treatment between the groups and at days 0 and 7.

Hydrocephalus is characterized by accumulation of CSF in the subarachnoid space and within the ventricle, and often with increased intracranial pressure.[14] By measuring ICP values on days 0 and 7 in all groups, our study examined the development of hydrocephalus and the correlation of acetazolamide activity with both histological and biochemical data.

Although the levels of cytokines such as AQ4 change due to the interaction of AQP channels, it has been observed that the affected blood-brain barrier consequently affects acute phase reactants.[15] We compared the changes in TNF-α and IL-1β values in the hydrocephalus models we created.

In this study, acetazolamide was shown to be more effective in the kaolin-induced hydrocephalus model, according to both ICP and ventricular size measurements. Biochemically, IL-1β and TNF-α values increased significantly in both groups with hydrocephalus. Although the biochemical values of both groups receiving acetazolamide treatment decreased compared to the untreated groups, they decreased more in the hydrocephalus model induced by autologous blood injection. Although acetazolamide treatment decreased IL-1β and TNF-α values, it was found to be more effective in the autologous blood-induced hydrocephalus model.

This study investigated the efficacy of medical treatment in animal hydrocephalus models. The next step may be to demonstrate the effectiveness of the surgical approach by using various types of shunts in the same experimental models.

CONCLUSION

Since the pathogenesis of hydrocephalus is not yet fully understood, examining the brain damage occurring in hydrocephalus and the underlying mechanisms is of vital importance in developing diagnostic, observational, and therapeutic tools that will affect the outcomes of hydrocephalus. In our study, 2 different hydrocephalus models were created and the animals' ICP values, ventricular sizes, TNF-α and IL-1β values and the effects of acetazolamide on these data were statistically demonstrated. We believe that it will contribute to the literature since we did not find a study in which all these data were compared together in the literature review. Moreover, this study will shed light on the mechanism of hydrocephalus development and the potentially effective treatment modalities and contribute to future research.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Elif Başaran Gündoğdu, Hasan Emre Aydın;* ***Design:*** *Elif Başaran Gündoğdu, Hasan Emre Aydın, Zehra Avcı Küpeli;* ***Control/Supervision:*** *Elif Başaran Gündoğdu;* ***Data Collection and/or Processing:*** *Elif Başaran Gündoğdu, Hasan Emre Aydın, Zehra Avcı Küpeli;* ***Analysis and/or Interpretation:*** *Elif Başaran Gündoğdu, Hasan Emre Aydın, Zehra Avcı Küpeli;* ***Literature Review:*** *Elif Başaran Gündoğdu, Hasan Emre Aydın, Zehra Avcı Küpeli;* ***Writing the Article:*** *Elif Başaran Gündoğdu;* ***Critical Review:*** *Elif Başaran Gündoğdu;* ***References and Fundings:*** *Elif Başaran Gündoğdu, Hasan Emre Aydın, Zehra Avcı Küpeli;* ***Materials:*** *Elif Başaran Gündoğdu, Hasan Emre Aydın, Zehra Avcı Küpeli.*

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