ORIGINAL RESEARCH

Med J SDU / SDÜ Tıp Fak Derg ▸ 2024:31(4):310-317 doi: 10.17343/sdutfd.1482851

Inflammation and Apoptosis-Related Damage in Lung, Liver, and Kidney Tissues due to Subarachnoidal Hemorrhage

Ali Serdar OGUZOGLU[1], Halil ASCI[2], Musa CANAN[1], Nilgün SENOL[1], Ozlem OZMEN[3]

[1] Department of Brain Surgery, Faculty of Medicine, Suleyman Demirel University, Isparta, Türkiye
[2] Department of Pharmacology, Faculty of Medicine, Suleyman Demirel University, Isparta, Türkiye
[3] Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Pathology, Burdur, Türkiye

Cite this article as: Oguzoglu AS, Asci H, Canan M, Senol N, Ozmen O. Inflammation and Apoptosis-Related Damage in Lung, Liver, and Kidney Tissues due to Subarachnoidal Hemorrhage. Med J SDU 2024;31(4):310-317.

Abstract

Objective
Oxidant and inflammatory substances released into the blood due to subarachnoidal hemorrhage (SAH) can pass into the peripheral compartment, causing distant organ damage due to blood-brain barrier permeability caused by oxidative stress, inflammation, and apoptosis. This study aimed to demonstrate the secondary damage to peripheral organs, including the lung, kidney, and liver, resulting from SAH.

Material and Method
Twenty rats were divided into sham and SAH groups, each consisting of ten animals. In the SAH group animals, 0.3 mL autologous blood taken from the tail artery was injected into the cisterna magna for 2 minutes. Seven days after SAH formation, all animals were euthanized under anesthesia. Following decapitation, brain tissues, lung, liver, and kidney tissues were placed in 10% formaldehyde for histopathological and immunohistochemical analysis.

Results
In the SAH group, neuronal degeneration in the cerebral cortex, and hyperemia and hemorrhage in the lung, kidney, and liver were observed histopathologically. In immunohistochemical examinations, decreased expression of brain-derived neurotrophic factor (BDNF) and neurofilament (NF) in the cerebral cortex, cerebellum, and hippocampus sections; In lung tissues, enhanced caspase (Cas)-3, hypoxia-inducible factor 1 alpha (Hif-1α) and nuclear factor kappa beta (NF-κB) expressions in the lung, Cas-5, cyclooxygenase-1 (Cox-1) and interleukin (IL)-1 expressions in the liver, Cas-3, Cox-1 and IL-3 expressions in the kidney were observed.

Conclusion
Following SAH, in addition to damage to brain tissue, peripheral tissues such as the lung, kidney, and liver can also be damaged through inflammation and apoptosis.

Keywords: Subarachnoid hemorrhage, liver, lung, kidney, inflammation, apoptosis

Correspondence: A.S.O / a.serdaroguzoglu@yahoo.co.uk
Received: 20.05.2024 • Accepted: 19.09.2024
ORCID IDs of the Authors: A.S.O: 0000-0002-1735-4062; H.A: 0000-0002-1545-035X; M.C: 0009-0005-7279-0026; N.S: 0000-0002-1714-3150; O.O: 0000-0002-1835-1082

Introduction

Subarachnoidal hemorrhage (SAH) is a highly progressive clinical condition that occurs secondary to damage to the vessels supplying brain tissue due to various causes, such as acquired or hereditary factors (1,2). Subarachnoidal hemorrhage (SAH) is a severe clinical condition resulting from damage to the blood vessels supplying brain tissue due to various acquired or hereditary factors (1,2). Bleeding is observed due to structural deterioration in the vessel wall, especially secondary to aneurysmal events in the vascular layer. Bleeding occurs due to structural deterioration in the vessel wall, often caused by aneurysmal events in the vascular layer. Increased levels of oxidant and inflammatory substances in the blood and brain tissue, triggered by the hypoxic environment distal to the bleeding area, are known to exacerbate the damage (3,4).

These substances can trigger mechanisms such as oxidative stress, inflammation, and apoptosis by binding to their receptors in the tissue they come into contact with (6). To investigate such damage mechanisms, oxidative stress index (OSI), total antioxidant status (TAS), total antioxidant status (TOS), levels for oxidative stress; interleukin 1 (IL-1β), caspase-5 (Cas-5), interleukin-3 (IL-3), hypoxia-induced factor 1 alpha (Hif-1α), cyclooxygenase-1 (Cox-1) and nuclear factor kappa beta (NFkβ) expressions to show inflammation and caspase-3 (Cas-3) expressions to show apoptosis. Oxidant and inflammatory molecules released into the bloodstream can penetrate the peripheral compartment, causing distant organ damage by increasing blood-brain barrier permeability (5).

This study aimed to demonstrate the damage in the brain and some peripheral tissues of SAH-induced rats by histopathologic and immunohistochemical methods.

Material and Method

Experimental Animals

Twenty adult male Wistar albino rats (250-350 g) were obtained from the Burdur Mehmet Akif Ersoy University Experimental Animal Laboratory. They were housed under controlled conditions (60% ± 5% humidity, 21-22 °C, and a 12-hour dark/light cycle) and provided with standard commercial water and feed.

Experimental Procedure

Twenty rats were divided into sham and SAH groups containing ten animals. In the SAH group animals, after aspiration of 0.3 mL CSF into the cisterna magna reached following neck dissection, 0.3 mL autologous blood taken from the tail artery was injected into the cisterna magna for 2 minutes (7). In the sham group, 0.3 mL of physiologic saline solution was applied to the cisterna magna after the aspiration of 0.3 mL of cerebrospinal fluid (CSF), to match the stress levels of the SAH group.

Seven days after SAH formation, all animals were euthanized under 8-10mg/kg xylazine (Xylazinbio 2%, Bioveta, Czech Republic), 90 mg/kg ketamine (Keta-control, Doğa İlaç, Turkey)anesthesia by surgical bleeding method by taking blood samples from the inferior vena cava through an abdominal incision. Following decapitation, brain tissues, lung, liver, and kidney tissues were placed in 10% formaldehyde for histopathological and immunohistochemical analysis.

Histopathologic Evaluation

Brain, lung, liver, and kidney tissues were collected and fixed in 10% buffered formalin for histopathological analysis at sacrifice. Following routine processing of tissues with a fully mechanized tissue processor, 5-µm-thick paraffin block pieces were cut using fully automatic rotary microtomes (Leica RM2155, Leica Microsystems, Wetzlar, Germany). Deparaffinization, rehydration with decreasing amounts of graded ethanol, staining with hematoxylin-eosin (HE), clearing in xylene, and sealing sections were the next steps. Histologic changes were evaluated under light microscopy.

Brain sections were stained with brain-derived neurotrophic factor (BDNF) and neurofilament (NF); lung sections with cas-3, Hif-1α, and NF-κB; kidney sections with Cas-3, Cox-1, and IL-3; and liver sections with Cas-5, Cox-1, and IL-3. Sections were mounted on polylysine-coated slides and stained with BDNF (Recombinant Anti-BDNF antibody [EPR1292] (ab108319)), NF (Anti-160 kD Neurofilament Medium antibody [EPR23510-76)) (ab254348)); Cas-3 (Anti-Cas-3 antibody [EPR18297] (ab184787)); Cas-5 (Recombinant Anti-Cas-5 antibody [EP876Y] (ab40887)); Hif-1α (Anti-HIF-1α) alpha antibody [mgc3] (ab16066)); NF-κB (Anti-NF-kB p65 antibody (ab16502)): Cox-1 (Anti-cox-1 antibody [EPR5866] (ab109025)); IL-1 (Anti-cox-1 antibody/[EPR5866] (ab109025)); -IL-3 antibody (ab190941); streptavidin was performed using the biotin technique. Primary and secondary antibodies were purchased from Abcam (Cambridge, UK), and primary antibodies were used at 1/100 dilution. After incubating the sections with primary antibodies for 60 minutes, they were stained using biotinylated secondary antibodies

and streptavidin-alkaline phosphatase conjugate through immunohistochemistry. The results were obtained using the EXPOSE Mouse and Rabbit Specific HRP/DAB Detection IHC kit (ab80436) as the secondary antibody, and diaminobenzidine was used as the chromogen (DAB). Negative controls were run with a dilution solution instead of primary antiserum. The slides were analyzed for immunopositivity for each marker, and the number of positive cells was determined by manually counting 100 cells for each rat under 20X magnification using ImageJ (National Institutes of Health, Bethesda MD). The microphotography was taken using the Database Guide Cell Sens Life Sciences Imaging Software System (Olympus Co., Tokyo, Japan).

Statistical Analysis

The variables were presented in the form of mean ± standard deviations. The Mann-Whitney U test was used to compare the histopathological and immunohistochemical scores between the groups. The statistical calculations were performed using the Graphpad Prism 8.0 program pack (Graphpad Software Inc., USA). A significance value of $p<0.05$ was set.

Results

Histopathologic Findings

Microscopic examinations showed no lesions in the control group. Nevertheless, subarachnoid hemorrhage led to neuronal degeneration characterized by SAH in the brain. Microscopic examinations showed no lesions in the control group. However, subarachnoid hemorrhage caused neuronal degeneration in the brain. SAH also caused hyperemia and hemorrhage in the lung, kidney, and liver (Figure 1).

Immunohistochemical Findings

Immunohistochemical examinations of the brain, cerebellum, and hippocampus sections showed decreased expression of BDNF and NF in the SAH group compared to the control group (Figures 2-3). Cas-3, Hif-1α, and NF-κB expressions were increased in lung tissues, which are generally localized in inflammatory and alveolar epithelial cells (Figure 4). In the liver of the SAH group, Cas-5, Cox-1, and IL-1 expressions were increased significantly in hepatocytes (Figure 5). Increased Cas-3, Cox-1, and IL-3 expressions localized in tubular epithelial cells in the renal cavities of the SAH group were observed (Figure 6)

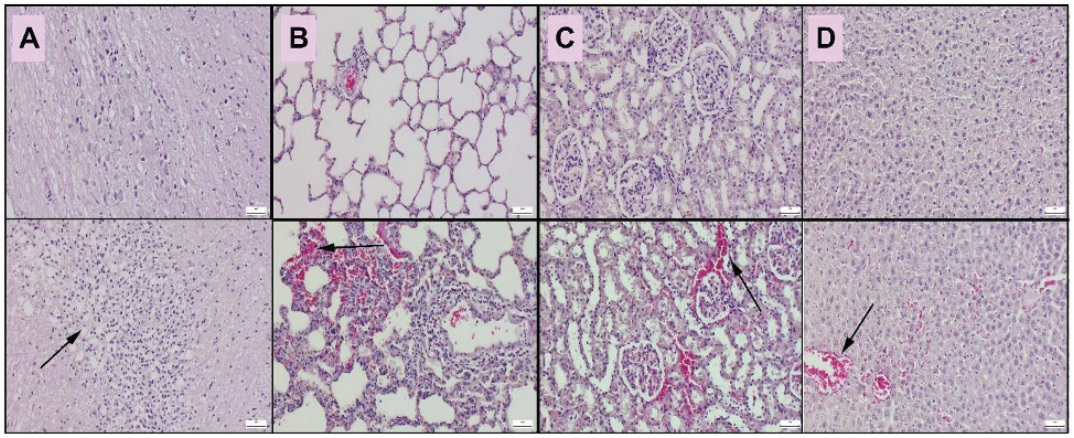

Figure 1

Histopathological findings of organs in control (upper row) and SAH (below row) between the groups. **(**A) Normal brain in normal brain and inflammatory reaction (arrow) brain in SAH group, (B) normal lung tissue in the lung in the control group and inflammatory cell infiltrations and hemorrhage (arrow) in SAH group, (C) normal kidney histology in control group and cortical hemorrhage (arrow) in SAH group, (D) normal heart tissue in control group and slight hemorrhage in (arrow) in SAH group, (E) normal liver histology in control group and hemorrhage (arrow) in SAH group HE, scale bars=50µm.

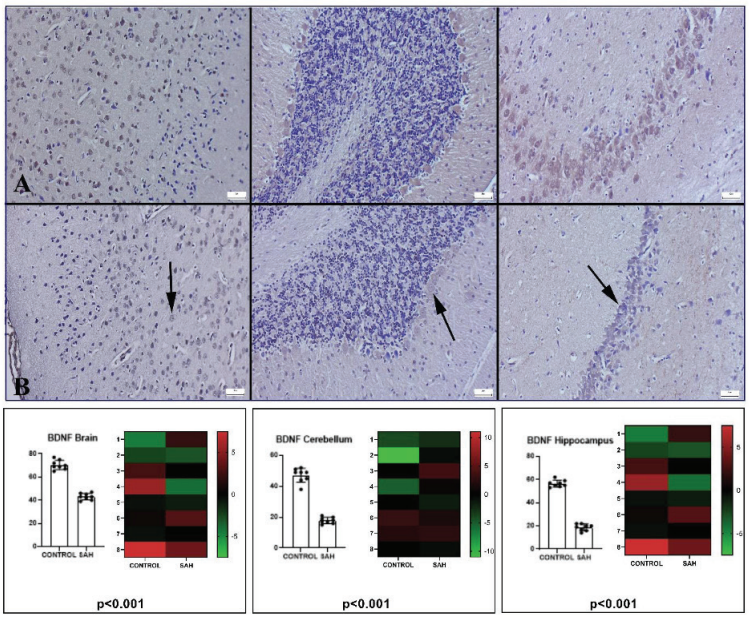

Figure 2

BDNF immunoexpression in the brain (first column), cerebellum (second column), and hippocampus (third column)

(A) BDNF expression in control and (B) SAH groups, arrows indicate decreased expression, Streptavidin biotin peroxidase method, Scale bars= 50µm

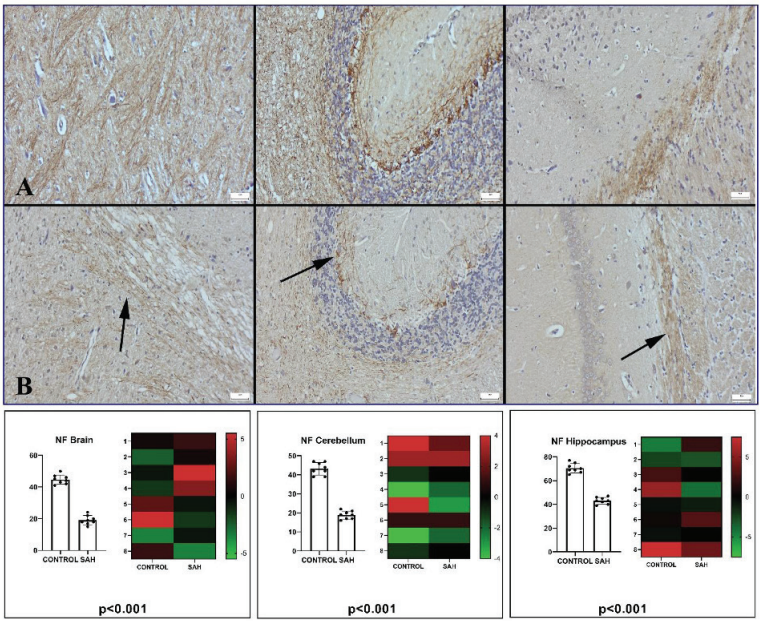

Figure 3

NF immunoexpression in the brain (first column), cerebellum (second column), and hippocampus (third column)

(A) Control and (B) SAH groups, arrows indicate reduced expression, Streptavidin biotin peroxidase method, Scale bars=50µm.

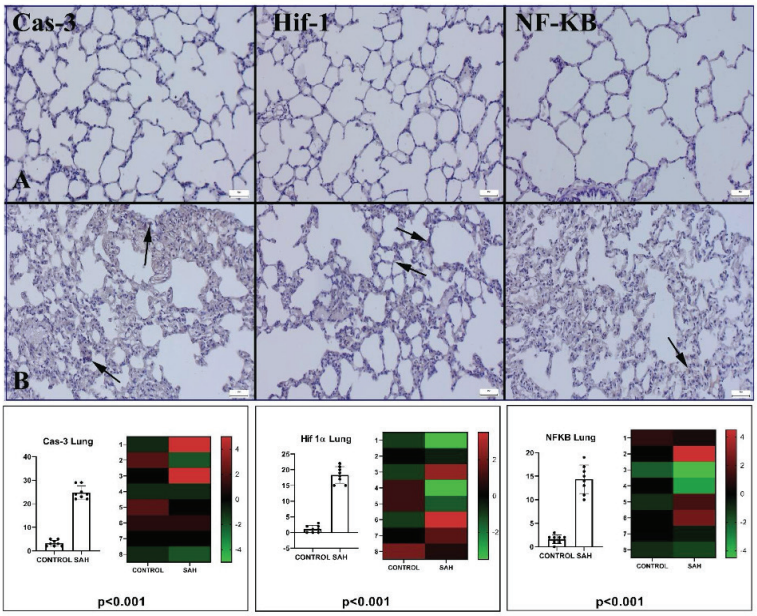

Figure 4

Cas-3, Hif-1α, NF-κB immunoexpressions in lung tissue

A) Control group, (B) SAH group, arrows indicate immunopositive cells, Streptavidin biotin peroxidase method, Scale bars=50μm.

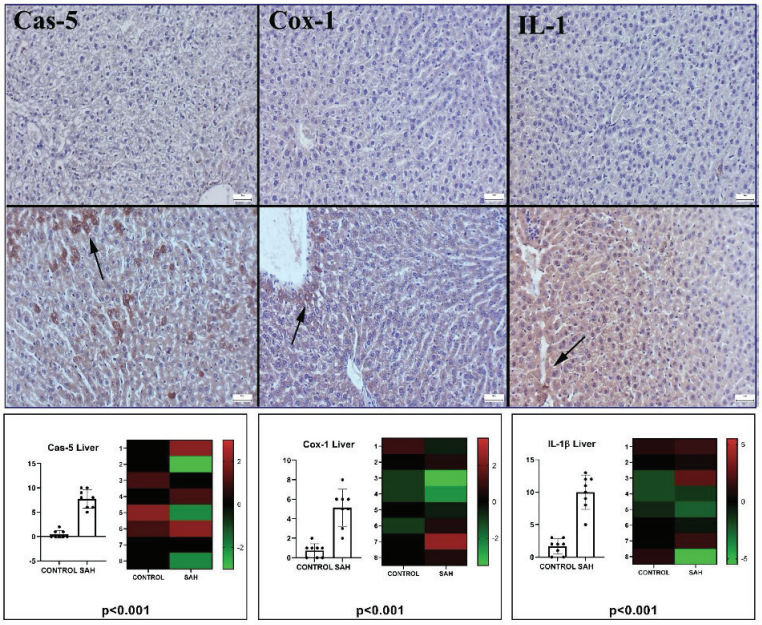

Figure 5

Cas-5, Cox-1, IL-1β immunoexpressions in liver tissue

(A) Control group, (B) SAH group, arrows indicate immunopositive cells, Streptavidin biotin peroxidase method, Scale bars=50μm.

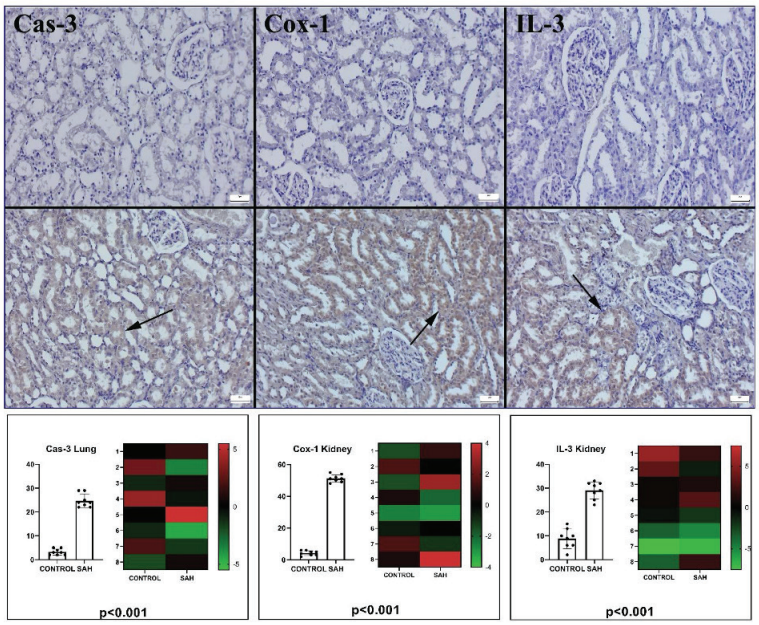

Figure 6

Cas-3, Cox-1, IL-3 immunoexpressions in kidney tissue

(A) Control group, (B) SAH group, arrows indicate immunopositive cells, Streptavidin biotin peroxidase method, Scale bars=50µm.

Discussion

Hypoxia-induced inflammatory reactions in the brain tissues distal to the hemorrhage area may cause local diffuse damage to the brain tissue. They may also cause damage to peripheral organs by passing into the peripheral blood due to increased blood-brain barrier permeability caused by cytokines released into the blood (9, 10). The histopathological findings from the brain tissue analyzed in the study indicate occurrences of SAH, evidenced by neuronal degeneration, gliosis, varying degrees of hyperemia, mild hemorrhage, and neuronal shrinkage. Hyperemia and hemorrhage findings in the lung, kidney, and liver tissues damaged by inflammatory cytokines passing to the periphery also indicate the development of peripheral organ damage. Consistent with the findings, Han et al. demonstrated that increased NF-κB levels following SAH lead to damage in the lungs (11).

In an observational study by Tujjar et al., it was reported that acute kidney injury developed in more than 10% of patients admitted to the intensive care unit following SAH. Additionally, Li et al. emphasized the development of fibrosis due to liver damage in some patients following SAH (12). SAH is a life-threatening disease with a severe prognosis due to its secondary effects (8).

For example, studies have shown that nephrotoxicity developing in patients with SAH increases mortality in the intensive care unit (2). It was also found that creatinine and BUN levels increased in these patients with SAH-dependent or independent mechanisms. Similarly, liver damage may also be observed in some patients following SAH (13). For example, a clinical study has shown that liver damage in patients with SAH is associated with rebleeding, intracranial infection, pneumonia, and acute kidney injury (13). There is also clinical data on the lung, one of the organs damaged after SAH, and it has been reported to increase over the years. For example, Veeravagu et al. showed in a study that the incidence of acute respiratory distress after SAH has increased since 1993 (14). Therefore, elucidating the cause-and-effect relationship of peripheral organ damages that develop after SAH will provide support for clinical studies to reduce mortality and morbidity in hundreds of patients with various complications. Clinical responses in patients with these organ damages, potentially linked to SAH, also corroborate these findings.

The expression of neurogenesis indicator BDNF and intermediate filaments that comprise the neuronal cytoskeleton indicator NF, whose expression was examined by immunohistochemical analysis to show the damage in brain tissue, decreased in the

cerebral cortex, cerebellum, and hippocampus (13). These damages may cause leukomotor dysfunctions, decreased memory and memory capacity, and balance disorders secondary to SAH (14, 15). In support of this, Chen et al. demonstrated in their study that the administration of exogenous BDNF reduced neurological deficits associated with SAH (16).

Immunohistochemical analyses were performed to determine the expression of various proinflammatory and apoptotic substances in lung, kidney, and liver tissues. In lung tissues, Cas-3 levels increased in inflammatory and alveolar epithelial cells as a common junction of many apoptotic pathways, indicating the development of apoptosis in the tissue. Also, NF-κB expression, which has a central role in cytokine production, and Hif-1α expression, which plays a role in hypoxia-induced inflammation, increased (17). Another study by Suresh et al. demonstrates that inflammatory damage occurring in the lungs is mediated through Hif-1α and NF-κB (18). Due to such cellular damage mechanisms in the lung tissue, the expansion capacity of the lung gradually decreases, and hypoxia-induced damage may be even more profound (19).

In this study, tissue responses in liver and kidney tissues, which are the organs of elimination, were also examined. Increased Cas-5, Cox-1, and IL-1 expressions in liver tissue of the SAH group were associated with increased prostaglandin synthesis due to arachidonic acid metabolism and activation of the nod-like receptor protein pathway (20). In their study, Galea et al. supported these findings by demonstrating that administering an IL-1 antagonist reduced inflammation associated with SAH (21). Increased apoptotic Cas-3 and proinflammatory IL-3 and Cox-1 expression in kidney tissue suggest that apoptosis and inflammation are also triggered in this tissue. Hvas et al. demonstrated that the damage caused by brain injury leads to an increase in COX-1 and COX-3 levels in the renal medulla (22). Damage to these organs, which are the organs of elimination, indicates that there may be problems in the excretion of the harmful substances produced in the disease and the drugs used in treatment (23, 24).

The critical aspect of this study, which includes the expression of very different markers in different tissues, is to help scientists who want to research these tissues in the future. In addition to these expressions, the lack of molecular genetic and biochemical analyses constitutes the study's limitation.

As a result, in SAH, which is a hazardous disorder, peripheral organ damage occurs in addition to brain tissue. The damage mechanisms induced by proinflammatory substances circulating in the blood by binding to their receptors in these organs may cause loss of function in tissues and aggravate the clinical response. More detailed molecular investigations including the expressions, must be conducted in future studies.

Conflict of Interest Statement
There is no conflict of interest between the authors.

Ethical Approval
In this study, all experiments were performed under the guidelines for animal research from the National Institutes of Health and were approved by the Committee on Animal Research of Mehmet Akıf Ersoy University, Burdur (Ethic No:24.04.2024/122-1298).

Funding
This study was supported by the Suleyman Demirel University Scientific Research Projects Coordination Unit (Project code: TSG-2022-8783).

Availability of Data and Materials
A data availability statement should include.

Authors Contributions
ASO: Conceptualization; Data curation; Investigation; Methodology; Validation; Visualization; Writing-original draft; Writing- review & editing.

HA: Methodology; Validation; Visualization; Writing-original draft; Writing- review & editing.

MC: Methodology; Validation; Visualization; Writing-original draft

NS: Methodology; Validation; Visualization; Writing-original draft, Writing- review & editing.

OO: Formal analysis; Validation; Visualization

References

1. Van Gijn J, Kerr RS, Rinkel GJ. Subarachnoid hemorrhage. The Lancet 2007;369(9558):306-18.
2. Tujjar O, Belloni I, Hougardy J-M, Scolletta S, Vincent J-L, Creteur J, Taccone FS. Acute kidney injury after subarachnoid hemorrhage. Journal of Neurosurgical Anesthesiology 2017;29(2):140-9.
3. Mukandala G, Tynan R, Lanigan S, O'Connor JJ. The effects of hypoxia and inflammation on synaptic signaling in the CNS. Brain Sciences 2016;6(1):6.
4. Nguyen A, Patel AB, Kioutchoukova IP, Diaz MJ, Lucke-Wold B. Mechanisms of mitochondrial oxidative stress in Brain Injury: from pathophysiology to therapeutics. Oxygen 2023;3(2):163-78.

5. Zhao Y, Gan L, Ren L, Lin Y, Ma C, Lin X. Factors influencing the blood-brain barrier permeability. Brain Research 2022;1788:147937.
6. Wang Z, Zhou F, Dou Y, Tian X, Liu C, Li H, et al. Melatonin alleviates intracerebral hemorrhage-induced secondary brain injury in rats via suppressing apoptosis, inflammation, oxidative stress, DNA damage, and mitochondria injury. Translational Stroke Research 2018;9:74-91.
7. Senol N, Oguzoglu AS, Erzurumlu Y, Ascı H, Savran M, Gulle K, et al. Modulation of salubrinal-mediated endoplasmic reticulum stress in an experimental subarachnoid hemorrhage model. World Neurosurgery 2021;153:e488-e96.
8. Chen S, Li Q, Wu H, Krafft PR, Wang Z, Zhang JH. The harmful effects of subarachnoid hemorrhage on extracerebral organs. BioMed Research is International 2014;2014(1):858496.
9. Bernardo-Castro S, Sousa JA, Brás A, Cecília C, Rodrigues B, Almendra L, et al. Pathophysiology of blood-brain barrier permeability throughout the different stages of ischemic stroke and its implication on hemorrhagic transformation and recovery. Frontiers in Neurology 2020;11:594672.
10. Jiang X, Andjelkovic AV, Zhu L, Yang T, Bennett MV, Chen J, et al. Blood-brain barrier dysfunction and recovery after ischemic stroke. Progress in Neurobiology 2018;163:144-71.
11. Han DW, Oh JE, Lim BJ, Han Y, Song Y. Dexmedetomidine attenuates subarachnoid hemorrhage-induced acute lung injury through regulating autophagy and TLR/NFκB signaling pathway. Korean J Anesthesiol 2022;75:518-29.
12. Li T, Wang P, Gong X, Chong W, Hai Y, You C, et al. Prevalence and prognostic significance of liver fibrosis in patients with aneurysmal subarachnoid hemorrhage. Frontiers in Neurology 2022;13:850405.
13. Mobed A, Charsouei S, Yazdani Y, Gargari MK, Ahmadalipour A, Sadremousavi SR, et al. Biosensors, recent advances in the determination of BDNF and NfL. Cellular and Molecular Neurobiology 2023;43(8):3801-14.
14. Zhou J, Guo P, Guo Z, Sun X, Chen Y, Feng H. Fluid metabolic pathways after subarachnoid hemorrhage. Journal of Neurochemistry 2022;160(1):13-33.
15. Alfonso M, Aftab S, Hamadneh T, Sherali N, Tsouklidis N. Understanding cognitive deficit after subarachnoid hemorrhage: a memory focused approach. Cureus 2020;12(11).
16. Chen H, Dang Y, Liu X, Ren J, Wang H. Exogenous brain-derived neurotrophic factor attenuates neuronal apoptosis and neurological deficits after subarachnoid hemorrhage in rats. Experimental and Therapeutic Medicine 2019;18(5):3837-44.
17. Demedts IK, Demoor T, Bracke KR, Joos GF, Brusselle GG. Role of apoptosis in the pathogenesis of COPD and pulmonary emphysema. Respiratory Research 2006;7:1-10.
18. Suresh MV, Yalamanchili G, Rao TC, Aktay S, Kralovich A, Shah YM, Raghavendran K. Hypoxia-inducible factor (HIF)-1α-induced regulation of lung injury in pulmonary aspiration is mediated through NF-kB. FASEB BioAdvances 2022;4(5):309.
19. Sargon MF. Lungs and hypoxia: a review of the literature. Anatomy 2021;15(1):76-83.
20. Pereira M, Liang J, Edwards-Hicks J, Meadows AM, Hinz C, Liggi S, et al. Arachidonic acid inhibition of the NLRP3 inflammasome is a mechanism to explain the anti-inflammatory effects of fasting. Cell Reports 2024;43(2).
21. Galea J, Ogungbenro K, Hulme S, Patel H, Scarth S, Hoadley M, et al. Reduction of inflammation after administration of interleukin-1 receptor antagonist following aneurysmal subarachnoid hemorrhage: results of the Subcutaneous Interleukin-1Ra in SAH (SCIL-SAH) study. Journal of Neurosurgery 2017;128(2):515-23.
22. Hvas C, Nørregaard R, Nielsen T, Barklin A, Tønnesen E. Brain death increases COX-1 and COX-2 expression in the renal medulla in a pig model. Acta Anaesthesiologica Scandinavica 2014;58(2):243-50.
23. Priante G, Gianesello L, Ceol M, Del Prete D, Anglani F. Cell death in the kidney. International Journal of Molecular Sciences 2019;20(14):3598.
24. Ratliff BB, Abdulmahdi W, Pawar R, Wolin MS. Oxidant mechanisms in renal injury and disease. Antioxidants & Redox Signaling 2016;25(3):119-46.