







ORIGINAL ARTICLE / ÖZGÜN MAKALE

## Evaluation of Serum Allopregnanolone Levels in Acute Stroke Patients Presenting to The Emergency Department and Undergoing Mechanical Thrombectomy

Acil Servise Başvuran ve Mekanik Trombektomi Uygulanan Akut İnme Hastalarında, Serum Allopregnanolon Düzeylerinin Değerlendirilmesi

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### Abstract

**Objectives:** Ischemic stroke is a disease that occurs as a result of impaired perfusion of the brain and is the second most common cause of death after cardiovascular diseases. Biochemical marker studies for early diagnosis of stroke patients and predicting the prognosis of patients have been the focus of the attention of researchers. Our primary aim in this study was to examine the change in serum Allopregnanolone levels in patients with ischemic stroke who underwent mechanical thrombectomy.

**Methods:** Patients diagnosed with ischemic stroke and underwent mechanical thrombectomy in Samsun Training and Research Hospital Emergency Service between December 2020 and February 2021 were included in the study. Serum Allopregnanolone levels from blood samples were measured by Enzyme-Linked Immuno Sorbent Assay (ELISA) method.

**Results:** Twenty-two stroke patients who underwent mechanical thrombectomy and 20 healthy volunteers were included in the study. The mean serum Allopregnanolone value at 0h was 56,439 (31.71-253.07), and the median serum Allopregnanolone value of the healthy control group was 51.219 (23.43-87.98), and no statistically significant difference was found ( $p=0.078$ ). No significant difference existed between the patient group's 0th-hour, 12th-hour, and 5th-day Allopregnanolone levels ( $p=0.554$ ). There was no significant relationship between the serum Allopregnanolone levels at the 0th hour, 12th hour, and 5th day of the patient group and the 3rd-month mRS scores ( $p=0.713$ ,  $p=0.616$ ,  $p=0.867$ , respectively).

**Conclusion:** Serum Allopregnanolone levels do not help diagnose, treat, and prognosis of patients with acute ischemic stroke who underwent mechanical thrombectomy. A clinical study with more patients should be updated with our findings.

**Keywords:** Ischemic Stroke, Mechanical Thrombectomy, Allopregnanolone, Diagnosis, Prognosis

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## Öz

**Amaç:** İskemik inme, beynin perfüzyonunun bozulması sonucu oluşan bir hastalıktır ve kardiyovasküler hastalıklardan sonraki ikinci en sık ölüm nedenidir. İnme hastalarına erken tanı koymak ve hastaların prognozlarını öngörmek amacıyla yapılan biyokimyasal belirteç çalışmaları araştırmacıların ilgi odağı olmuştur. Bu çalışmadaki primer amacımız mekanik trombektomi uygulanan iskemik inme hastalarındaki serum Allopregnanolon düzeylerindeki değişimi incelemektir.

**Yöntem:** Çalışma Aralık 2020 ile Şubat 2021 tarihleri arasında Samsun Eğitim ve Araştırma Hastanesi Acil Servisinde iskemik inme tanısı almış ve mekanik trombektomi yapılan hastalar dahil edildi. Alınan kan örneklerinden serum Allopregnanolon düzeyleri Enzyme-Linked Immuno Sorbent Assay (ELISA) yöntemiyle ölçüldü.

**Bulgular:** Çalışmaya mekanik trombektomi yapılan 22 inme hastası ve kontrol grubunu oluşturan 20 sağlıklı gönüllü dahil edildi. 0.saat serum Allopregnanolon ortanca değeri 56.439 (31.71-253.07), sağlıklı kontrol grubunun serum Allopregnanolon ortanca değeri 51.219 (23.43-87.98) olup istatistiksel olarak anlamlı fark saptanmamıştır ( $p=0.078$ ). Hasta grubunun 0.saat, 12.saat ve 5.gün Allopregnanolon düzeyleri arasında anlamlı fark yoktu ( $p=0.554$ ). Hasta grubunun 0.saatteki, 12.saatteki ve 5.gündeki serum Allopregnanolon düzeyleri ile 3. ay mRS skorları arasında anlamlı ilişki yoktu ( $p=0.713$ ,  $p=0.616$ ,  $p=0.867$ ; sırasıyla).

**Sonuç:** Serum Allopregnanolon düzeyleri, mekanik trombektomi uygulanan akut iskemik inmeli hastaların tanı, tedavi ve prognozuna yardımcı olamaz. Daha fazla sayıda hasta içeren bir klinik çalışma ile bulgularımız güncellenmelidir.

**Anahtar Sözcükler:** İskemik İnme, Mekanik Trombektomi, Allopregnanolon, Tanı, Prognoz

## INTRODUCTION

Ischemic stroke is a clinical condition due to insufficient cerebral blood flow (1). Anamnesis, physical examination, non-contrast computed tomography (CT), and magnetic resonance imaging (MRI) are used to diagnose patients presenting to the emergency department with stroke symptoms. However, CT without contrast can give negative results in the first 24 hours, and MRI shows 80% of strokes in the first 24 hours (2). Stroke treatments include antiaggregants, anticoagulants, intravenous thrombolytic agents (iv-tpa), and endovascular interventions. In selected patients after stroke, mechanical thrombectomy can be performed in the presence of contraindications for intra-arterial or systemic intravenous-tissue plasminogen activator in patients who come to the emergency department in the first 24 hours (3).

Steroid hormones secreted from endocrine organs such as gonads, adrenal cortex, and placenta can reach the Central Nervous System by crossing the blood-brain barrier thanks to their

lipophilic structure. Hormones synthesized from cholesterol in the central nervous system and acting on neurons are called neurosteroids (4). It is known that steroid hormones generally exert their effects as transcription factors in regulating gene expression by binding to intracellular receptors. Due to these effects on gene transfer, they affect cell division (5). One of these neurosteroids,  $3\alpha$ ,  $5\alpha$  tetrahydro progesterone (Allopregnanolone), acts through nuclear steroid receptors and GABA receptors. Allopregnanolone is a vital regulator that affects neuron excitability via GABA-A receptors (6,7). Its sensitivity to hypoxia and its high concentrations in brain tissue make Allopregnanolone a biomarker that can be used in stroke patients.

Our primary outcome in this study was to determine the change in serum Allopregnanolone levels in patients with ischemic stroke who underwent mechanical thrombectomy. Our secondary outcome is determining the value of serum allopregnanolone in the diagnosis and

prognosis of the disease.

## METHODS

Our study was approved by the Ondokuz Mayıs University Clinical Research Ethics Committee on 14.01.2021 with protocol number 2021/015. The Declaration of Helsinki, Good Clinical Practices, and Good Laboratory Practices carried it out.

Our study was planned as a prospective, descriptive study. The study was conducted between 01.12.2020-01.03.2021 in Samsun Training and Research Hospital emergency service and neurology clinic. Patients over 18 who applied to the emergency department, were diagnosed with ischemic stroke clinically and radiologically, and underwent mechanical thrombectomy due to large vessel occlusion were included in our study. Those under 18, Patients with a previous history of cerebrovascular disease, cardiopulmonary arrest before or after admission to the emergency department, and a history of last intracranial surgery and epilepsy were excluded from the study. A control group consisting of healthy volunteers with demographic characteristics similar to the patient group was also included in the study. Serum Allopregnanolone levels in the patient group at the time of admission (0. hour), 12th hour, and 5th day after mechanical thrombectomy; In the control group, a single peripheral venous blood sample was taken and evaluated.

At the same time, age and gender, time from symptom onset to recanalization, and Modified Rankin Scale (mRS) score at the third month were recorded. mRS evaluated a stroke's prognosis and functional recovery at 3 months. Good results for mRS were defined as mRS 0-2, bad results as 3-6, and death of the patient within 90 days was defined as mRS=6 (8).

Peripheral venous blood samples from all participants were centrifuged at 1500 rpm for 10 minutes. Samples were stored at -80 °C until analysis. The commercially available Enzyme-Linked immunosorbent assay (ELISA) method was used to determine serum Allopregnanolone level. Allopregnanolone was detected in serum with the DetectX® Allopregnanolone ELISA Kit (Bioassay Technology Laboratory, Zhejiang, China). Analysis was performed following the instructions given by the manufacturer in the kit insert. An ELISA reader recorded

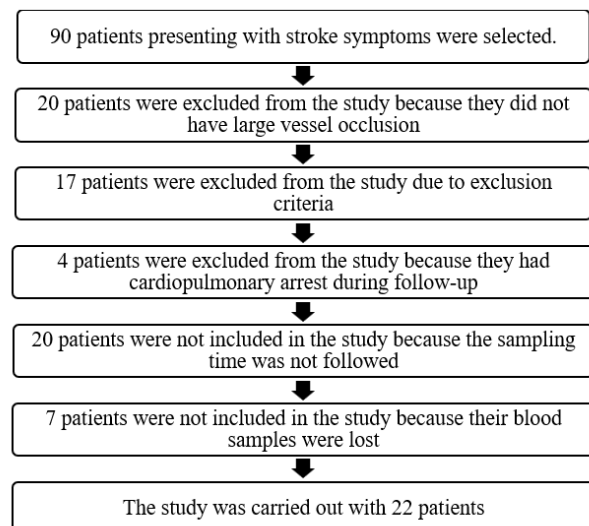
results at 450 nm (nanometers) (Tecan®, Infinite M200 pro, Austria).

## Statistical Analysis

The collected data were analyzed using the IBM SPSS 24.0 (Chicago, USA) package program. The conformity of the data to the normal distribution was determined by the Shapiro-Wilks test. Normally distributed numerical data were expressed as mean  $\pm$  standard deviation, and non-normally distributed numerical data were expressed as median (minimum-maximum). For normally distributed values, a t-test was used for parametric analyses, Mann Whitney U test, and a Friedman two-way analysis of variance were used for non-parametric analyses for non-normally distributed values. The chi-square test was used to compare categorical variables. The correlation of alloregnonal levels in temporal processes was evaluated with Spearman correlation analysis. All studies were analyzed at a 95% confidence level. The statistical significance level for all analyses was accepted as  $p < 0.05$ .

## RESULTS

In our study, 22 patients who underwent mechanical thrombectomy constituted the patient group, and 20 healthy volunteers formed the control group. The study flowchart is presented in Figure 1.



**Figure 1.** Study Flow Chart

The mean age of the patient group was  $69.04 \pm 11.23$ , while the mean age of the control group was  $68.38 \pm 14.60$ . The patient and control groups were similar in age and gender (Table 1).

**Table 1.** Comparison of age and gender characteristics between patient and control groups

	Patients Group (n=22)	Control Group (n=20)	p
Age	69.04 ± 11.23	68.38 ± 14.60	p=0.761
Gender	Male	8 (40)	p=0.286
	Female	12 (60)	

The comparison of Allopregnanolone levels of the patient and control groups is presented in Table 2. Accordingly, serum Allopregnanolone levels obtained at admission were similar in the patient and control groups (p=0.078).

**Table 2.** Comparison of serum Allopregnanolone levels between patient and control groups

Allopregnanolone	Group	Median	Minimum	Maximum	p
0. hour	Patients (n=22)	56.438	31.71	253.07	0.078
	Controls (n=20)	51.219	23.43	87.98	

The temporal variation of serum Allopregnanolone levels at 0 hours, 12 hours, and 5 days for the patient group is presented in Table 3. The median value of serum Allopregnanolone levels at 0 hours (at admission) was 56.439 (31.71-253.07), the median value at 12th hours was 52,989 (34.5-261.03), and the median value at day 5 was 52.672 (35.35-250.3). Accordingly, there was no significant difference between serum Allopregnanolone levels measured at 0, 12, and 5 days in ischemic stroke patients who underwent mechanical thrombectomy (p=0.554).

**Table 3.** Evaluation of Allopregnanolone levels of the patient group at the time of admission (0.hour), 12.hour, and 5th day

Allopregnanolone	Median	Minimum	Maximum	p
0.hour	56.439	31.71	253.07	0.554
12.hour	52.989	34.50	261.03	
5.day	52.673	35.35	250.30	

The correlation of serum Allopregnanolone levels of these temporal processes with each other is presented in Table 4. Accordingly, a moderate positive correlation was found between the 0th-hour Allopregnanolone levels and the 12th-hour and 5th-day Allopregnanolone levels.

The median value of symptom-recanalization time for the patients in our study was 309 (120-617) minutes.

**Table 4.** Correlation of Allopregnanolone levels with each other in temporal processes

Allopregnanolone	0.hour	12.hour	5.hour
0.hour	r*	1.000	
	p	.	
12.hour	r*	0.528	1.000
	p	<b>0.012</b>	.
5.day	r*	0.431	0.625
	p	<b>0.045</b>	<b>0.002</b>
	n	22	22

\*: correlation coefficient

The relationship between serum Allopregnanolone levels at 0, 12, and 5 days of the patient group and the 3rd-month mRS scores are presented in Table 5. Accordingly, it was determined that Allopregnanolone levels measured at the time of application and afterward were not statistically significantly correlated with the 3rd-month mRS score (p=0.713, p=0.616, p=0.867; respectively).

**Table 5.** Evaluation of Allopregnanolone levels of the patient group according to the 3rd month MRS score

Allopregnanolone	3.month mRS Score	Median	Minimum	Maximum	p
0.hour	Good (n=9)	81.285	33.18	252.01	0.713
	Worse (n=13)	77.484	31.71	253.07	
12.hour	Good (n=9)	58.324	34.50	261.02	0.616
	Worse (n=13)	51.041	36.97	261.03	
5.day	Good (n=9)	68.449	47.33	250.3	0.867
	Worse (n=13)	49.655	35.35	247.3	

## DISCUSSION

Since emergency services are the first point of contact for acute stroke patients, early diagnosis of stroke patients becomes essential. However, robust diagnostic and prognostic biomarkers are currently unavailable for stroke patients. In addition, there are limited marker studies on mechanical thrombectomy, which is critical for treating stroke.

In this study, where we aimed to measure the diagnostic and prognostic value of serum Allopregnanolone levels in stroke patients who underwent mechanical thrombectomy, we found that the serum Allopregnanolone

levels of the patients who underwent mechanical thrombectomy were similar to those of the control group consisting of healthy volunteers. Stroke patients requiring mechanical thrombectomy are patients with large vessel occlusion. In this context, allopregnanolone levels are not a valuable parameter in diagnosing the disease.

Age has an important place among stroke risk factors—the risk of stroke increases, especially over 65 (9,10). In the study by Kocatürk et al., the mean age of patients diagnosed with ischemic stroke was 65 (11). In our study, the mean age was higher than the literature data. The reason for this difference may be that the patient group in our study consisted of patients who required mechanical thrombectomy.

According to the Ministry of Health data, it has been reported that patients with ischemic stroke in our country are generally female (12). In our study, there was a female predominance in the patient group. Our findings are compatible with the literature.

When the serum Allopregnanolone levels of the patient group were evaluated in the following time period, we found that the values at the time of admission and the 12th hour and 5th day after mechanical thrombectomy were similar. These findings suggest that allopregnanolone levels do not change with thrombectomy treatment and are not suitable parameters for the follow-up of these patients.

When the correlations between the 0th-hour serum Allopregnanolone levels and the 12th-hour post-thrombectomy and 5th-day post-thrombectomy values of the patients were examined, A moderate positive correlation was found between the 0th-hour Allopregnanolone level and the 12th hour and 5th-day Allopregnanolone levels. According to these data, the Allopregnanolone levels of the patients at the time of admission affect the Allopregnanolone levels in the following period.

It has been observed that the longer the time between the onset of stroke symptoms and the start of treatment in stroke patients, the longer the time between mortality and morbidity. In studies in the literature, the duration of symptom recanalization is 270 minutes (11). The median value of symptom-recanalization time for the patients in our study was 309 (120-617)

minutes. It was observed that the patients participating in our study had higher symptom-recanalization times. Since our hospital's stroke center serves the region, it can receive referrals from different hospitals. This situation is effective in the high symptom-recanalization time.

The prognosis of patients after stroke is evaluated by 3rd monthly mRS scores (8). Our study found no statistically significant correlation between serum Allopregnanolone levels at admission (0. hour), 12. hours and 5th days after mechanical thrombectomy, and mRS scores at the 3rd month. In this context, serum allopregnanolone levels cannot be used as an indicator of the prognosis of the disease.

### Limitations

The main limitations of our study are the small number of patients and the fact that it is a single-center study.

### CONCLUSION

Serum Allopregnanolone levels do not help diagnose, treat, and prognosis of patients with acute ischemic stroke who underwent mechanical thrombectomy. A clinical study with a higher number of patients should support our findings.

### ACKNOWLEDGEMENT

#### Conflict of Interest

The authors declare no conflict of interest

#### Support Resources

This research received no specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

#### Ethical Declaration

Our study was approved by the Ondokuz Mayıs University Clinical Research Ethics Committee on 14.01.2021 with protocol number 2021/015. The Declaration of Helsinki, Good Clinical Practices, and Good Laboratory Practices carried it out.

#### Authorship Contributions

Concept: HTY, MY, Design: MY, MG, MY, Supervising: HTY, MG, MY, SG, Data collection and entry: HTY, ÇKA, MY Analysis, and interpretation: MY, HTY, Literature search: HTY, MG, MY, Writing: HTY, MG, ÇKA, Critical review: MY, SG, ÇKA

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