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The role of allopregnanolone level in the diagnosis of the disease in transient ischemic attack patients

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Abstract

Aim: The risk of stroke increases in patients developing transient ischemic attack (TIA) in the short term, and early initiation of treatment significantly reduces this risk. Therefore, serum biomarkers that confirm or exclude TIA will be very valuable in clinical practice. This study aimed to investigate the relationship between the Allopregnanolone (AP) levels of TIA patients.

Methods: We conducted a prospective observational study of consecutive emergency department patients with TIA (December 2018–October 2019) and age/sex-matched healthy controls. Serum allopregnanolone (AP) was measured by ELISA at presentation and compared between groups, and diagnostic performance was assessed using ROC analysis.

Results: 40 patients (17 women 42.5%, 23 men 57.5%) who met the inclusion criteria, and 40 healthy volunteers were included in the study. AP levels were found to be significantly higher in both men and women according to their gender compared to the control group ($p<0.001$). AP levels were significantly higher in the patients compared to the control group (197.04±32.15 vs. 128.75±12.66, $p<0.001$). The optimum cut-off value of AP in detecting TIA was determined as AUC: 0.740 (0.630-0.850), sensitivity: 70%, and specificity: 72.5% at 134.5.

Conclusion: Our study demonstrates that serum allopregnanolone levels are significantly elevated in patients diagnosed with transient ischemic attack. These results, obtained from a controlled cohort with rigorous exclusion criteria, provide a foundation for future studies investigating the diagnostic role of neurosteroids in cerebrovascular events.

Keywords: Allopregnanolone, transient ischemic attack, emergency care, biomarkers

Introduction

Transient ischemic attack (TIA) is a clinical syndrome characterized by short-term symptoms of acute, focal cerebral, or monocular dysfunction due to insufficient blood flow, and its clinical diagnosis may be difficult for both emergency physicians and neurologists (1). It is known that the risk of stroke increases in patients developing TIA in the short term, and early initiation of treatment significantly reduces this risk (1,2). Therefore, serum biomarkers that confirm or exclude TIA will be very valuable in clinical practice. Rapid and accurate exclusion of TIA will save unnecessary patient referrals and may provide quick and effective treatment for early diagnosis (3).

Neurosteroids are a subset of neuroactive steroids that can be synthesized in glia and neurons in many brain regions, including the neocortex and hippocampus. These steroids include hormonal steroids from the peripheral glands, steroids synthesized de novo by the nervous system, and synthetic steroids that can alter nervous system functions (4-6). Allopregnanolone (AP), the most important neuroactive steroid, is an allosteric modulator of both g-aminobutyric acid A (GABA-A) and serotonin (5-HT3) receptors and is likely to have neuroprotective, and psychiatric properties. In experimental studies, AP has been shown to inhibit the expression of vasopressin and corticotropin-releasing hormone, which affect the reproductive activity of the hypothalamic-pituitary-adrenal system (4-7). AP has been shown to be neuroprotective in different experimental models such as trauma, ischemia and neurodegenerative diseases, and it has been shown to be effective in reducing volume infarction in ischemic lesion (6-10).

However, the relationship between AP levels in TIA patients has not been examined in any previous study to the best of our knowledge. Therefore, this study aimed to investigate the relationship between the AP levels of TIA patients at the time of admission.

Materials and Methods

The study was performed in accordance with the principles of the Declaration of Helsinki. Ethical approval was obtained from the Süleyman Demirel University, Faculty of Medicine local ethics committee (protocol code: 2019/290721), and written informed consent was obtained from all participants.

This study was supported by Süleyman Demirel University Scientific Research Projects Coordination Unit (project no: TTU-2019-6929).

Sample Size

Based on prior literature evaluating serum allopregnanolone levels in neurological disorders, a large effect size was anticipated between TIA patients and healthy controls. Assuming an effect size of d = 0.8, with a two-sided alpha level of 0.05 and a power of 80%, a minimum of 26 participants per group was required for statistical significance using the Mann-Whitney U test.

To enhance reliability and account for potential deviations from normality or data loss, we included 40 participants in each group, which exceeds the calculated minimum and ensures sufficient statistical power for detecting clinically meaningful differences.

Study design

This prospective observational study enrolled patients admitted to a tertiary university emergency department and diagnosed with TIA between December 2018 and October 2019. Consecutive adults (≥18 years) presenting within 24 hours of symptom onset with suspected transient focal neurological deficits were prospectively screened. The initial assessment and eligibility screening were performed by an emergency physician. All suspected cases were then evaluated by a board-certified neurologist, and the final TIA diagnosis was confirmed according to AHA/ASA criteria.

In the event of disagreement between the emergency physician and the neurologist, cases were adjudicated by consensus; if consensus could not be reached or an alternative diagnosis was favored, the patient was excluded.

The control group comprised age- and sex-matched healthy volunteers. Eligibility required the absence of known vascular risk factors—including physician-diagnosed hypertension, diabetes mellitus, coronary artery disease, atrial fibrillation—or prior TIA/stroke, and no active endocrine or inflammatory disease. Individuals who were pregnant or using oral contraceptives/hormone therapy, as well as those taking antiplatelet, anticoagulant, statin, or antihypertensive medications, were excluded. Eligibility was verified by a structured medical history and medication review at enrollment.

Inclusion criteria were age ≥18 years, presentation within 24 hours of the first symptom, and a diagnosis of TIA based on American Heart Association/American Stroke Association criteria.

Exclusion criteria included evidence of increased intracranial pressure or clinical/imaging signs suggestive of a structural brain lesion; prior neurosurgical intervention; chronic conditions affecting steroid metabolism (e.g., sex-hormone disorders, thyroid dysfunction), liver or hematologic disease; pregnancy; or oral contraceptive use. Demographics (age, sex), presenting complaints and timing, electrocardiography findings, comorbidities, and medication history were recorded on a standardized case report form.

The detailed patient selection process is illustrated in Figure 1.

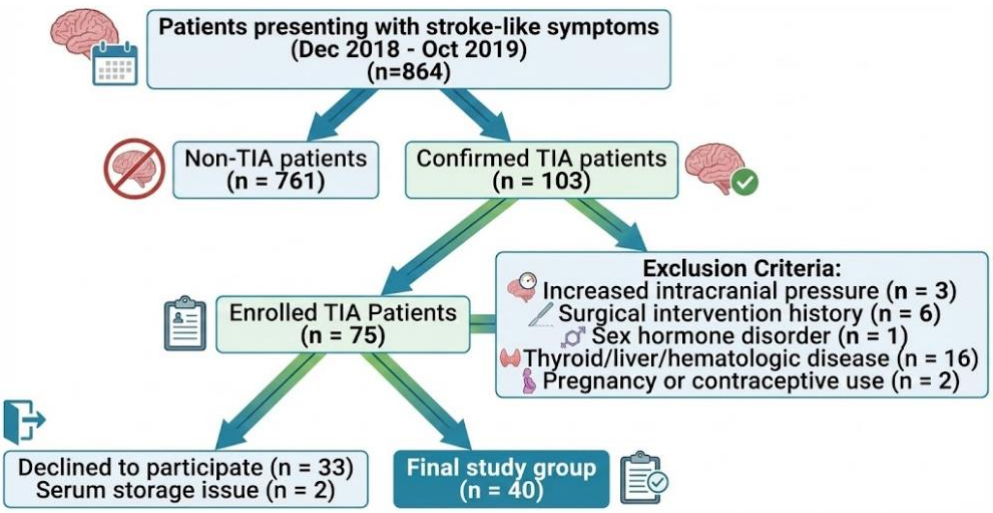

Figure 1. Flow chart of the study.

Data collection

Venous blood was drawn before any treatment and transferred to additive-free tubes. Samples were centrifuged at 4,000 rpm for 5 min, and the resulting serum was aliquoted and stored at −80 °C until analysis. For the assay, serum aliquots (≈1 mL) were thawed on ice and mixed gently to avoid foaming.

Serum allopregnanolone (AP) concentrations were expressed in ng/mL and quantified using a commercial ELISA kit (Shanghai YL Biotech Co., Ltd., Shanghai, China; Cat. No.: YLA4065HU). A calibrator series was prepared by serial two-fold dilutions to yield 20, 10, 5, 2.5, 1.25, 0.625, 0.313, and 0 ng/mL standards (the undiluted stock served as the highest standard; sample diluent as 0 ng/mL). Following the manufacturer's instructions, 50 µL of each calibrator or serum sample and 50 µL of the biotinylated detection antibody were dispensed into the microplate wells and incubated at 37 °C for 45 min, after which the plate was washed three times. The horseradish peroxidase conjugate (100 µL/well) was then added and incubated at 37 °C for 30 min, followed by five wash cycles. Next, 100 µL of substrate solution was dispensed into each well and incubated at 37 °C for 5 min; the reaction was read immediately at 450 nm.

All measurements were performed in duplicate, and the mean value was used for analysis; samples with duplicate CV >15% were re-run. Concentrations were derived from a four-parameter logistic (4-PL) standard curve.

Statistical Analysis

Statistical analysis of our study was performed using SPSS 23.0 software (IBM Inc., Armonk, NY, USA). Continuous variables in the patient and control groups were given as mean±standard deviation, and categorical data were given as frequency and percentage (%). Student t-test was used to compare TIA patients and the control group for normally distributed variables, while the Mann-Whitney U test was used for non-normally distributed variables. The optimum cut-off value of AP in detecting TIA will be examined by Receiver Operating Characteristic (ROC) analysis, and $p<0.05$ was considered statistically significant.

Results

Of the 864 patients who presented with stroke-like symptoms between December 2018 and October 2019, 103 were diagnosed with transient ischemic attack (TIA). After applying the exclusion criteria, 75 eligible patients remained. Of these, 35 were excluded due to refusal to participate (n = 33) or serum storage issues (n = 2), resulting in a final study cohort of 40 patients (17 women [42.5%], 23 men [57.5%]). In addition, 40 healthy volunteers (16 women [40%], 24 men [60%]) with similar demographic characteristics were included as the control group.

The mean age of the TIA patients was 70.15±13.71, and the mean age in the control subgroup was 70.25±13.52. The most common symptoms were slurred speech or difficulty understanding in 21 patients (52.5%), weakness/numbness or paralysis in 9 patients (22.5%), and syncope in 8 patients (20%), respectively. Hypertension, coronary heart disease, and atrial fibrillation were the most common risk factors. 25 (62.5%) of the TIA patients included in the study applied within the first 4 hours. The most common time of admission was nighttime (00:00-08:00) with 19 patients (47.5%). The main characteristics of the patients were given in Table 1.

Table 1. The clinical characteristics of patients with TIA.

Variables	Total (n=40)
Age (years)	70.15±13.71
Male gender n(%)	23 (57.5)
Symptoms n(%)	
Weakness /numbness or paralysis	9 (22.5)
Slurred speech or difficulty understanding	21 (52.5)
Blindness in one or both eyes	4 (10)
Dizziness	6 (15)
Syncope	8 (20)
Others	2 (5)
Clinical history n(%)	
Hypertension	19 (47.5)
Atrial fibrillation	10 (25)
Coronary artery disease	15 (37.5)
Others	8 (20)
Medications	
Antiplatelet agents	8 (20)
Oral anticoagulants	0 (0)
Antihypertensive drugs	19 (47.5)
Time of onset of the symptom	
<4 h	25 (62.5)
4-24h	15 (37.5)
Time of arrival	
Daytime (08:00-16:00)	6 (15)
Evening (16:00-00:00)	15 (37.5)
Nighttime (00:00-08:00)	19 (47.5)

TIA: Transient ischemic attack

AP levels were found to be significantly higher in TIA patients at <65 years of age compared to the control group (184.10±28.31 vs. 146.39±27.66, p<0.001). AP levels were also found to be statistically significantly higher in TIA patients at ≥65 years of age compared to the control group (204.01±47.45 vs. 120.26±69.33, p<0.001). AP levels were found to be significantly higher in both men and women according to their gender compared to the control group (p<0.001). A statistically significant difference was found when the mean AP levels of the TIA patients and the control group were compared. AP levels were significantly higher in the patients compared to the control group (197.04±32.15 vs. 128.75±12.66, p<0.001) (Fig. 2). There was no significant relationship between the clinical history and symptom duration of the patients and AP levels (p>0.05). Comparison of the AP values of the patient and control groups between the groups was given in Table 2. However, the efficacy of AP in predicting TIA was calculated by drawing ROC curves (Fig. 3). The optimum cut-off value of AP in detecting TIA was determined as AUC: 0.740 (0.630-0.850), sensitivity: 70%, and specificity: 72.5% at 134.5.

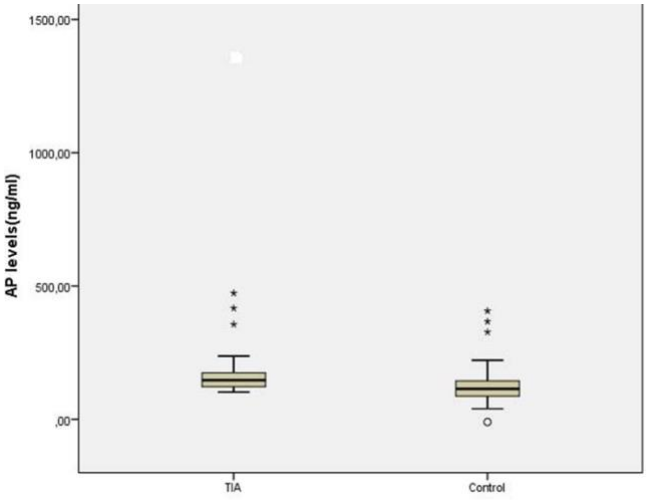

Figure 2. Comparison of Allopregnanolone (ng/ml) levels between TIA and control groups.

Table 2. Baseline characteristics of patients and AP (ng/ml) levels

Variables	Patients AP (ng/ml) mean ± SD	Controls AP (ng/ml) mean ± SD	p
Age (years)			
<65	184.10±28.31	146.39±27.66	<0.001
≥65	204.01±47.45	120.26±69.33	<0.001
Gender			
Female	179.79±26.02	121.37±14.82	<0.001
Male	209.79±52.98	139.82±22.89	<0.001
Mean AP level	197.04±32.15	128.75±12.66	<0.001
Clinical history			
Hypertension		-	0.101
Had	188.44±22.44		
Absent	204.82±26.81		
Atrial fibrillation		-	0.333
Had	159.16±9.68		
Absent	209.67±23.38		
Coronary artery disease		-	0.476
Had	165.58±23.62		
Absent	215.91±24.76		
Time of onset of the symptom		-	0.586
<4 h	212.87±49.87		
4-24h	182.79±29.52		

AP: Allopregnanolon

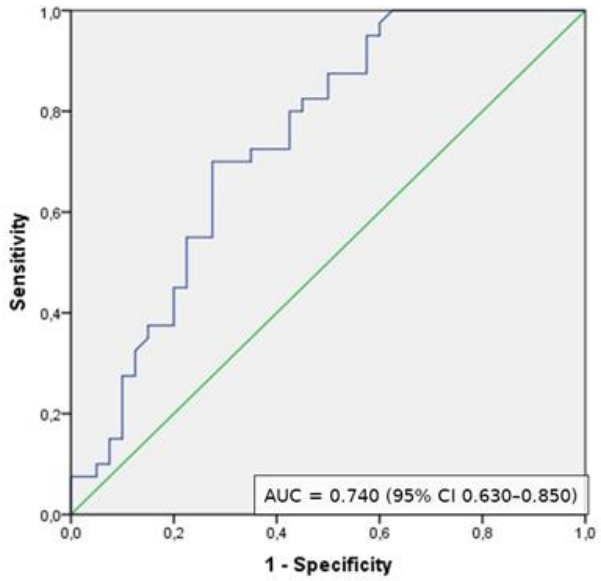

Figure 3. Receiver operating characteristic (ROC) curve of AP levels for predicting TIA.

Discussion

Stroke still maintains its importance due to its sequelae, high mortality and impact on quality of life. This situation brings a serious financial and moral burden to the whole society (8). Early diagnosis and treatment of this disease, which has high mortality and morbidity, is coming into prominence. Early diagnosis and treatment of TIA become an important issue as it is a precursor in terms of all thromboembolic events (9). AP was used for the first time in the literature in our study, and it was concluded that it may be useful in diagnosis since there is no routinely applicable biomarker for TIA. A diagnostic biomarker useful for TIA should first be detectable in the blood from the early hours of symptoms and even sensitive to low-grade ischemia (10). Many parameters have been used in the diagnosis and prognosis of TIA in the literature, and the main ones are plasminogen activator inhibitor-1, ubiquitin fusion degradation protein-1, nucleoside diphosphate kinase-A, fibrinogen, and D-dimer (11,12). In a recent study by Tian et al. soluble ST2 was associated with short- and long-term poor prognosis in patients with TIA (13). Blek et al. revealed the association of Copeptin with stroke in their study and reported that it may be a strong tool for early risk classification in TIA patients (14). Wang et al. found high CRP levels in TIA patients and reported their diagnostic usability (15). In a study conducted by George et al. platelet basic protein was defined as a candidate serum biomarker for TIA by using proteomics based on mass spectrometry (16). Our study revealed the usability of AP in predicting TIA for the first time in the literature. Mechanistically, allopregnanolone is an allosteric modulator of GABA-A (and 5-HT3) receptors and influences hypothalamic–pituitary–adrenal axis signaling; experimental models of ischemia have shown neuroprotective effects and reduced infarct volumes, which may underlie the elevated AP observed in our TIA cohort (7,10).

Neurosteroids are important neuromodulators that regulate many brain functions, activate and inhibit neuronal communication and interaction. The effect of neurosteroids on neurons by easily crossing the blood-brain barrier has been known for many years (17). The role of AP, one of the neuroactive steroids, in neuropsychiatric diseases has been reported by studies (18). In a study conducted by Rustichelli et al. AP levels were found to be significantly lower in menstrual-related migraine and postmenopausal migraine groups compared to control groups (19). It was reported in a study conducted by Coskun et al. that low AP levels could be used to show pathological results on tomography in patients with head trauma (20). Luisi et al. reported that serum AP levels were higher in patients with pre-eclampsia in their study (21). It has been shown to increase AP levels in patients with alcohol intoxication in another study conducted by Torres et al. AP is a derivative of progesterone and its concentrations may vary in many physiological and pathological conditions as progesterone can be synthesized in the ovaries, placenta and adrenal glands as well as neurons and glia as neurosteroids (22). In addition, since the mechanisms, targets and effectors of the neuroprotective effects of progesterone and allopregnanolone have not been adequately clarified, their relationship with many diseases and conditions continues to be studied. Our study has shown that AP level increases in TIA patients and AP is a diagnostic marker.

Although serum AP showed moderate discriminative ability (AUC 0.74), these data do not support its use as a stand-alone diagnostic. In clinical practice, AP is more likely to be useful as an adjunct, ideally as part of a multi-marker panel combined with bedside assessment and neuroimaging to improve diagnostic accuracy and triage. The kinetics of AP after TIA remain undefined. Future studies should include serial sampling (e.g., 0–6–24–72 h) and comparisons with established candidates (e.g., ST2, copeptin, hs-CRP and others) to determine whether AP provides incremental value over clinical variables and imaging. Multicenter prospective cohorts with multivariable modeling, decision-curve analysis, and reclassification metrics are warranted.

Limitations

This study had some limitations. The first of these is that our study is a single-center study. Secondly, other neurosteroids and cortisol levels along with AP levels were not included in the study. Thirdly, AP levels taken at the time of admission to the emergency department were included in the study, and basal and follow-up AP levels could not be examined. Finally, the lack of diagnostic comparison with biomarkers such as

plasminogen activator inhibitor-1, ubiquitin fusion degradation protein-1, nucleoside diphosphate kinase-A, fibrinogen, and D-dimer are one of our most important limitations. To minimize potential sources of bias, strict inclusion and exclusion criteria were applied, and all AP measurements were performed using the same ELISA kit under standardized laboratory conditions. However, as the study was observational and single-centered, selection bias and measurement bias could not be entirely excluded.

Conclusion

This study provides the first evidence that serum allopregnanolone levels are significantly elevated in patients diagnosed with transient ischemic attack (TIA) at the time of emergency department admission. The consistency of this elevation across age and sex subgroups strengthens the reliability of the findings. Although several serum biomarkers have previously been investigated for their diagnostic utility in TIA, none have gained widespread clinical application due to limited specificity, accessibility, or reproducibility. Allopregnanolone, as a neurosteroid with known roles in neuroprotection, appears to respond to transient cerebral ischemia in a measurable and statistically meaningful manner.

The data presented here suggest that allopregnanolone has potential as a diagnostic adjunct in the early identification of TIA, particularly in cases where clinical and radiological findings remain inconclusive. Its measurement in the acute phase may contribute to timely decision-making and more accurate risk stratification. Nevertheless, the limitations of the current study—namely its single-center design and the absence of comparative evaluation with other candidate biomarkers—indicate a need for broader, multicenter investigations. Future research should explore the dynamic profile of allopregnanolone levels over time and assess its integration into biomarker panels to improve diagnostic precision in TIA.

Disclosures

Ethical Approval: Ethics committee approval was received for this study from the Süleyman Demirel University, Faculty of Medicine local ethics committee, in accordance with the World Medical Association Declaration of Helsinki, with the approval number: 2019/290721.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – A.Ş., N.G.B., H.H.A., A.Y.H., İ.G.Y., F.Ç.O., C.B.; Design – N.G.B., A.S., H.H.A., A.Y.H., İ.G.Y., F.Ç.O., C.B.; Supervision – C.B., F.S.; Materials – İ.G.Y., F.Ç.O.; Data collection &/or processing – A.S., H.H.A., A.Y.H., İ.G.Y., F.Ç.O., C.B.; Analysis and/or interpretation – N.G.B., A.S., H.H.A., C.B.; Literature search – N.G.B., A.S., H.H.A., A.Y.H., İ.G.Y., F.Ç.O., C.B.; Writing – A.Ş.; Critical review – A.Y.H., İ.G.Y., F.Ç.O., C.B.

Conflict of Interest: There is no any conflict of interest.

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