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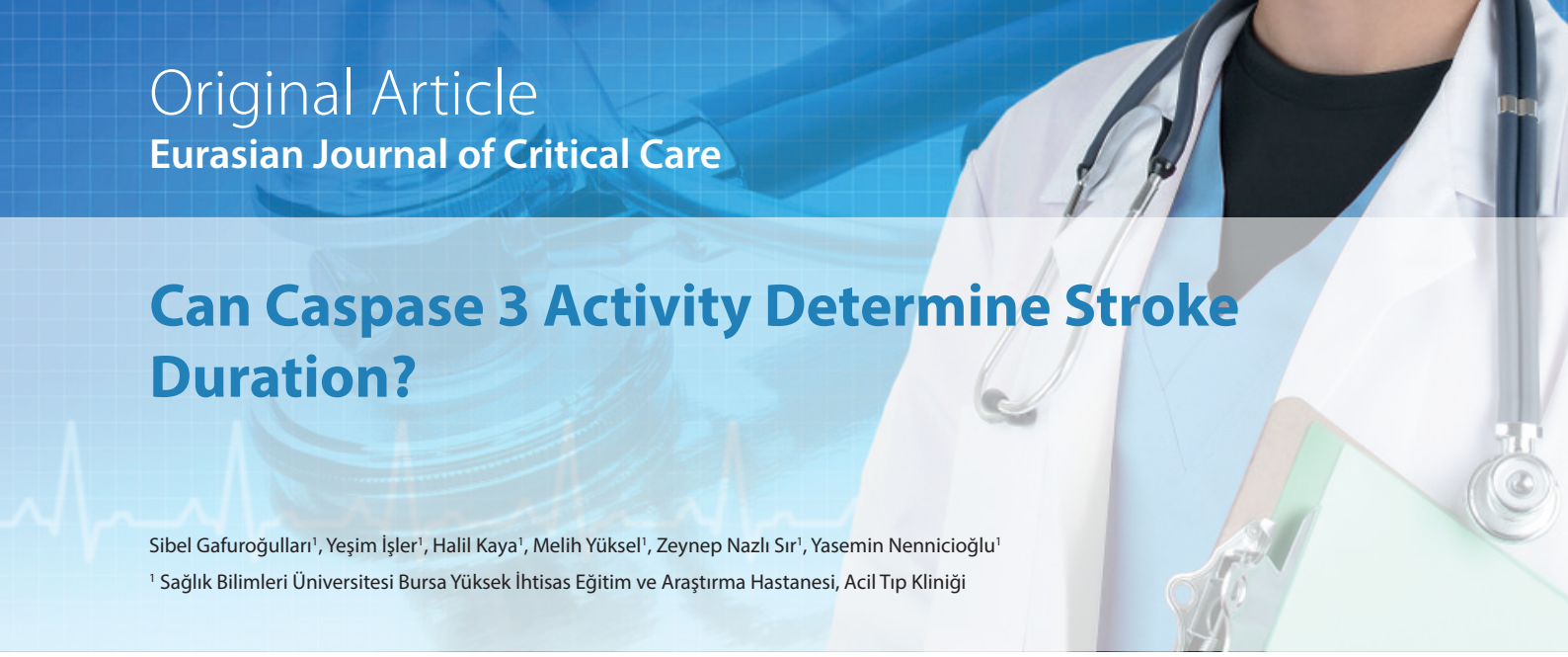

Can Caspase 3 Activity Determine Stroke Duration?

Sibel Gafuroğulları[1], Yeşim İşler[1], Halil Kaya[1], Melih Yüksel[1], Zeynep Nazlı Sır[1], Yasemin Nennicioğlu[1]
[1] Sağlık Bilimleri Üniversitesi Bursa Yüksek İhtisas Eğitim ve Araştırma Hastanesi, Acil Tıp Kliniği

Introduction

Stroke is one of the most important emergencies in neurological diseases. Ischemic stroke has an important place among emergency department admissions. One of the most important questions in assessing the thrombolytic treatment suitability of patients is to know the onset duration of stroke. Door - needle time for thrombolytic therapy is 4.5 hours. Therefore, time is very important for thrombolytic treatment. If there is no any person present with the patient at the time of stroke, or stroke occurred while asleep, the patient loses the chance of thrombolytic therapy because the onset duration of stroke is unknown. Despite the emerging technology and many biochemical parameters for such patients, a biomarker that still provides information about time has not been found.

Depending on the duration and severity of ischemia, necrosis or apoptosis, or both, may cause death of brain cells[1]. Apoptosis is a programmed, organized cell death process. Activation has been described after brain ischemia[2]. Apoptosis occurs after a complex caspase cascade that results in DNA fragmentation, in which Caspase 3 plays a critical role in the final stage. However, other Caspaz-3-independent mechanisms have been described to occur in the ischemic penumbra and are also associated with neuronal apoptosis. Caspase-3 is synthesized as procaspase. Apoptotic cell death in neurons returns to the active form in the cascade. Neuronal cell death as a result of experimental ischemia and traumatic brain injury occurs when caspase-3 is activated. There are many methods used in the determination of apoptosis in tissue. Identification of caspase 3 expressing cells is one of these methods[3]. In animal studies, caspase inhibitors have been used to reduce neuronal damage[4]. In animal models of focal cerebral ischemia, apoptotic cells have been shown to increase Caspase 3 activity and decrease infarct width with Caspase 3 inhibition[5]. Caspase 3 deficient rats were found to be more resistant to ischemic stress[6].

Only a few studies have been associated with Caspase 3 in caspase-associated apoptosis in ischemic brain tissue in humans. Based on this information, we designed our study to investigate the relationship between Caspase 3 level and stroke duration, which we measured from patients with known stroke onset, and to estimate the stroke onset.

Materials and Methods

This study was conducted with the approval of Clinical Research Ethics Committee of Uludağ University, Faculty of Medicine dated 05.12.2017, numbered 52588837-000 / 659.

Between December 2017 and April 2018, patients with a diagnosis of acute ischemic stroke, known to have an onset of acute ischemic stroke, were included in the study at Bursa Yüksek Ihtisas Training and Research Hospital, Emergency Medicine Clinic. The control group included volunteers over 18 years of age, no history of chronic disease, infectious or inflammatory disease, who agreed to participate in the study, and had a similar age distribution to stroke. Under the age of 18, unknown stroke onset time, the patients who had a stroke during sleep, history of previous stroke or lacunar infarction, hemorrhagic stroke, transischemic attack or seizure, history of malignancy, infectious process and could not the patients information were excluded.

During the study, 1233 patients were diagnosed as stroke and 246 of them were evaluated as hemorrhagic stroke. The remaining 987 patients were diagnosed with ischemic stroke. When 100 of these patients met the inclusion criteria, our study was terminated.

Corresponding Author: Yeşim İşler **e-mail:** yesimisler@gmail.com
Received: October 20, 2021 • **Accepted:** December 6, 2021
Orcid: https://orcid.org/0000-0002-6389-5361

Sibel Gafuroğulları **e-mail:** dr.sibelg@gmail.com
Yeşim İşler **e-mail:** yesimisler@gmail.com
Halil Kaya **e-mail:** drhalilkaya@gmail.com
Melih Yüksel **e-mail:** melihdr@gmail.com
Zeynep Nazlı Sır **e-mail:** nazlizeynepsir@gmail.com
Yasemin Nennicioğlu **e-mail:** dr_yasemin_blk@hotmail.com

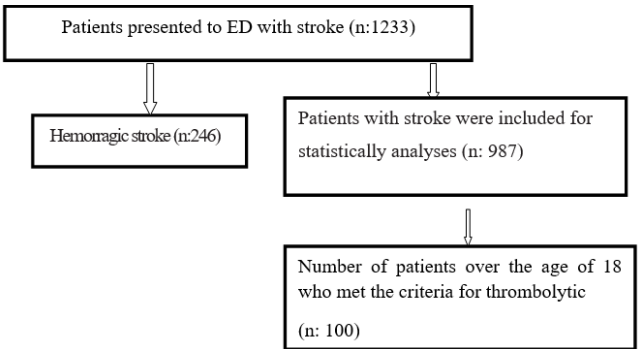

Figure 1: Flow chart of patients

Study Planning

The study was conducted prospectively in patients admitted to Bursa Yüksek Ihtisas Training and Research Hospital Emergency Department and diagnosed as acute ischemic stroke. 55 female, 45 male patients and 27 female and 23 male controls were included in the study. Demographic characteristics, smoking and duration of the patients, hypertension, diabetes mellitus (DM), coronary artery disease, rhythm disturbances and other chronic diseases were recorded. Drugs used by the patient were also recorded. The NIHSS (National Institutes of Health Stroke Scale) score was calculated for each patient and the time of occurrence of stroke was recorded.

Blood was collected from each patient who agreed to participate in the study at the time of admission for other analyzes, and 5 ml of blood was collected into the tube containing separator gel and the time from the onset of stroke to the collection of blood was recorded as stroke time.

Stroke times were divided into three groups. Patients who applied within the first 30 minutes were enrolled in the 30th minute group. The arrivals between 3-4 hours were recorded in the 4th hour group and the arrivals after 4 hours were recorded in the> 4,5th hour group. Stroke times were divided into two groups. Patients who applied the first the arrivals were recorded in the 4th hour group and the arrivals after 4 hours were recorded in the> 4,5th hour group.

Blood was centrifuged. The obtained serum was taken into eppendurf tubes and stored at -20°C. Total Caspase 3 levels were measured in the biochemistry laboratory of our hospital (standard range 0.31-20 ng / mL) following the manufacturer's instructions by ELISA method. Blood samples were taken from healthy volunteers in the control group and Caspase 3 level was recorded.

All patients included in the study underwent tomography. Patients with bleeding on tomography were excluded from the study. MRI was not performed in all patients because of thrombolytic or thrombectomy procedure. Data on the involved side, ischemia-inducing vessel and ischemic brain tissue volume of patients with MRI were recorded, and patients without MRI were not subjected to these analyzes. 1.5 T Sıgna Excite GE Magnetic Resonance System was used for MRI imaging. Exponential ADC images were used for volume measurement to determine infarct areas with greater accuracy.

Volumetric images are obtained from selected Exponential ADC images on selected patients, using three dimensional imaging models, on a point-by-point basis and optimal calibration, with paint, using a detailed modeling system to measure volumes in four planes: oblique, axial, sagittal and coronal. The volume of ischemic tissue was extracted. The volume area of the infarcted tissue resulting from this extraction was obtained in three dimensions on the calculations of the machine. The measurement was performed by a radiologist.

Statistical Analysis

In summarizing the data obtained from the study, descriptive statistics were given in tables as mean ± standard deviation or median - quartile width for continuous variables. Categorical variables were summarized as numbers and percentages. Normality test of numerical variables was checked by Kolmogorov Smirnov Test. Mann Whitney U Test was used for comparison of two independent groups when numerical variables were not normally distributed. Kruskall Wallis Test was used for independent comparisons of more than two groups. Pearson Chi-Square was used in 2x2 tables and Fisher Freeman Halton Test was used in RxC tables. Spearman Rho Correlation Coefficient was used to investigate the relationships between numerical variables when the variables were not normally distributed. In statistical analysis, Jamovi Project (2017) (Jamovi (Version 0.8) [Computer Software] was used and statistical significance was considered as 0.05 (p-value). In the comparison of two independent groups, t test was used for the Independent Groups, which is a parametric test, and Mann Whitney U test was used in cases where the numerical variables showed normal distribution.

Results

In table 1 gender, age and caspase 3 levels of stroke patients and control groups were compared and no significant difference was found.

In table 2, risk factors and caspase 3 level were compared and no significant difference was found.

In table 3, stroke patients were evaluated for the NIHSS score, caspase 3 levels, stroke onset duration, stroke occurence time, ischemic volume, affected artery, affected side and MR imaging. Since some of the patients included in the study were thrombolytic or thrombectomy candidates because of a stroke unit in our hospital, MRI was not performed for each patient. It was seen that 55% of the patients underwent MRI and 45% did not undergo MRI.

In table 4, caspase 3 median values was compared with stroke side, level of consciousness, speech impairment, upper extremity weakness, lower extremity weakness, and facial paralysis. When the comparisons were examined, the difference between Caspase 3 median values only according to the presence or absence of facial paralysis was statistically significant (p = 0.033). The median of those without facial paralysis was higher than those with facial paralysis. When the other comparisons were examined, it was found that the difference between the median values was not statistically significant.

In table 5, relationship between stroke occurrence time and NIHSS Score and stroke onset duration were compared and no significant difference was found.

In table 6, relationship between stroke onset duration and ischemic volume, NIHSS score and caspase 3 values were compared and no significant difference was found.

Discussion

The aim of this study was to investigate whether there is a relationship between Caspase 3 levels and stroke duration obtained from patients with acute ischemic stroke symptoms and to predict the time of stroke for patients whose stroke time is not determined based on Caspase 3 level. Thus, it was aimed to determine whether stroke patients with thrombolytic indication but not thrombolytic stroke could not be identified because stroke time is not known. At the same time, since Caspase 3 is an apoptosis enzyme, it is aimed to investigate whether there is an association between ischemic brain volume and the relationship between NIHSS score and Caspase 3 which measures the clinical severity of stroke.

Male gender ratio was 45%, female ratio was 55% and the mean age was 68.57 ± 13.25. There was no statistically significant difference between the mean age and sex between the patients and the control group. In the literature, male dominance in stroke patients has been shown in many studies, but in our study the rate of women was found to be higher. In a study by Jesper et al., the rate of male was 54% and female was 46% [7]. In the study of Bustamante et al., the rate of male patients was 53.9%, female patients was 46.1%, and the average age was 70[8].

In a study by Rosell et al., no significant relationship was found between Caspase 3 levels and risk factors other than atrial fibrillation. In stroke patients with atrial fibrillation, Caspase 3 level increased in the first 24 hours[9]. In another study by Montaner et al.,the etiology of biochemical markers and stroke was investigated and caspase 3 levels were found to be significantly higher in patients with AF[10]. In our study, we found no possible increase in Caspase 3 in the late period because we looked at Caspase 3 levels in the early hours. In our study, no statistically significant relationship was found between the risk factors and Caspase 3 levels. When the patients included in the study were grouped based on stroke duration, no correlation was found between Caspase 3 level and stroke duration.

In a study by Koç et al., patients aged 65 years or older who presented to the emergency department with neurological symptoms were examined for their final diagnosis. In their study, the most frequent presenting complaint was speech disorder with a rate of 37.5%. hemiparesis 2.9%; facial paralysis was found to be 1 % [11].The most common finding in our study was speech disorder with a rate of 62 %, and 15 % change in consciousness and 49 % facial paralysis. No significant correlation was found between these neurological findings and Caspase 3 activity except for patients with facial paralysis. The median Caspase 3 levels of the patients with facial paralysis were lower than those without facial paralysis.

When the duration of admission to the emergency department was evaluated, it was observed that 66% of the patients presented before 4.5 hours. In the study performed by Kıyan et al., the application rate was recorded as 20.5% in the first three hours[12].Their study was carried out in 2009. During the 9-year period, ambulance services have been developed in our country, training on stroke has been increased and awareness on stroke has increased. In addition, since our hospital had a stroke unit, it was suggested that the patients could be brought to the emergency room by ambulance in the early period.

In the present study, 74% of the patients had a stroke between 06:00 and 18:00 in the evaluation of stroke time zone. In a study by Kıyan et al., 60.5% of the patients were admitted between 18:00-08:00, the most common application time was between 18:00-21:00 and 22: 00-01:00[15]. In our study, it is seen that the majority of the patients came to the emergency department between 06:00-18:00. The reason for this time may be that the ambulance is called and the patient is informed about the clinical change as soon as there are signs of stroke, as the hours in which the patient and his relatives are together are within this interval.

There was no significant difference between the time zone of stroke and the NIHSS score of the patients. In addition, a statistically significant result was not obtained in the comparison between the time of emergency admission and the time zone where the stroke occurred.

MRI was performed in 55% of the patients in our study group. 56.36% of the patients had left side of the brain, 34.55% right and 9% had bilateral involvement. The ischemic areas detected in the evaluation were detected as MCA involvement with a rate of 50.91%. In the study performed by Morita et al., MCA was the most commonly affected artery[13]. In our study, the most affected MCA infarction was observed similar to the rates in the literature.

The mean value of ischemic tissue volume obtained from Exponential ADC maps of the patients included in the study and MRI was calculated as 4.78 cm^3. In the study of Youn et al. ischemic brain tissue volume was found to be 3.91[14].

The mean NIHSS score of the patients in our study was 7.9. In the study of Appelros et al., the average NIHSS score was found to be 6[15]. When the ischemic tissue volume and NIHSS score of our patients were compared, a significant, linear and same-way relationship was observed. In the study of Morita et al., the relationship between ischemic volume and NIHSS could not be established[13].

In a study by Linfante et al., no correlation was found between NIHSS score and lesion volume, and it was thought that the study was due to the fact that the study was performed on patients with posterior circulation stroke[16].

Caspase 3 increased after ischemia in ischemic human brain tissue[17]. In the study performed by Montaner et al to determine the biochemical marker in the differential diagnosis of acute stroke, Caspase 3 level was found to be the most predictive marker[18].

Animal models of cerebral ischemia have been shown that in the first 24 hours after ischemia, Caspase 3 is activated early in apoptotic cells on the border of ischemic infarction[19]. It was found that Caspase 3 is rapidly activated with neuronal death due to apoptosis in ischemic brain tissue[20]. In studies conducted in patients with ischemic stroke, Caspase 3, an apoptosis enzyme, was found to be higher in stroke patients than in control groups[9]. In animal studies, Caspase inhibitors have been used to reduce neuronal damage[4]. In animal models with focal cerebral ischemia, Caspase 3 activity is increased in apoptotic cells and Caspase 3 inhibition has been shown to decrease infarct width[5]. Caspase 3 deficient rats were found to be more resistant to ischemic stress[6]. However, most of these studies are animal model studies. It was found that Caspase 3 is rapidly activated with neuronal death due to apoptosis in ischemic brain tissue[20]. In 2004, Qi et al. conducted a study investigating the relationship between neuron injury and Caspase 3 in ischemia in the human hippocampus. Immunohistochemical method was used and Caspase 3 increased slowly after 8 hours, showed a dramatic increase after 24 hours and started to decrease after 72 hours[21]. Lynch et al. conducted a series of caspase measurements to investigate a diagnostic test for acute stroke. In the first three hours after the onset of stroke, the mean Caspase 3 level was 10.9 ± 3.9 ng / mL; 18.2 ± 9.8 hours in 3-6 hours; the mean 39.6 ± 18.6; it was measured as 88.9 ± 33.6 in 12-24 hours on average[22].

Based on the findings in the literature, we can interpret the reason for the difference between the patients in our study and the control group because the majority of our patients were measured before 4.5 hours. Our aim was to investigate the level of Caspase 3 in patients admitted for the first 4.5 hours of thrombolytic therapy and whether this would give an idea about infarction. Perhaps there would be a marked increase in the Caspase 3 level in our patients in the following hours.

In our study, no statistically significant difference was observed between Caspase 3 activity and the control group ($p = 0.556$). In the study conducted by Rosell et al., the median Caspase 3 level obtained from the stroke group was 1.66 ng / ml, and 1.08 ng / ml in the control group, and the difference between the two groups was found to be significant[9]. In a study by Montaner et al., caspase 3 and nine other biomarkers were used to differentiate between stroke and other neurological events mimicking the stroke clinic. It was concluded that the combination of caspase 3 and D-dimer was the strongest predictor of stroke[23].The data of our study did not support these findings. On the other hand, Bustamante et al. compared the Caspase 3 level with ischemic stroke, hemorrhagic stroke and other diseases that mimic stroke and found no significant difference between them[8].

In a study by Montaner et al., it was concluded that there was no significant relationship between caspase 3 and NIHSS score[10]. Similarly in our study, no significant correlation was found between Caspase 3 level and NIHSS score.

In the study performed by Rossel et al., repeated caspase 3 levels were measured from stroke patients and control diffusion MRI images were obtained and it was found that caspase 3 levels were increased in patients whose infarct area increased compared to the first MRI image[9]. In our study, no significant relationship was found between caspase 3 levels and ischemic brain tissue volume calculated from MRI images of stroke patients. This may be due to the fact that the Caspase 3 level was examined in the early period rather than in the late period. (before 4.5 hours)

In our study, we evaluated the patients in two groups according to the stroke onset period before and after 4.5 hours. There were no significant difference between the groups in terms of NIHSS scores, ischemic brain tissue volume and Caspase 3 levels.

Our aim was to determine whether the patient was in the time zone where we could use thrombolytic therapy based on Caspase 3 level. But our research has not found such a relationship.

Limitations

We included patients with known stroke duration in our study. Since these patients are mostly brought to the hospital in less than 4.5 hours, we think that blood samples taken from these patients before there is a significant increase in Caspase 3 levels may be the reason for the low levels in our cases. The fact that we have not repeatedly investigated caspase 3 levels can be considered as a limitation in this regard.

Conclusion

In our study, caspase 3 levels were not significant in terms of both the presence or absence of stroke and the duration of stroke onset. The caspase 3 level is not useful for early detection of ischemic stroke duration for thrombolytic treatments.

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