Diagnostic Value of Cyclophilin A in Acute Ischemic Stroke

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Abstract

Aim: The early diagnosis and treatment of patients presenting to the emergency department symptoms of stroke can significantly reduce mortality and morbidity rates associated with it. This study aimed to investigate the diagnostic value of serum yclophilin A levels in our study group who presented to the emergency department with symptoms of acute ischemic stroke.

Materials and Methods: In total, 114 patients diagnosed with acute ischemic stroke between October 2013 and October 2014 and a control group of 66 healthy volunteers were included. Cyclophilin A levels in the patient and control groups were compared.

Results: The median cyclophilin A levels in the patient and control groups measured at the time of presentation were 13.47 (11.97–17.92) ng/mL and 11.54 (8.48–16.22) ng/mL, respectively. These levels were significantly higher in the patient group than in the control group ($p<0.05$).

Conclusion: Plasma cyclophilin A levels were significantly higher in the patient group than in the control group.

Keywords: Acute ischemic stroke, cyclophilin A, emergency department

Introduction

The most important cause of ischemic stroke is atherothrombotic events. Atherosclerosis is a systemic disease of medium- and large-diameter elastic and muscular arteries and can lead to ischemia and infarction in the brain, heart, and extremities. Systemic inflammation has been shown to be involved in every stage of the development of atherosclerosis (1, 2). Hypoxia and, therefore, oxidative stress increase in patients with acute ischemic stroke. Cyclophilin A is a protein exhibiting peptidyl–prolyl cis–trans isomerase activity because of which it assists in protein folding (3). Cyclophilin A, which is an intracellular protein, is released from smooth muscle cells and macrophages in response increased oxidative stress. It exhibits pro-inflammatory effects on endothelial cells and plays an important role in the pathogenesis of inflammatory diseases (4). Cyclophilin A is regarded as a biomarker whose levels rise in platelet activation during acute thrombotic complications. The current study aimed to determine cyclophilin A levels in patients presenting to the emergency department and with the diagnosis of acute ischemic stroke and to investigate this parameter in terms of its diagnostic value in acute ischemic stroke.

Materials and methods

Study design and settings

This research was planned as a multicenter, prospective, time-limited study. Following local ethical committee approval, 114 patients presenting to a tertiary university education and research hospital with symptoms of stroke and diagnosed with acute ischemic stroke following brain diffusion magnetic resonance imaging over a

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12-month period were included. Sixty-six healthy volunteers with similar demographic characteristics to patients in the patient group were enrolled in the control group. Patients aged 18 years or over, with a confirmed diagnosis of acute ischemic stroke in the emergency department, and who provided consent were enrolled. Patients with acute coronary syndrome, hemorrhagic stroke, liver failure, acute kidney failure, sepsis, acute pulmonary edema, acute peripheral artery disease, pulmonary thromboembolism, acute mesenteric ischemia, cardiopulmonary arrest, or multi-trauma were excluded.

Blood sample measurement of cyclophilin A levels

In total, 5 mL of blood was placed into vacuum separator tubes without an anticoagulant. After being stored at room temperature for approximately 30 min, the samples were centrifuged at 3000 rpm for 10 min. Separated serum samples were then placed into 1.5 mL Eppendorf tubes and kept at −80°C until measurement. Cyclophilin A levels were calculated using a commercial CUSABIO (catalog no: CSB-E09920h) enzyme-linked immunosorbent assay (ELISA) kit in line with the manufacturer's instructions. Levels are expressed as ng/mL.

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences 13.0 for windows v.13.0 (SPSS Inc.; Chicago, IL, USA). Non-parametric variables were calculated as median (interquartile range). Parametric variables were calculated as mean and standard deviation. Non-parametric tests were used to analyze data. The Mann–Whitney U test was used to compare the median values in the groups. Correlations between variables were evaluated using Spearman correlation analysis. Receiver operating characteristic (ROC) curves were used to assess the sensitivity and specificity of serum cyclophilin A levels. P values of <0.05 were considered to be statistically significant.

Results

The demographic and biochemical characteristics of the patient and control groups are shown in Table 1. The median cyclophilin A levels in the patient group measured at the time of presentation to the emergency department were 13.47 (11.97–17.92) ng/mL; the median levels were 11.54 (8.48–16.22) ng/mL in the control group ($p<0.05$). The median levels in the ischemic stroke and control groups are shown in Figure 1. Spearman correlation analysis performed to examine the relationship between age and cyclophilin A levels revealed no significant correlation between the patient group and the control group ($p>0.05$). In ROC analysis that was performed to investigate the diagnostic value of cyclophilin A in patients with acute ischemic stroke, the area under the curve was determined to be 0.630 (95% confidence interval, 0.579–0.720) (Figure 2). When the level of cyclophilin A at 12.2 ng/mL, the sensitivity was calculated 55% and specificity was calculated 70%. while diagnosing for acute ischemic stroke.

Table 1. Demographic and biochemical features of the study population

	Stroke	Control	p
Age, years	68.2±15.3	61.7±9.7	0.001
Gender, female (%)	51.8	42.4	0.23
Cyclophilin A (ng/mL)*	13.47 (11.97–17.92)	11.54 (8.48–16.22)	0.004
*Values are reported as median (25%–75%)			

Discussion

This study showed that plasma cyclophilin A levels increase in the event of ischemic stroke. Recent studies have shown changes in the levels of cyclophilin A in coronary artery disease and aortic aneurysm (5-7). Some studies have shown that cyclophilin A levels increase during oxidative stress and inflammation (8-10). Studies on the subject refer to the interaction between endothelial cells in the arterial wall and vascular smooth muscle cells playing an important role in ensuring vascular integrity. Reactive oxygen species (ROS) have been reported to be released from vascular smooth muscle cells

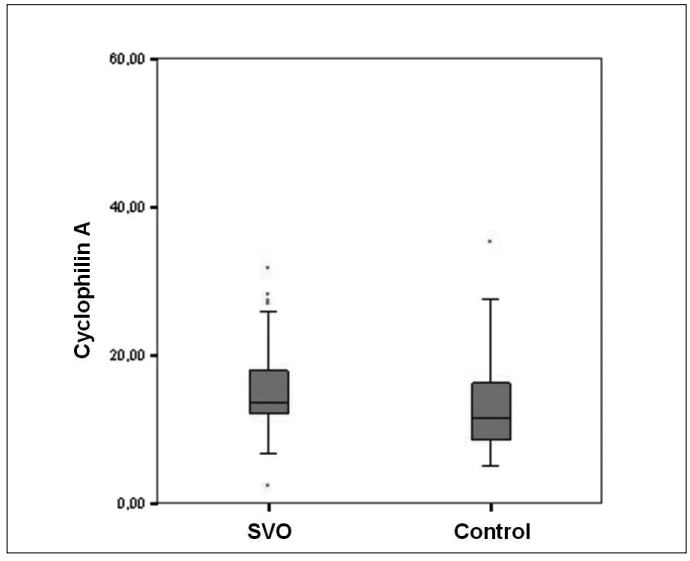

Figure 1. Median levels in the ischemic stroke and control groups shown in a box plot

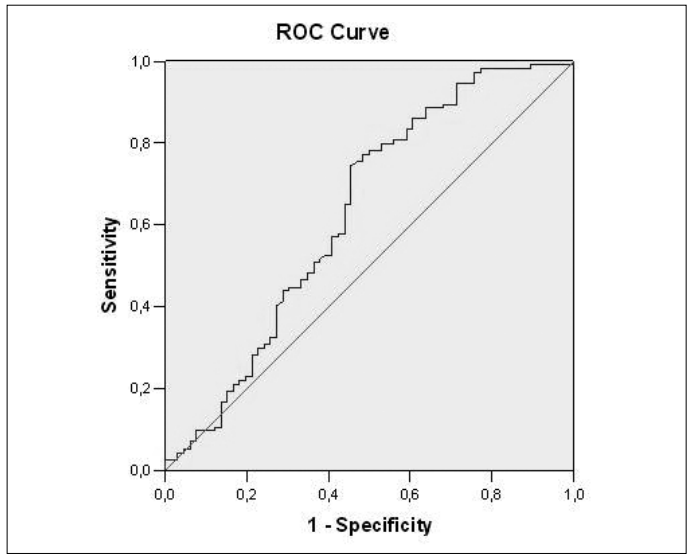

Figure 2. Receiver operating characteristic curve analyses of the diagnostic value of plasma cyclophilin A in ischemic stroke

under oxidative stress (11, 12). ROS induce the release of cyclophilin A from endothelial cells (10, 11). Cyclophilin A induces the formation of adhesion molecules from endothelial cells and causes övascular smooth muscle cells to multiply and migrate (4). Cyclophilin A is also involved in the activation of matrix metalloproteins, which are chemoattractants for inflammatory cells (13, 14). Cyclophilin A therefore plays a key mediator role in affecting endothelial cell, vascular smooth muscle cell, and inflammatory cell functions in oxidative stress. In a study on patients with diabetes mellitus (DM), Ramachandran et al. (15) determined higher cyclophilin A levels in patients with DM than in controls. In their immunohistochemical investigation of synovial fluid from patients with rheumatoid arthritis, Ho Kim et al. detected cyclophilin A in endothelial cells, particularly macrophages, lymphocytes, and smooth muscle cells. (14). A study by Satoh et al. (16), support the idea that cyclophilin A levels increases during inflammatory processes. One study involving patients with coronary artery disease had significantly higher cyclophilin A levels in patients with stenosis than in those without (16). Yan et al. (17) investigated the prognostic value of cyclophilin A in patients with coronary syndrome and reported significantly higher levels of cyclophilin A in patients with acute myocardial infarction and unstable angina pectoris than in patients with stable angina pectoris and healthy subjects. The same study also identified a powerful correlation between cyclophilin A levels and complex coronary stenosis (17). In their histopathological examination of patients with inflammatory or non-inflammatory cardiomyopathy, Seizer et al. (18) did not detect cyclophilin A in patients with non-inflammatory cardiomyopathy but detected it in patients with inflammatory cardiomyopathy. In our study, we attributed the significantly higher cyclophilin A levels in the patient group than in the control group to occlusion occurring due to inflammation against an atherosclerotic background.

Study limitations

One of the limitations of this study is the small number of patients. Patients were also differentiated in terms of etiology. As not all ischemic strokes occur against an atherosclerotic and inflammatory background, cyclophilin A levels may be normal in patients with cardioembolic stroke in particular. Further studies examining cyclophilin A levels in ischemic stroke subgroups may therefore be needed.

Conclusion

Evaluation of the studies in the literature and our research reveals a significant increase in plasma cyclophilin A levels due to atherosclerosis and inflammation. Our study may be considered a pioneering work in the field of acute stroke management and can serve as a basis for further comprehensive studies. Cyclophilin A levels can assist in the diagnosis of patients presenting to the emergency department with ischemic stroke.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Karadeniz Technical University (The approval no was 2013-92).

Informed Consent: Written informed consent was obtained from the patients and patients' parents who participated in this study.

Peer-review: Externally peer-reviewed.

Conflict of Interest: No conflict of interest was declared by the authors.

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