The importance of cytochrome c in the physiopathology of acute ischemic stroke

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Abstract

Aim: The neurons are the cells most susceptible to ischemic events. The exact mechanisms of cellular damage due to hypoxia and ischemia and the role of cytochrome c in this case have not been fully explained in detail. The aim of this study was to investigate cytochrome c in patients with acute ischemic stroke.

Materials and Methods: The cytochrome c levels in venous blood received from 56 patients with acute ischemic stroke admitted to our hospital neurology clinic and 30 healthy volunteers have been studied. Infarct volume and arrival of patients with acute ischemic stroke were also recorded in the NIHSS.

Results: There was no significant difference between the two groups. In addition, there was no statistical association between cytochrome c levels and infarct volume and arrival NIHSS in patients with acute ischemic stroke.

Conclusion: Cytochrome c plays a very important role in complex cascades in the process leading to neuronal death from acute brain vascular occlusion. Brain ischemia; endogenous neuroprotective mechanisms are activated, and cytochrome c is removed from the environment and kept within normal limits. In addition, blood cytochrome c levels in patients with acute ischemic stroke are not a biochemical marker for the diagnosis and the severity of disease.

Introduction

Brain tissue needs absolute energy. Because of sudden occlusion of the arteries feeding the brain, the oxygen and glucose required for the cell cannot be delivered. In this cerebral ischemia, the damage starts from the center, i.e. the core region. Then the center-to-environment damage wave begins to spread through a gradient in which the maximum damage is at the center [1]. In general, the ischemic core region is a region driven to die by less than 12 ml / 100 g / min. because it has collateral support to the peripheral region. The areas near the core region are hypoperfused critically (15-18 ml / 100 g / min). The cells perfused in this way are electrically silent due to the dysfunction of Na / K pumps in the membrane [2, 3]. This ischemic brain injury results in a complex interplay of multiple pathways including excitotoxicity, acidotoxicity, ionic imbalance, peri-infarction depolarization, oxidative and nitrative stress, inflammation and apoptosis [4]. Amplification of the ongoing processes in the cellular and humoral immune system following acute focal ischemic stroke causes numerous brain damage such as blood brain barrier deterioration and microvascular stasis [5]. This confusion is further increased by including gene expression and hormonal system as well.

Cytochrome c, a water-soluble protein that is not capable of producing ATP, is released into the cytosol. Cytochrome c is proapoptotic and triggers cell death. Thus, in normal healthy individuals, cytochrome c, which is only present in the cell, is added to the systemic circulation after the cell membrane is ruptured. This events directory develops very fast. In ischemic core, excitotoxic and necrotic cell death occurs within minutes. Marenzi et al. [6] showed that the cytochrome c was detectable in the circulating blood during the initial stages of patients with ST-segment elevation myocardial infarction. In addition, systemic inflammatory response, cardiac arrest, fulminant hepatitis, influenza-associated encephalopathy and chemotherapy have also been shown to increase the level of systemic circulation. Thus, cytochrome c in all the pa-

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tients seems to be a potential biomarker of mitochondrial dysfunction that may provide diagnostic [7, 8].

In this study, we will examine the importance of cytochrome c in the pathophysiology of ischemic injury in acute ischemic stroke patients. We will also investigate the use of cytochrome c as a biomarker in the diagnosis of these patients and in determining the severity of the disease.

Materials and Methods

Patient population

This study was performed in 56 patients and 30 healthy control grous who were hospitalized with the diagnosis of acute ischemic stroke in the Neurology Department of Yakutiye Research Hospital of Atatürk University Medical Faculty. Age and gender of the patient and control groups were consistent. The inclusion and exlusion criteria of the patients are shown in Table 1. Of all patients included in the study; age, gender, comorbid diseases, ischemic stroke story through their own and family background were recorded. Systemic and neurological examinations of all patients were carried out. The National Institutes of Health Stroke Scale (NIHSS) score was used to determine the clinical severity of stroke. This scale was performed on admission to the patients. NIHSS levels were divided into 3 groups. Patients with NIH stroke score between 0 and 6 were grouped as mild, 7-15 years as moderate, and 16 and above as severe [9].

Biochemical analysis

Peripheral venous blood samples were taken at the first 24 hours after the acute ischemic stroke and at any time from the control group. The serum was separated by centrifugation of blood samples, at + 4°C, 1500 rpm during 15 min. It was stored by small volumes in ependorf tubes at -80C until analysis. When the samples were taken at -80°C, kept at -20°C for one night, they could dissolve gradually the next day first to + 4°C and from + 4 ° C to the room temperature. The cytochrome c levels were measured using the commercially available enzyme-linked immunosorbent assay, QuantiGlo (R&D System Inc., Minneapolis, Minnesota, USA).

Calculation of infarct volume

In qualitative evaluation of stroke; magnetic resonance imaging (MRI) device was used. MRI studies were performed with Siemens® Sonata 1.5-T and GE-HD 3-T MR devices with echo planar imaging system. Imaging protocol was defined with DWI weighted images. The parameters used for imaging were: TR / TE = 3300/84 ms for 1.5 T MR examination; field of view (FOV) = 240 mm; section thickness = 5mm; cross-sectional gap = 1.5 mm. 3 for MRI MR / TE = 6000/80 ms; field of view (FOV) = 240 mm; section thickness = 5mm; cross-sectional gap = 1.5 mm. The infarcted areas were determined after all sections being examined. We used the Cavalieri method to measure the volumes of infarct areas. To calculate the surface area of the sections and infarct areas, we used image analysis system equipped with the special Stereo Investigator software and the point grid field measurement scale method.

Sample size determination

The sample size was calculated as a minimum of 78 people with a 5% margin of error, 80% power and an effect size of 0.7 and a ratio of $\frac{1}{2}$ of the groups based on the difference between the two groups with the G power programme.

t tests - Means: Wilcoxon-Mann-Whitney test (two groups)

Options: A.R.E. method

Analysis: A priori: Compute required sample size

Input:

- Tail(s) = Two
- Parent distribution = Normal
- Effect size d = 0.7
- α err prob = 0.05
- Power (1-β err prob) = 0.80
- Allocation ratio N2/N1 = 0.5

Output:

- Noncentrality parameter δ = 2.8479003
- Critical t = 1.9932359
- Df = 72.4845134
- Sample size group 1 = 52
- Sample size group 2 = 26
- Total sample size = 78
- Actual power = 0.8023425

Statistical analysis

The SPSS software package 20.0 program was used for all analyses. Kolmogrov-Smirnov test was used for normal distribution of variables used in the study. Continuous variables were expressed as mean ± SD or median (minimum-maximum) and categorical variables as numbers and percentages. Categorical variables were compared with Chi-square and Fisher Exact test, and continuous variables were compared with Student-t and Mann-Whitney U test. The relationships between the variables were evaluated by Pearson and Spearman correlation analyzes. ROC (Reciever Operating Characteristic) curve analysis was used to determine the cut-off point of the CYC that could differentiate the patient group and the control group. ROC curves based on the logistic regression model were constructed. Sensitivity, specificity and AUC of the ROC based on the trapezoidal rule were calculated. Values of AUC closer to 100% indicate better discrimination power of a biomarker between cases versus controls. The slope of the ROC curve represents a likelihood ratio for a diagnostic test. Potential risk factors for SVO were analyzed by inserting into an univariate model. P values less than 0.05 were considered significant.

Results

The mean age of the 56 patients in the patient group was 72 (range 48-87) and 31 of them were women. The mean age of the 30 subjects in the control group was 67 (range 54-80) and 14 of them were female. There was no statistical difference between the two groups (p = 0.06). The mean NIHS score in the patient group was 12.9 ± 8.3 and the mean infarct volume was 737314285.7 ± 1002151857. Since these values were not present in the control group, no statistical evaluation could be made between the two groups. But in terms of other baseline values, both groups were similar (Table 2). While the mean cytochrome c level in the patient group was 31.8 ± 36.6 ng / ml, the mean cytochrome c level in the control group was 34.6 ± 35.6 ng / ml (p = 0.73).

Results of univariate analysis are presented in Table 3. In univariate logistic regression analyses; age, CYC level, di-

Table 1. Inclusion ve Exclusion criteria.

Inclusion criteria
Patients with acute ischemic stroke between 18-80 years of age
Volunteers

| Exclusion criteria |
| --- |
| Patients with ischemic stroke admitted after the first 24 hours |
| Patients with transient ischemic attack |
| Patients with brain tumors or systemic malignancy |
| The cause of stroke is those with intracerebral and subarachnoid hemorrhage |
| Rheumatic and autoimmune diseases |
| Those with acute head trauma |
| Patients using daily anti-inflammatory |
| Patients with near term and simultaneous myocardial infarction |
| Patients with chronic obstructive pulmonary disease and asthma |
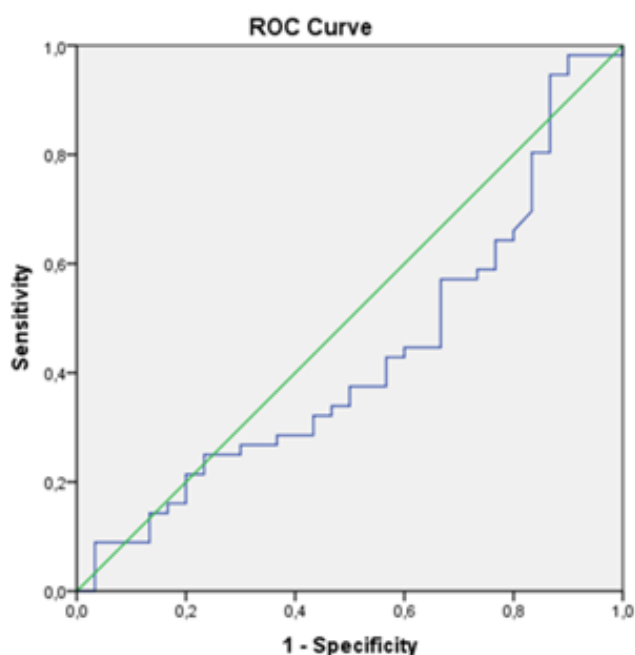
Figure 1. These specificity and sensitivity values are too low.

abetes mellitus, hypertension, atrial fibrillation, coronary heart disease, transient ischemic attack, carotid artery lesion (%50-70) and hyperlipidemia were all included in the model. There was no statistically significant association between these factors and SVO in our study.

Pearson correlation analysis was performed to investigate the relationship between cytochrome-C level and NIHS score and infarct volume in the patient group. The relationship between the increase in cytochrome c level and NIHS score was studied with r= -0.05 and p = 0.73. The relation between cytochrome c level and increase in infarct volume was studied with r = -0.1 and p = 0.46.

ROC analysis revealed that using a cut-off point of 12.73, CYC predicts SVO with a sensitivity of 42.9% and specificity of 43.3%. The area under the curve for this relationship is 0.429 and the 95% CI is 0.301–0.558. These specificity and sensitivity values are very low. There is a relationship that can not be considered as meaningful (Figure 1).

Discussion

Penumbra, which is a reversibly damaged site in the treatment of acute ischemic stroke, is seen as pharmacological, diagnostic, biochemical, brain plasticity, neuronal preservative and neuronal repairing target [10, 11]. Recently there have been major advances in the treatment of acute ischemic stroke. This development started with tissue plasminogen activator (t-PA). However, large vessel occlusions could not provide enough recanalization. Then, reperfusion was achieved in most of the patients who were transferred to the hospital in the early period after the combination of mechanical thrombectomy [12, 13]. However, there should be a recoverable penumbra area for patients to benefit clinically from recanalization. To maintain blood supply to the penumbra area, cerebral autoregulation increases blood pressure and, thus; cerebral perfusion pressure. In acute ischemic stroke; ischemia triggers necrosis and apoptosis in nerve cells. This leads to the "core" where the cell death takes place. Clinically, it can result in death or disability of the patient. In the aim to minimize these problems, the patient should be brought to the relevant center immediately and diagnosed quickly. Although the diffusion-weighted MRI in the acute phase informs about the affected zone and its degree; we encounter problems such as time consuming, costly, limited access and unability to make shooting in some patients

In addition, there is no biomarker to show the degree of influence on brain. To determine the diagnosis and prognosis of acute myocardial infarction; cardiac troponin (cTn) is widely used as biomarker [14, 15]. Cytochrome c is a proapoptotic signal molecule [1]. It is found in the inner membrane of mitochondria and cannot be detected in the circulating blood of normal healthy people. When the cell is exposed to ischemia, the permeability in the mitochondrial breast increases and the mitochondria begin to swell. Cytochrome c in the inner membrane is mixed into the cytosol [16, 17]. But there are some uncertainties about the role of cytochrome c in this complex of systems and events. Accumulation of cytochrome c in the cytosol and activation of caspase-3-like protease was already detected during ischemia and before reperfusion. Cytochrome c, which

Table 2. Basal values of patient and control group.

	SVO Group (n=56)	Control Group (n=30)	p value
Age (year)	72 (48-87)	67 (54-80)	0.06
Gender (Male/Female)	25/31	16/14	0.59
Diabetes Mellitus (n, %)	11 (19.6)	5 (16.7)	0.96
Hypertension (n, %)	39 (69.6)	16 (53.3)	0.21
Hyperlipidemia (n, %)	16 (28.6)	8 (26.7)	1
Congestive Heart Failure (n, %)	11 (19.6)	4 (13.3)	0.66
Chronic Renal Failure (n, %)	13 (23.2)	5 (16.7)	0.66
Coronary Artery Disease (n, %)	17 (30.4)	10 (33.3)	0.97
Carotid Stenosis	5 (8.9)	1 (3.3)	0.66
AF (n, %)	18 (32.1)	8 (%6.7)	0.78
NIHSS	12.9±8.3	-	-
Infarct Volume	737314285.7±1002151857	-	-
CYC	31.8±36.6	34.6±35.6	0.73

Table 3. Results of univariate analysis.

	Univariate Analysis		
Age	1.04	0.98-1.1	0.15
CYC	1.01	0.99-1.01	0.91
DM	1.45	0.41-5.12	0.56
HT	1.74	0.61-4.96	0.3
AF	1.43	0.49-4.13	0.51
CAD	0.81	0.27-2.39	0.7
TIA	1.7	0.36-8	0.5
Carotid Stenosis	4.9	0.45-52.8	0.19
Hyperlipidemia	1.03	0.35-3	0.95

goes through the cell membrane that is destroyed, enters the systemic circulation. Especially in myocardial infarction studies, it has been shown that there is a relationship between cytochrome c levels taken from venous blood and myocardial infarction, CK-MB level, ejection fraction and this relationship is correlated with poor clinical performance [18]. Also; systemic circulation, cardiac arrest, fulminant hepatitis, influenza-associated encephalopathy and chemotherapy have also been shown to increase in the systemic circulation [7, 8]. There was no significant statistical difference between the cytochrome c levels obtained from the systemic circulation of our patient group and volunteers. But theoretically, our expectation was a significant increase in the level of cytochrome c in the nerve tissue, which is more susceptible to ischemia, in the destruction-dependent environment.

NIHS score is widely used in emergency and neurology services. It is a simple method that is accepted by everyone on the expression of clinical severity of the disease. It is made easily and in a brief time [19]. The mean NIHS score applied for our patients was 12.9 ± 8.3. Therefore, they had generally moderate clinical effects. However; there was no meaningful relationship between NIHS score and cytochrome c levels in these patients.

Although NIHS score is used to show clinical severity in patients with acute ischemic stroke; it is inadequate to show the exact location of the ischemia and the magnitude of the region that objectively affects it. Neurora-diological methods are currently in use for this purpose. However; the calculation of the ischemic region in brain tissue is not simple. This is since the geometric boundaries are not regular. Cavalieri found that the volumes of three-dimensional objects that do not have a regular geometric shape can be calculated by dividing them into parallel slices. In addition, we need to calculate the surface area of the sections to calculate the infarct volume volume [20]. We used this method to calculate the infarct volume of our patients with acute ischemic stroke. The dead nerve cell is also increased when the infarct volume is increased. In parallel with this, we expect cytochrome c levels to increase in the environment too. However; there was no meaningful relationship between the levels of infarct volume and cytochrome c levels.

In the light of this work we have seen that the cytochrome c plays a very important role in complex cascades in the process leading to neuronal death from acute brain embolism. The rise of cytochrome c is an indicator of cellular destruction. In other tissues except the brain; despite its high incidence of ischemia and inflammation in clinical trials, we showed that in patients with stroke it was not at important level and that a different and protective system was activated in the brain and the cytochrome c was removed from the environment and kept within normal limits. A good understanding of these endogenous neuroprotective mechanisms will contribute significantly to the development of new and effective treatment approaches either in the treatment or prevention of ischemia. Considering neuronal death, dysfunction and reperfusion injury prevention in the development of therapeutic approaches in SI are among the considerations. However, the effectiveness of many complex mechanisms in the pathogenesis of SI can evoke that more than one treatment process can be used in the treatment of SI. Also, in this study we showed that; in patients with acute ischemic stroke, the level of cytochrome c in the blood is not a biochemical marker for the diagnosis and the severity of the disease. But this study had some limitations. This study had a single-center experience and relatively few patients.

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