European Review for Medical and Pharmacological Sciences 2013; 17: 2773-2777

Acute-phase reactants and cytokines in ischemic stroke: do they have any relationship with short-term mortality?

M. SAHAN, A. SEBE[1], A. ACIKALIN[2], O. AKPINAR[3], F. KOC[4], M.O. AY[2], M. GULEN[5], M. TOPAL[2], S. SATAR[2]

Emergency Medicine Service, Elazıg Education and Research Hospital, Elazig, Turkey
[1]Department of Emergency Medicine, School of Medicine, Cukurova University, Adana, Turkey
[2]Department of Emergency Medicine, Adana Numune Education and Research Hospital, Adana, Turkey
[3]Department of Cardiology, Adana BSK Metropark Hospital, Adana, Turkey
[4]Department of Neurology, School of Medicine, Cukurova University, Adana,Turkey
[5]Department of Emergency Medicine, Eskisehir Yunus Emre State Hospital, Eskisehir, Turkey

Abstract. – BACKGROUND: Many unknown risk factors play a role in the etiopathogenesis of stroke. The appearance of inflammatory cells within the damaged tissue after cerebral ischemia suggests that an inflammatory response may play a role in stroke pathogenesis. In our study, we examined whether an association exists between the acute-phase reactants and the levels of cytokines, the volume and diameter of the stroke, and short-term mortality in patients who were diagnosed as acute ischemic a stroke after admission to the Emergency Department.

PATIENTS AND METHODS: A total of 50 consecutive patients who applied to the Emergency Service with acute ischemic stroke were enrolled in the study. Their stroke volume were calculated and serum samples were obtained as soon as they arrived into the Emergency Service. The patients were evaluated according to the Glasgow Coma Scale (GCS) and National Institutes of Health Stroke Scale (NIHSS).

RESULTS: There was no significant correlations between stroke volume and levels of cytokine and acute-phase reactants in dead patient group or in living patient group. A correlation and statistical significance was found between stroke volume and hospital stay time in living patient group. In addition, GCS and NIHSS scores were correlated with stroke volume and was found a significant stastistically.

CONCLUSIONS: Scales such as GKS and NIHHS, which evaluate the functional state of patients, are the best indicators for defining prognosis in our daily practices. In addition, we found a positive correlation between levels of CRP (C reactive protein) and prognosis. However, we did not observe a statistically significant correlation between prognosis and other acute-phase reactants such as TNF-alpha, IL-6, IL-8, IL-10, fibrinogen, and leukocytes.

Key Words:
Acute-phase reactant, Cytokine, Emergency, Mortality, Stroke.

Introduction

Stroke is a disease that is associated with a high rate of morbidity and mortality. The World Health Organization has defined stroke as a vascular event that occurs suddenly, causing focal or global cerebral dysfunction, which may last at least 24 hours and may lead to death. Stroke is the third leading cause of death worldwide, it causes disabilities and psychosocial problems in family members of those affected by stroke, and it is also an economical burden. Therefore, prevention and therapy of strokes is a significant public health concern[1].

Eighty-five percent of stroke events are ischemic, and 15% are hemorrhagic. The appearance of inflammatory cells (macrophages, neutrophils, monocytes) within the damaged tissue after cerebral ischemia suggests that an inflammatory response may play a role in stroke pathogenesis. Several inflammatory mediators such as cytokines, chemokines, leukocytes, and adhesion molecules have been found in ischemic and surrounding tissues[2].

In this study, we examined whether an association exists between the acute-phase reactants and the levels of cytokines, the volume and diameter of the stroke, and short-term mortality in patients who were diagnosed as acute ischemic stroke after admission to the Emergency Department.

Corresponding Author: Salim Satar, MD; e-mail: salim.satar@yahoo.com

Patients and Methods

This prospective study was designed between June 1, 2007 and March 30, 2009 after the Ethic Committee approval. Data collection forms were created to collect information from investigators in a standard format. The data collection form included information about age, gender, date of presentation to the Emergency Room, whether the patient had been referred to the hospital as an in- or outpatient, whether the patient arrived on his/her own, the amount of time the patient waited before being admitted to the Emergency Room, the patient's previous medical records (history of chronic disease or cerebrovascular events), the Glascow coma scale (GCS), the National Institutes of Health Stroke Scale (NIHSS), brain tomography results, the amount of time the patient waited until being released (discharged or exitus), the prognosis, and the volume of ischemic area.

Medical treatment was administered to all patients. However, thrombolytic treatment was not administered because their situations did not meet the appropriate conditions.

Estimation of the Total Infarction Volume

The lesion volume in a single slice was calculated using the formula: lesion volume in one slice = lesion area x slice thickness. The total lesion volume was calculated from the sum of the volumes of the slices within the lesion.

Laboratory Parameters

Blood samples were collected from the patients who participated in the study when they diagnosed ischemic stroke in the Emergency Department, and before being discharged from the hospital. The levels of C-reactive protein (CRP), white blood cells (WBC), fibrinogen, thrombocyte, interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 10 (IL-10), and tumor necrosis factor-alpha (TNF-) were obtained from patient blood samples. The blood samples for WBC and thrombocyte (normal range: 4-10 uL and 150-500 uL respectively) were placed in tubes with EDTA, and the samples were evaluated using a Sysmex XT-2000i (Sysmex Corporation of America, Long Grove, IL, USA), which performs an automatic hematology analysis. The analysis was performed by laser impedance flow cytometry method using BD FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA, USA). The blood samples for fibrinogen (normal range: 200-200 mg/dL) were evaluated by adding 1.2 ml of blood to a tube filled with 0.8 ml citrate using a MDA Fibriguik device and enzymatic polymerization. C-reactive protein (CRP) (normal range: 0-8.2 mg/L) was evaluated using a monoclonal antibody and a Dade Behring BN II (Dade Behring, Deerfield, IL, USA) device, and the reference range was standardized according to BCR CRM 470. The levels of interleukin and TNFα were obtained by the ELISA (enzyme linked immunosorbent assay) method using cytoscreen immunoassay kits (Pierce, Rockford, IL, USA).

Statistical Analysis

The data were analyzed using the SPSS 17 program for statistical analysis (SPSS Inc, Chicago, IL, USA). Continuous variables were expressed as the average ± standard deviation, and discrete variables were expressed as numbers and percentages. The Chi-square test was used to analyze discrete variables, and the Mann-Whitney U-test was used to analyze continuous variables. A *t*-test was used to calculate the input and output values of continuous variables, and a Wilcoxon test was used in cases of non-normal distribution. $p < 0.05$ was considered significant.

Results

A total of 115 patients who received emergency treatment and who were diagnosed with ischemic stroke between June 2007 and March 2009 were included in this study. Eighteen patients who had recurrent stroke during the hospitalization period, 10 patients who developed a hemorrhagic component, and 12 patients with accompanying chronic diseases (intracranial tumour, chronic renal failure, intraabdominal mass, liver failure) were excluded from the study. Therefore, 75 patients were included in the study.

Thirty-six of the patients included in the study were women (48%) and 39 were men (52%). The mean age was 66.8 ± 15.8 years; the mean age of women was 70.4 ± 11 and of men was 63.4 ± 12.9 years ($p = 0.005$). The patients presented for emergency care an average of 13.5 ± 15.8 hours (mean 6 hours) after they had a stroke. Forty-eight patients (64%) were directly admitted, and 27 (36%) were admitted at another health institution. Ten patients (13.3%), including five women and five men, died during their follow-up treatments.

Statistically significant differences in CRP and IL-10 levels were found in blood samples taken from patients who were hospitalized for varying

Table I. General evaluation of the died and discharged patients from Hospital.

	Died			Discharged from Hospital			
	Mean	Median	Min-max	Mean	Median	Min-max	p
Age (years)	68.8 ± 8.5	69	55-85	66.4 ± 13.0	70	26-88	0.791
WBC	11.6 ± 3.8	10.9	6.6-18	101.1 ± 3.3	9.4	4.5-22.5	0.168
Platelets	228 ± 60	212	158-312	268 ± 104	253	76-759	0.193
Fibrinogen	568 ± 24	477	374-1000	490 ± 188	445	158-1000	0.296
CRP	39.2 ± 45.9	24	3.3 -149	20.0 ± 32.6	6.3	3-133	0.029
IL-6	51.6 ± 86.9	11.1	0.5-238	35.5 ± 104.0	7.2	0-500	0.296
IL-8	10.7 ± 21.4	3.5	1.6-71	91.3 ± 248	2.4	0-1400	0.417
IL-10	3.7 ±2.6	3	0.3 -9.7	12.3 ± 86.6	1.1	0-700	0.005
TNF-α	4.0 ± 2.6	3.8	0-8	12.5 ± 46.5	2.1	0-324	0.299
Hospital stay (day)	5.9 ± 4.1	5	0.5-13	12.2 ± 5.7	12	5-38	**0.001**
Elapsed time (hour)	12.2 ± 9.8	10	4-36	13.7 ± 16.6	6	1-72	0.472
GCS	9.6 ± 1.6	9	8-12	13.5 ± 2.1	15	9-15	**< 0.001**
NIHSS	22.4 ± 6.7	24	7-30	8.8 ± 6.4	7	0-27	**< 0.001**
Volume (mm^3)	71537 ± 177278	4233	272-569910	40509 ± 68837	6280	224-381510	0.338

lengths of time (Table I). Because the statistical significance of CRP was high, ROC analysis was performed. This analysis showed the cut-off value for CRP to be 8.3 (sensitivity, 80%; specificity, 58.5%).

No significant correlations were found between stroke volume and levels of cytokine and acute-phase reactants in dead patient group or in living patient group. In addition, there is no statistical relation on obtained blood samples studied WBC, platelets, CRP, IL-6, IL-8, IL-10 and TNF levels in the admission to emergency service and discharge from hospital in the living patients group (n=65) (Table II).

A correlation and statistical significance was found between only stroke volume and hospital stay time analyzed that relationship of the studied cytokine and acute phase reactants levels obtained blood samples on admission to Emergency Department between stroke volume and hospital stay time in living patient group. In addition, GCS and NIHSS scores were correlated with stroke volume and was found a significant statistically correlation (p = 0.008, r = –0.302 and p = 0.010, r = 0.297, respectively).

Discussion

Many independent risk factors play a role in the etiopathogenesis of stroke. Among these, age, gender, genetic background (history of stroke in the family), and race are factors that cannot be changed, while hypertension, hypercholesterolemia, sedentary lifestyle, smoking and alcohol use, heart disease, diabetes mellitus, and obesity are factors that can be changed[3,4]. Age, which cannot be changed, is a significant risk factor for stroke. Studies have shown that approximately 70% of people who have a stroke are over the age of 65 years[5,6]. In our series, the mean age was 66.8 ± 15.8 years.

In addition to the standard risk factors listed above, numerous risk factors affect the prognosis

Table II. The Evaluation between cytokine levels and acute phase reactants in survived patients group on the admission to Service and discharged from Hospital.

	Mortality			Discharged from Hospital			
	Mean	Median	Min-max	Mean	Median	Min-max	p
WBC	10.1 ± 3.3	9	5-23	10.0 ± 3.6	9	4.5-22.5	0.739
Platelets	268 ± 105	253	76-759	282 ± 100	276	5-24	0.053
CRP	20.0 ± 32.5	6	3-133	29.0 ± 31.5	16	32-637	0.087
IL-6	35.5 ± 104	7	0-500	28.6 ± 88	4	3-115	0.624
IL-8	91.3 ± 248	2	0-1400	41 ± 240	2	0-600	0.073
IL-10	12.3 ± 86.6	1	0-700	1.4 ± 1.4	1	0-1800	0.348
TNF-α	12.5 ± 46.5	2	0-325	10.3 ± 30.3	2	0-6	0.972

for and severity of tissue damage that secondarily appears following an ischemic stroke. One such risk factor is the presence of acute-phase reactants. It has been shown that stroke triggers an acute-phase response. An increase in acute-phase proteins such as CRP and fibrinogen is found in approximately one-fourth of patients with ischemic stroke[7].

In ischemic stroke, as a response to ischemia, several cells such as neurons and astrocytes become active and secrete cytokines[8]. Initially, a transient inflammatory response appears, which is caused by interleukin (IL)-1β and tumour necrosis factor-alpha (TNF-α). Subsequently, a long-lasting inflammatory response appears that is mediated by IL-1β and TNF-α, and then by IL-6 and IL-8. These cytokines play a role in the increased levels of acute-phase reactants such as CRP and fibrinogen and in leukocyte aggregation and changes in cell adhesion molecules, which contribute to adhesion to the vascular wall[8]. Previous work performed in animal models and humans has shown that inflammatory mechanisms contribute to neuronal damage[9,10]. In these experiments, it was shown that the peripheral leukocytes migrate to the brain parenchyma within 12 hours, and this process is facilitated by cerebral endothelial cells and adhesion molecules on the surface of leukocytes[11].

Epidemiological studies have shown that an increase in leukocytes is associated with higher occurrences of myocardial infarction and ischemic stroke. Additionally, it has reported that the number of leukocytes is associated with ischemic stroke and that an increase in leukocytes is associated with increased probability of stroke events in people who are predisposed to having a stroke[12,13]. In our series, the average WBC values of patients who died during the study and of those who survived, measured at the time of presentation were 11.6 ± 3.8 and 10.1 ± 3.3, respectively. This difference was not statistically significant ($p = 0.168$).

It has been shown that high fibrinogen levels are associated with cardiovascular disease and ischemic stroke[12]. Genetic variation may change the characteristics of fibrinogen. In a population-based study, the fibrinogen genotype was studied in patients with myocardial infarction and ischemic stroke and in normal controls. In these cases, the associations between fibrinogen-A alpha (FGA Thr312Ala) and fibrinogen-B beta (FBG-455G/A) and arterial thrombosis were examined. Results from this study demonstrated that the level of plasma fibrinogen was a greater risk factor for ischemic stroke than for myocardial infarction[14,15]. In our series, the average fibrinogen values of patients who died during the study and of patients who survived, measured at the time of presentation, were 568 ± 24 and 490 ± 188 respectively. No statistically significant difference was detected between the two groups ($p = 0.296$).

CRP is a serum protein that is produced in the livers of individuals with acute and chronic systemic inflammation. Increased CRP levels are associated with an increased risk of stroke[16], impending acute coronary syndrome, and peripheral vascular disease. According to the Framingham Risk Score, CRP is a more important risk factor for coronary disease than are low-density lipoprotein cholesterol levels[17]. Studies have shown a relationship between plasma CRP levels and the short-termed prognosis in ischemic stroke[18]. It was shown that increased CRP level is an independent prognostic factor that identifies the existence and degree of carotid stenosis[17,18]. Thus, it is presumed that elevated CRP level is a risk factor for carotid stenosis in individuals who have normal LDL cholesterol levels without a thrombotic risk factor[15]. In studies performed on postmenopausal women, inflammation markers were shown to be very important indicators of clinical and subclinical atherosclerosis, independent of vascular risk factors such as hypertension. Additionally, an association was shown between increased fibrinogen and CRP levels and carotid stenosis[19,20]. Furthermore, it has been suggested that an association exists between high CRP level and non-stabilized atheromatous plaques, and a liability to atherothrombotic cases in individuals with high CRP levels. In the first week following the onset of symptoms, body temperatures exceed 37.5°C in more than 60% of stroke patients. Indicators of poor prognostic include fever and increased serum levels of CRP and fibrinogen concentrations[7]. The clinical data show that the risk of death increases in patients with high CRP plasma levels within the first 72 hours following an ischemic stroke.

In our series the comparison of average CRP values in patients who died during the study with those who survived showed a significant association between prognosis and CRP levels ($p = 0.029$). It is noteworthy that CRP is not only associated with increased cerebrovascular disease mortality, but also with cardiovascular disease mortality. Therefore, CRP is an important component in determining prognosis whether the ischemic stroke is cardio embolic or atherothrombotic. Additionally, it should be considered that

these patients may die due to cardiovascular disease. Like fibrinogen, CRP polymorphisms are important in detecting cardiovascular risk, and an association between the CRP genotype and ischemic stroke may exist.

Cerebral ischemia induces the release of IL-6 from neurons and astrocytes. IL-6 levels increase in patients within a few days following a stroke. In patients with ischemic stroke, high IL-6 levels found in the cerebrospinal fluid and blood are associated with the presence of neurological deterioration and poor functional outcome[8]. In our series, the average IL-6 values of patients who died during the study and of patients who survived, measured at the time of presentation, were 51.6 ± 86.9 and 35.5 ± 104, respectively. No significant association between mortality and IL-6 levels was found ($p = 0.296$).

We examined the relationship between individual prognosis and levels of IL-8, TNF-α, and IL6. The average IL-8 values of patients who died and of patients who survived, measured at the time of presentation, were 10.7 ± 21.4 and 91.3 ± 248 ($p = 0.417$), respectively. The average IL-10 values of the same groups were 3.7 ± 2.6 and 12.3 ± 86.6 ($p = 0.005$), respectively, and the average TNF-α values of those groups were 4 ± 2.6 and 12.5 ± 46.5 ($p = 0.299$), respectively. The three factors were not found to be significantly associated with patient prognosis.

Conclusions

Scales such as GKS and NIHHS, which evaluate the functional state of patient, are the best indicators for defining prognosis in our daily practices. In addition, we found a positive correlation between levels of CRP and prognosis. However, we did not observe a statistically significant correlation between prognosis and other acute-phase reactants such as TNF-α, IL-6, IL-8, IL-10, fibrinogen, and leukocytes.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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