Tumor necrosis factor alpha serum levels and inflammatory response in acute ischemic stroke

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ABSTRACT

Objective: To assess the implication of tumor necrosis factor alpha (TNF-α) and interleukine-6 (IL-6) in acute ischemic stroke and to correlate this with lesion size, vascular risk factors, and neurological impairment.

Methods: We included 70 patients consecutively admitted to the Department of 1st Neurology, Haydarpasa Numune Educational and Research Hospital, Istanbul, Turkey, between September 2001 and April 2002, with first-ever ischemic cerebral infarction within the first 24 hours from onset. The TNF-α, IL-6, fibrinogen, C-reactive protein, erythrocyte sedimentation rate (ESR) and leukocytes were determined in plasma on admission. Neurological impairment was evaluated with the modified Rankin Scale.

Results: We found higher baseline levels of TNF-α and IL-6 in the plasma of patients with acute ischemic stroke and neurological impairment in comparison to control subjects. In the large infarct group, TNF-α, IL-6, low-density lipoprotein-cholesterol and fibrinogen were found significantly higher compared to the small infarct group. While an association between TNF-α and IL-6 values and lesion size were determined, no relation was found between localization and etiology. The TNF-α level was found to be in positive correlation with IL-6, fibrinogen, and ESR. The IL-6 level was found to be in positive correlation with ESR fibrinogen, and leukocytes.

Conclusion: Inflammatory findings are associated with the early stage of ischemic stroke. The TNF-α and IL-6 were also higher in patients with clinical worsening. The release of proinflammatory cytokines after focal cerebral ischemia indicates a step leading to tissue necrosis or reflects the amount of ischemic brain injury, since the higher concentrations of TNF-α and IL-6 are found in patients with large infarctions.

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The onset of cerebral ischemia triggers the cascade of proinflammatory molecular and cellular events.[1] Activated astrocytes, microglia, and endothelial cells become immunologically reactive and play an important role in the development of ischemic damage by producing proinflammatory cytokines such as, interleukine-1 (IL-1), tumor necrosis factor alpha (TNF-α), interleukine-6 (IL-6), and adhesion molecules, so they can be determined in body fluids such as serum and CSF.[2-6] The TNF-α, with potent stimulatory effects in immune and vascular response, increases rapidly in the brain lesion and the surrounding tissue after experimental brain ischemia.[7,8] The TNF-α causes cerebral edema and a procoagulatory state by increasing leucocyte invasion to the ischemic area, and potent vasoactive agents such as endothelin-1 and nitric oxide increase brain damage.[9,10] After passing over a certain threshold, TNF-α induces the release of other proinflammatory cytokines such as IL-6 from astrocytes and microglia.[11-14] The release of IL-6 and TNF-α after cerebral ischemia shows a pathogenical step causing tissue necrosis, and it was found that increased serum cytokine levels were associated with infarct size, neurological history, and bad prognosis independent of the type of stroke.[15-19] The TNF-α and especially IL-6 causes the synthesis of acute phase reactants with hepatocyte activation by initiating the acute phase stress response.[20-24] The presence of an increase in fibrinogen (fib), C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) and leucocyte count with cytokine induction in acute inflammation plays an important role in the development of ischemic damage and clinical prognosis.[25-28] In our study, we investigated the correlation between serum TNF-α levels, secreted as a response to acute inflammation in patients with acute ischemic stroke and the size of ischemic lesion, localization, clinical prognosis and IL-6,

and acute phase reactants, which have a role in the development of ischemia.

Methods. Seventy consecutive male and female patients admitted to the Department of 1st Neurology, Haydarpasa Numune Educational and Research Hospital (HNERH), Istanbul, Turkey, between September 2001 and April 2002 with the diagnosis of acute ischemic stroke were included in this study and were compared with 22 control age-matched subjects. Control subjects were chosen from various outpatient clinics and they had no findings related to atherosclerosis in either examinations or laboratory investigations. Patients with intracerebral and subarachnoid hemorrhage, temporary ischemic attack, a history of previous stroke, a history of infection within the last 2 weeks, an acquired infection after the admission, a head trauma within the last month, an autoimmune and immunosuppressive disease or use of immunosuppressive drugs, newly acquired heart disease, rheumatic, hepatic or renal disease and a brain tumor or systemic cancer disease were excluded. All patients were examined by a neurologist and they had routine biochemical and hematological tests, CT or MRI, electrocardiography, echocardiography, and high resolution B-mode Doppler ultrasonography (DUSG). Clinical information included age, sex, and risk factors. Patients were estimated as hypertensive if the average systolic blood pressure (BP) was ≥140 mm Hg and average diastolic BP was ≥90 mm Hg, or if they were taking antihypertensive medication. Diabetes mellitus (DM) was defined in 2 ways; by history if the patient had this diagnosis and if there were at least 2 fasting glucose concentrations of ≥140 mg/dl. Ischemic heart disease (IHD) was defined by a history of angina or myocardial infarction. A patient was defined as a smoker if he/she was a current smoker in the last 12 months. Transient ischemic attack (TIA) was defined as an acute loss of ocular or focal cerebral function lasting less than 24 hours that was presumed to be due to ischemic vascular disease. Cross-examination of alcohol consumption and other similar risk factors (history of migraine, oral contraceptive usage, and so forth). was also performed. Blood for chemistry and basic hematology determination was drawn at the time of emergency admission. After an overnight fast of at least 12 hours, venous blood samples were collected in tubes with EDTA for serum TNF-α and IL-6 serums were centrifuged and separated into serums, and then they were stored at -80°C until they were used. The serum TNF-α and IL-6 values were assessed by the ELISA method using Immunotech enzyme immunoassay kit (Immunotech, Marseille/France). The TNF-α and IL-6 determinations were performed blinded to clinical and radiological data. Serum total cholesterol, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C), and triglycerides were measured by Boehringer commercial kits using standard enzymatic procedures. Borderline for normal values were: total cholesterol ≥200 mg/dl, HDL-C ≤35 mg/ dl, LDL-C ≥130 mg/dl and triglyceride ≥150 mg/dl. According to the results of cranial CT or MRI at admission and day 3, dimensions of the infarct were assessed in all cases and defined as follows; large infarct (LI) if the widest diameter is ≥ 3 cm; and small infarct (SI) if the widest diameter is <3 cm. The infarct domains, which were assessed as cortical or subcortical according to their localization in the imaging, were all supratentorially located. The cases were divided into 4 groups: large cortical infarct (LCI), small cortical infarct (SCI), large subcortical infarct (LSI), and small subcortical infarct (SSI). On the basis of clinical evaluation and results of imaging studies, the neurologist classified all strokes into 4 major etiologic subtypes according to the following criteria; 1. Large-artery disease (LAD): ischemic stroke with (a) evidence of extracranial or intracranial occlusive large-artery disease and (b) no cardioembolic source, and (c) clinical opinion that the most likely cause of brain infarction was atherothrombosis involving the aortic arch, carotid arteries or major branches, or vertebral, basilar, and posterior cerebral arteries; 2. Small-artery disease (SAD, lacuna): ischemic stroke with (a) consciousness and higher cerebral function maintained plus (b) one of the classic lacunar syndromes or nonlacunar small-artery syndromes, and (c) CT or MRI brain scan, performed within 3 weeks of symptom onset that is either normal or shows a small deep infarct in the basal ganglia, internal capsule, or brain stem; 3. Cardioembolic (CE) disease: ischemic stroke with (a) a major cardioembolic source plus (b) no definite evidence of occlusive large-artery disease, and (c) clinical opinion that the most likely cause of brain infarction was embolism from the heart; 4. Other causes (OC): ischemic stroke that did not meet criteria for one of the categories outlined above or where there was more than one likely explanation.[29] The prognosis was determined by the neurological examination of the patients, which was assessed by the modified Rankin Scale (mRS) 2 months after stroke onset. According to this scale, mRS point of 0, 1, and 2 was described independent, and those of 3 and more as dependent.

For data assessment, SPSS-For Windows, version 10.0 was used, and for descriptive statistical values arithmetical mean, standard deviation, minimum and maximum values and percentage values were used. In analytical evaluations, Kruskall Wallis test was used for comparing the median values of more than 2

groups, while Mann Whitney U test was employed for comparing the median values of 2 groups. The Wilcoxon sign test was used to determine the prognosis and the relation between the unstable variables was evaluated with Pearson's correlation test. This study was approved by the research ethics committee of the HNERH.

Results. Seventy patients with ischemic stroke (37 female and 33 male) and 22 control subjects (10 male and 12 female) were included in the study. The mean age of the patient group was within the range of 68.5±12.4 and the mean age of the control group was within the range of 65.9±8.7. There were 16 patients in the SCI group, 19 patients in the LCI group, 19 patients in the SSI group, and 16 patients in the LSI group. Hypertension (HT), was determined in 67.1%, heart disease (HD) in 65.7%, DM in 35.7%, smoking in 17.1%, hyperlipidemia (HL) in 11%, hematocrit (Hct) level in 0.08%, alcohol consumption in 0.07%, oral contraceptive use in 0.02%, and snoring in 0.02%. In respect of the risk factors, HT, HD, DM, and high level of triglycerides displayed significantly higher rates of prevalence in the patient population (p<0.05). Leucocytes (white blood cells-WBC), erythrocyte sedimentation rate, CRP, and fib levels were determined statistically significantly higher compared to the control group (p<0.0001). When the patients were evaluated according to the etiology of stroke as LAD, CE, SAD, and OC; the mean TNF-α of the LAD (n=18) was 42.3±16.3, and of the IL-6 was 44.1±16.5. In the CE group (n=46) the level of TNF-α was 44.2±25.2 and the level of IL-6 was 49.2±25.5. No association was determined between etiology and TNF-α and IL-6 levels (p>0.05). Statistical analysis was not carried out between the other etiological groups due to the inadequate number of the patients (**Table 1**). The mean serum TNF-α values were determined as 50.1±14.8 pg/ml in LCI, 46.3±34.5 pg/ml in LSI, 39.2±25.3 pg/ml in SCI, 41.7±26.3 pg/ml in SSI, and 16.7±5.5 pg/ml in the control groups. Serum IL-6 levels were determined as 58.1±29.7 pg/ml in LCI, 56.03±15.2 pg/ml in LSI, 34.1±17.05 pg/ml in SCI, 43.8±18.5 pg/ml in SSI, and 15.1±4.9 pg/ml in the control groups. When TNF-α and IL-6 values were determined between the patient groups and the control group, statistical significance was determined (p<0.0001). The size of the difference was in order of LCI, LSI, SSI, SCI. Patients were divided into 2 groups as large infarct group (LCI and LSI) and small infarct group (SCI and SSI). In the large infarct group LDL, fib, TNF-α, and IL-6 values were found significantly higher compared to the small infarct group (p<0.05). No correlation was determined between other parameters (p>0.05). When the subcortical and cortical groups were compared with each other, there was no significant difference between TNF-α, IL-6 levels, and other values by means of localization (p>0.05). When the patients were divided into 2 groups as under 65 years (n=20) and over 65 years (n=50) and the values of each group were compared with each other, no significant difference was determined between the groups by means of TNF-α and IL-6 values (p>0.05) according to age. The groups were also evaluated by means of gender, and no statistically significant difference was determined in female and male patient groups (p>0.05) (**Table 1**). Patients were evaluated by the mRS 2 months after stroke onset according to the neurological prognosis. Thirty-four patients from the large infarct group and 23 patients from the small infarct group had bad prognosis. Twenty-two of these patients had worsening in the neurological condition and 8 out of these patients died. Serum TNF-α and IL-6 values were found significantly higher in the group with bad prognosis (p<0.05). In the determination of correlation between the TNF-α level and all parameters in the patient group, the TNF-α level was found to be in positive correlation with IL-6 at the rate of 38.4%, fib at the rate of 30.8%, ESR at the rate of 25.4%, and early prognosis at the rate of 25%. The IL-6 level was found to be in positive correlation with ESR at the rate of 23.4%, fib at the rate of 43.8%, WBC at the rate of 31.4%, and early prognosis at the rate of 39%.

Table 1 - The comparison of patients by means of gender, age, etiology, and mRS with TNF-α and IL-6.

Variable	n	TNF-α	*P-value*	IL-6	*P-value*
Gender					
Female	37	46.3 ± 2.53	0.256	43.8 ± 20.3	0.165
Male	33	42.1 ± 24.5		53.2 ± 249	
Age					
<65 years	20	41.7 ± 206	0.868	55.4 ± 23.8	0.149
>65 years	50	45.5 ± 26.2		45.4 ± 22.1	
Etiology					
LAD	18	42.3 ± 16.3	0.933	44.1 ± 16.5	0.545
CE	46	44.2 ± 25.2		49.2 ± 25.5	
mRS					
Good prognosis	13	31.1 ± 9.1	0.025*	36.0 ± 19.5	0.027*
Bad prognosis	57	47.09 ± 26.1		51.1 ± 22.9	

*Significant values, LAD - Large artery disease, CE - Cardioembolic disease, mRS - modified Rankin Scale.

Discussion. The TNF-α is a proinflammatory cytokine with potent stimulatory effects in immune and vascular response.[30] It is released from astrocytes, microglia, activated monocytes, neutrophiles, fibroblasts,

mast cells, eosinophils, epithelial and endothelial cells, B and T cells, and mainly from macrophages.[9,31,32] The TNF-α is activated by the convertase enzyme (TACE).[33] Its effects occur with the binding of receptors expressed by glial cells and infiltrating leucocytes. Its role in normal homeostasis is not exactly known. It is thought that the main function of TNF-α is related with the mature immune system such as the control of acute phase reaction with the release of acute phase reactants from the liver and the induction of cytokines.[30] The role of TNF-α on the brain is not exactly known. In some animal models, neuroprotective and in some neurotoxic effects were determined.[34] It was suggested that neurotoxic effects occurred via microglia and astroglia activated by TNF-α and the direct effect of TNF-α on neurons produced a neuroprotective effect.[35-37] In a clinical study performed on a patient group with acute ischemic stroke, it was stated that ischemic tolerance developed against TNF-α in patients with a previous transient ischemic attack and after ischemic stroke occurred, the neuroprotective effect of TNF-α took place.[38]

Various biological stimuli such as lipopolysaccharide, interferon-α (IFN-α), IL-1 beta and ischemia induces astrocytes and microglia to produce TNF-α.[2,31] After intraventricular lipopolysaccharide injection in rats, the detection of TNF-α was higher in CSF compared to serum, and this shows that TNF-α is synthesized in the brain.[10] After TNF-α passes over a certain threshold level, it induces astrocytes and microglia to secrete proinflammatory cytokines such as IL-6, which increases tissue damage.[2,39-41]

In our study, when the serum TNF-α and IL-6 values within 24 hours in patients with acute ischemic stroke were examined, a 38.4% positive correlation was determined. The TNF-α was thought to induce the release of IL-6. When the serum and CSF TNF-α levels in patients with acute ischemic stroke were compared with the control group, they were found significantly high.[42-44] Serum and CSF TNF-α levels were found correlated with lesion size.[45-47] While the levels of IL-6 in serum and CSF correlated with the lesion size in the brain, its level being higher in brain than in CSF at the early stage of ischemia shows intrathecal release.[6,17,47,48] In some studies, there were no statistically significant increase in the TNF-α levels in CSF and sera of patients with ischemic stroke and control groups.[32,49] The reason for this was thought to be the high dilution of TNF-α in peripheral blood. We found higher levels of TNF-α and IL-6 in the plasma of patients with acute ischemic stroke and neurological impairment ($p<0.001$). There was a significant correlation between the lesion size and serum TNF-α and IL-6 values. In the patient groups with a large infarct, serum IL-6 and TNF-α values were found to be higher compared to the ones with a small infarct. We think that the fact of the correlation of serum IL-6 and TNF-α levels with lesion size in ischemic stroke reflects the synthesis in brain tissue supporting the previous studies. In our study, as we could not perform TNF-α and IL-6 measurements in CSF, we could not show the correlation between serum and CSF TNF-α and IL-6 values. It was determined that IL-6 and TNF-α levels in serum and CSF of patients with cortical infarcts were higher compared to the ones with subcortical infarcts.[17,48] The reason for higher TNF-α levels in patients with a white matter lesion compared to the ones with grey matter lesion has been suggested as the damage given by TNF-α to myeline and oligodendrocytes.[50] In our study, the cortical and subcortical groups had no significant difference determined by means of serum TNF-α and IL-6 values ($p>0.05$). The IL-6 and TNF-α levels in CSF and serum was found lower in lacunar infarct.[17,22] Although, the serum IL-6 levels of acute ischemic stroke patients with cardio emboli and large artery disease were higher, this difference was not found statistically significant.[18] In our study group, no difference was found between serum TNF-α and IL-6 values in etiological subgroups. The TNF-α and IL-6 levels that were found to be increased in serum and CSF in acute ischemic stroke reflected the severity of neurological condition and functional disability.[17,50] We found serum IL-6 and TNF-α levels of the group with bad prognosis to be higher. In the determination of correlation between the bad prognosis and TNF-α and IL-6 levels in the patient group, bad prognosis was found to be in positive correlation with TNF-α at the rate of 25%, and IL-6 levels at the rate of 30.8%.

In experimental studies, it was observed that TNF-α injected intraventricularly increased infarct size and brain edema in relation to the dose.[17,51-53] The IL-6 and TNF-α synthesized during the ischemic process in the brain increased ischemic injury by activating the synthesis of acute phase reactants and showed correlation with the size of the lesion and long-term recovery.[13,20,54-56] Fibrinogen, ESR, CRP and WBC number from acute phase reactants were found to be increased after ischemic stroke, correlated with the severity of stroke and size of the lesion and to be higher in patients with a large infarct compared to the ones with a small infarct.[22,57,58] In the present study, CRP, fib, WBC, and ESR levels were found significantly higher compared with the control group. No association was determined between acute phase reactants and prognosis, localization and etiology. The TNF-α level was found to be positive in correlation with fib and ESR, while IL-6 level was found to be in positive correlation with ESR, fib, and WBC. It was

thought that fib, ESR and WBC were related with lesion size; on the other hand, although CRP is a significant indicator in ischemic stroke, it did not affect lesion size.

As a result, inflammatory findings are associated with the early stage of the ischemic stroke and the serum levels of TNF-α and IL-6 are measured in higher levels. While the lesion size is associated with serum TNF-α and IL-6 levels, the etiological subgroups have no effect on TNF-α and IL-6 levels. The TNF-α and IL-6 may effect the severity of stroke and clinical prognosis. The release of proinflammatory cytokines after focal cerebral ischemia indicates tissue necrosis or amount of ischemic brain injury.

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