The study of serum C-reactive protein, serum cystatin C, and carbohydrate antigen 125 in patients with acute ischemic stroke

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ABSTRACT

Stroke is the most common, deadly, and complicating neurological disease. Many studies have shown that the levels of some acute inflammatory reactants in people with ischemic stroke are higher than average. Therefore, in this study, three acute inflammatory reactants, i.e., C-reactive protein, Serum cystatin C, and carbohydrate antigen 125, were evaluated in patients with acute ischemic stroke to consider the association between these serums with intra and extra-cerebral vessels stenosis. In this cross-sectional study, 90 patients with non-embolic ischemic stroke were evaluated. The diagnosis was by physical examination, rejection of emboli, and brain imaging. Blood samples were taken in the first 24 hours of a stroke. ELISA test was used to measure CRP, Serum cystatin C, and CA125. Doppler ultrasound of cerebral arteries was also performed in the first five days. Independent chi-square and t-tests were used to analyze the data. The result of CRP level in patients with stenosis was $7.58 \pm 1.33 \mu g/ml$ and in patients without stenosis was $4.10 \pm 1.75 \mu g/ml$ (p = 0.004). Also, there was a significant relationship between serum CRP level and stenosis (p = 0.003). In patients with abnormal CRP, the internal carotid artery, middle cerebral artery, and anterior cerebral artery were the most involved. In patients with normal CRP, the most involved arteries were the anterior cerebral artery, internal carotid artery, and middle cerebral artery, respectively. There was a significant relationship between serum CRP level and the location of internal carotid artery stenosis (p = 0.015) and middle cerebral artery (p = 0.006). The amount of cystatin C between the normal CRP and abnormal CRP groups was statistically significant so that its concentration in the normal group was less than in the abnormal group (p = 0.04). The results of measuring the serum concentration of carbohydrate antigen 125 showed that the serum level in the normal group was statistically lower than in the abnormal group (P = 0.02). The results showed that stenosis of the internal carotid artery and middle cerebral artery is more common in patients with ischemic stroke with high serum CRP levels. This finding suggests that abnormal CRP may be more associated with narrowing some cerebral arteries.

Introduction

Stroke is the most common and, at the same time, the most deadly and complicated neurological disease. The frequency of ischemic cases reaches 75% (1). Many studies have shown that the level of some acute inflammatory reactants in people with ischemic stroke is higher than average (2). One of the most important of these reactants is the C-reactive protein (CRP), which varies from 74% in different studies (3-5). Recent studies have shown that mortality rates in patients with stroke with abnormal CRP levels are significantly higher (6, 7). This finding also applies to the complications of a stroke. Currently, the growing evidence of the role of inflammation in the development of vascular atherosclerosis has led to the use of CRP as a marker. The potential for prognosis in patients with myocardial infarction has been noted in some studies, although the exact relationship is unclear (8).

So far, various researches have been published on which cerebral artery stenosis is related to CRP levels (3-7). Some radiological and ultrasound evidence suggests that carotid stenosis is more common in people with abnormal CRP levels (9). This finding has also been reported in asymptomatic cases (10). However, there is disagreement about the existence of such a strong association between CRP and intracranial stenosis. For example, some studies suggest a link between CRP and middle cerebral artery stenosis (11), and some deny such a link (12).

Recent studies have attempted to identify potential biomarkers associated with regeneration and brain dysfunction after acute ischemic stroke (13). Carbohydrate antigen 125 (CA125) is a glycoprotein

belonging to the mucin family and a valuable marker for diagnosing ovarian cancer (14). Mucins are high molecular weight glycoproteins that protect epithelial surfaces through lubrication and hydration. Its half-life is approximately seven days. Mechanical stress and inflammatory stimuli cause its secretion from mesothelial cells (15). Therefore, CA125 has been introduced to indicate inflammatory status in heart and brain diseases. Inflammation may be an essential factor in explaining the serum association of CA125 with these diseases (16). Some studies have reported that CA125 is a strong predictor of stroke severity (15-18). In addition, is a potent cysteine protease inhibitor that plays an important role in human vascular pathophysiology by regulating cathepsins S and K (19). Cathepsins are overexpressed in atherosclerotic lesions and human aneurysms, causing rupture-prone plaques by destroying the extracellular matrix. Epidemiological studies show a strong association between circulating levels of cystatin C and the risk of coronary heart disease, ischemic stroke, and heart failure (20). In addition, genetic analysis shows that the concentration of cystatin C has a common polygenic background with stroke. Hence cystatin C has been introduced as a valid therapeutic target (21).

Due to the lack of a study that evaluated all intracranial and extracranial vessels at the same time, and due to disagreement in this field and the emphasis of previous studies on the inadequacy of current results, in this study, serum C-reactive protein, Serum cystatin C, and carbohydrate antigen 125 were considered in patients with acute ischemic stroke. It was also evaluated how C-reactive protein is associated with intracranial and extracranial artery stenosis.

Materials and methods
Studied patients

This cross-sectional study was performed using a simple sampling method on 90 patients diagnosed with acute ischemic stroke. The patients had a stroke for the first time, and all were admitted in the first 24 hours after the onset of the disease. The diagnosis was confirmed by paraclinical CT-Scan and MRI methods, and if necessary, contrast material was used, and suspected cases were removed from the study. Then, cardiac examinations, including ECG,

echocardiography, and cardiologist visit, were performed for all patients, and embolism cases were excluded from the study. If the patient has a history of surgery, a trauma in the last three months, a history of other diseases (such as kidney failure, liver, heart, vasculitis, infection, and fever) were also excluded from the study at the time of admission or use of antibiotics in a recent month. Drug consumption (except drugs that control myocardial ischemia, diabetes, hypertension, and hyperlipidemia) was also excluded.

CRP measurement and TCD (Transcranial Doppler) study

Blood samples were taken to measure quantitative CRP for patients in the first 24 hours after the onset of clinical symptoms. Individuals who had more than 24 hours after signs were also excluded from the study. ELISA measured serum CRP levels, and values above 3 μg / ml were considered abnormal.

Also, for all patients, transcranial and extracranial cerebrovascular Doppler was performed using a two-way CW / PW Doppler device connected to DWL software (Sipplingen, Germany) in the cerebrovascular Doppler room. This device uses two separate 4 MHz probes to examine the Common Carotid Artery and Internal Carotid Artery. 2 MHz probes were used to analyze Arterial Cerebral Artery, Middle Cerebral Artery, Posterior Cerebral Artery, Ophthalmic Artery, Vertebral Artery, and Basilar Artery. Peak Systolic Velocity (PSV), Velocity End Diastolic (EDV), Mean Flow Velocity (MFV), Pulsatility Index (PI), and resistance index (RI) were measured for each artery. They were calculated automatically by the device, and to remove the artifact and improve accuracy, the data was also calculated manually, and in case of discrepancies between the obtained data, manual items were determined as the study criterion.

A questionnaire containing demographic information (age and sex) was prepared for each patient. Patients were divided into two groups based on the normal and abnormal CRP, and the location of stenosis was compared in the two groups. The location of the stenosis was also assessed based on the CRP mean.

Serum cystatin C and carbohydrate antigen 125 evaluations

Serum carbohydrate antigen 125 was measured by the ELISA test method (BioSource kit, China). The coefficients of in-test and out-test changes were 6% and 5%, respectively, with an accuracy of 1.0 U/ml. Serum cystatin C was measured by the ELISA method (BioSource kit, China). The in-test and out-test changes coefficients were 8% and 6%, respectively, with 18.0 pg/ml accuracy.

Statistical analysis

After confirming the normal distribution of data using the Shapiro-Wilk test, the independent t-test was used for statistical analysis. All data were presented as mean + standard deviation. Calculations were performed using SPSS software version 18, and the significance level of the tests was considered p≤0.05.

Results

In this study, 90 patients were evaluated. Fifty-eight (4.64%) patients were male, and 32 (6.35%) patients were female. The mean age for men was 67.48 ± 14.10 years, and for women was 68.25 ± 11.61 years. The minimum age of patients was 32 years, and the highest age of patients was 90 years. The mean age of individuals with normal CRP was 63.42 years, with a standard deviation of 11.25. The mean age of individuals with abnormal CRP was 65.75 years, with a standard deviation of 13.52. Fifty-five percent of people with normal CRP and 60% of people with abnormal CRP were male. The mean serum CRP level in all patients was 5.92μg/ml with a standard deviation of 5.89. The mean serum CRP level was 7.58 ± 1.33 in patients with stenosis and 4.10 ± 1.75 in those without stenosis. This difference was statistically significant (p = 0.004). Thirty-four percent of people with normal CRP and 66% of those with abnormal CRP had stenosis. Cases of cerebrovascular stenosis were more common in people with abnormal serum CRP levels (p = 0.003). The most involved vessels in all patients were ICA, MCA, ACA, BA, and VA, respectively. The most involved vessels in patients with abnormal CRP were ICA, MCA, ACA, BA, and VA, respectively. The most involved vessels in patients with normal CRP were ACA, ICA, MCA, and BA, respectively. There was a significant relationship

between serum CRP level (i.e., between normal and abnormal cases) and the site of ICA stenosis (p = 0.015) and MCA (p = 0.006), but there was no significant relationship between BA, ACA, and VA vessels. In the case of PCA and CCA vessels, no significance could be calculated due to the lack of stenosis (Table 1). There was no significant relationship between age and serum CRP level (p = 0.815). Also, no significant association was observed between stroke risk factors and serum CRP level (p> 0.05) (Table 2).

Table 1. The frequency of normal and abnormal CRP cases depends on the type of vessel

Vessel		Normal CRP	Abnormal CRP	P-value
CCA	With Stenosis	0 (0%)	0 (0%)	-
	Without Stenosis	45 (50%)	45 (50%)	
ICA	With Stenosis	6 (26.1%)	17 (73.9%)	0.015
	Without Stenosis	39 (58.2%)	28 (41.8%)	
MCA	With Stenosis	3 (17.6%)	14 (82.8%)	0.006
	Without Stenosis	42 (57.5%)	31 (42.5%)	
ACA	With Stenosis	6 (40%)	9 (60%)	0.573
	Without Stenosis	39 (52%)	36 (48%)	
PCA	With Stenosis	0 (0%)	0 (0%)	-
	Without Stenosis	45 (50%)	45 (50%)	
BA	With Stenosis	2 (25%)	6 (75%)	0.266
	Without Stenosis	43 (52.4%)	39 (47.6%)	
VA	With Stenosis	0 (0%)	1 (100%)	1
	Without Stenosis	45 (50.6%)	44 (49.4%)	

The results have been reported frequently. Chi-square was the statistical test, and p ≤ 0.05 was significant.

Table 2. Frequency of normal and abnormal CRP cases according to risk factors for ischemic stroke

Risk Factor		Normal CRP	Abnormal CRP	P-value
Hypertension	With Stenosis	22 (51.2%)	21 (48.8%)	1
	Without Stenosis	23 (48.9%)	24 (51.1%)	
Diabetes Mellitus	With Stenosis	14 (46.7%)	16 (53.3%)	0.823
	Without Stenosis	31 (51.7%)	29 (48.3%)	
Hyperlipidemia	With Stenosis	9 (50%)	9 (50%)	1
	Without Stenosis	36 (50%)	36 (50%)	
Ischemic Heart Disease	With Stenosis	12 (66.7%)	6 (33.3%)	0.187
	Without Stenosis	33 (45.8%)	39 (54.2%)	
Smoking	With Stenosis	12 (52.2%)	11 (47.8%)	1
	Without Stenosis	33 (49.3%)	34 (50.7%)	

The results have been reported frequently. Chi-square was the statistical test, and p ≤ 0.05 was significant.

Serum levels of cystatin C in both normal and abnormal CRP groups are shown in Figure 1. According to the results of this section, the amount of cystatin C between the normal CRP and abnormal CRP groups was statistically significant so that its concentration in the normal group was less than in the abnormal group (p = 0.04).
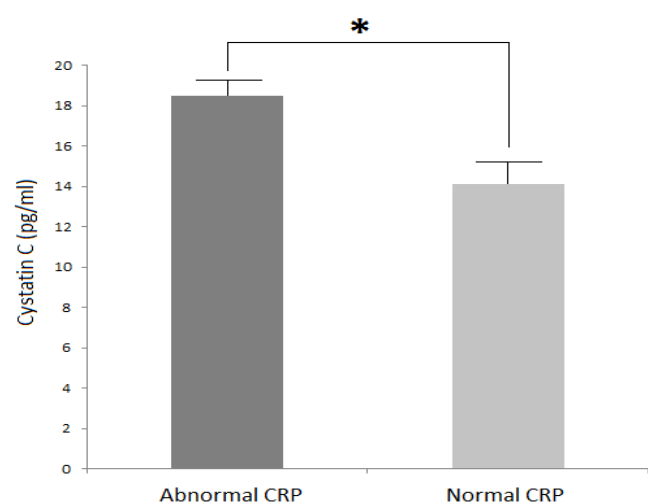

Figure 1. Comparison of cystatin C levels in the studied groups;*indicates a significant difference (p< 0.05) between normal CRP and abnormal CRP groups

The results of measuring the serum concentration of carbohydrate antigen 125 showed that the serum level in the normal group was statistically lower than in the abnormal group (P = 0.02).
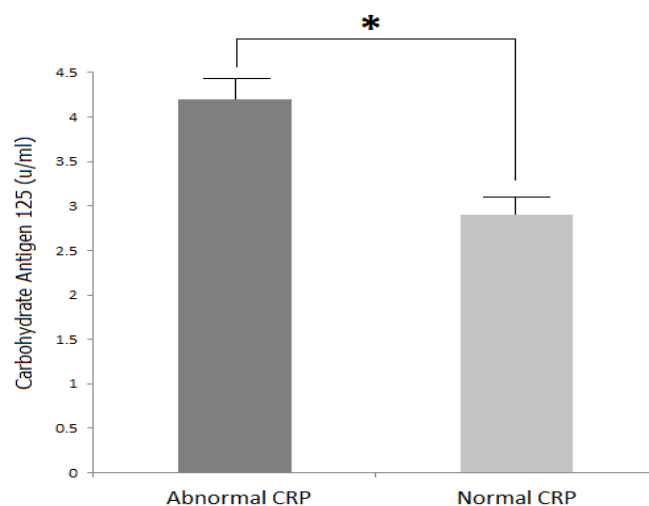

Figure 2. Comparison of carbohydrate antigen 125 levels in the studied groups; * indicates a significant difference (p< 0.05) between normal CRP and abnormal CRP groups

Discussion

In this study, we evaluated the pattern of cerebrovascular stenosis in terms of serum CRP levels in patients with ischemic stroke. We also compared the serum levels of cystatin C and carbohydrate antigen 125 in the two groups of normal CRP and abnormal CRP. We made two critical findings in our research. First, cases of cerebrovascular stenosis were more common in people with abnormal serum CRP levels (p = 0.004). Also, the mean serum CRP level was higher in patients with stenosis than in patients without stenosis (p = 0.004). Many studies confirm our findings, and all show that cerebrovascular stenosis in stroke patients is higher in people with abnormal serum CRP levels (3-7). This finding suggests that serum CRP levels may be used to screen people prone to cerebrovascular stenosis. Because this laboratory method is easy and cheap, it doubles its importance.

The second important finding of our study was that people with abnormal serum CRP levels had both extracranial and intracranial stenosis. However, this association was significantly more significant with the narrowing of some arteries, such as the internal carotid artery and middle cerebral artery. This finding indicates that abnormal CRP is associated with more stenosis in specific arteries and may also be used to predict the type of artery prone to stenosis. Almost all studies that have examined the relationship between CRP and carotid arteries have reached our conclusion (6, 7). Their results confirmed the association between

CRP and carotid stenosis. These studies are based on both vascular ultrasound and imaging techniques, in which indices such as intima-media thickness, blood flow velocity, and stenosis progression were evaluated. Even when asymptomatic individuals were studied, high levels of CRP were associated with greater frequency and severity of stenosis. Studies show that this relationship is independent of age (22). In the case of intracranial stenosis, some studies confirm our findings, including research of Bang *et al*. (23) and Liu *et al*. (24). However, these studies were only on the middle cerebral artery, and all found a direct relationship between serum CRP levels and stenosis of this artery.

Contrary to our findings and the above studies, two studies did not show a significant difference in intracranial stenosis in patients and only confirmed the association of serum CRP levels with extracranial stenosis (12, 25). We did not find any study that evaluated all arteries, but what we can conclude from our findings is that the incidence of stenosis in some intracranial and extracranial arteries is higher due to increased serum CRP levels. But a definite statement about other arteries needs further investigation.

The frequency of abnormal serum CRP levels in patients with stroke has been reported from 25 to 75%, partly due to the time of sampling and test method (26). The association of high serum CRP levels with the greater incidence of stenosis in patients with stroke is unknown. CRP is essentially a blood protein whose function is not yet well understood and is associated with interleukins. Some studies have shown an adverse effect of CRP by impairing the coagulation process of fibrinogen or platelet levels (27, 28). Cytokines may also mediate the negative impact of CRP (29). LDL binding and vascular wall damage is other functions of CRP (30). These findings suggest that high serum CRP levels may directly affect the course of atherosclerosis.

Our study also had some limitations that need to be considered. First, CRP was measured only once, and serial measurements may increase accuracy. Second, in this study, the results were purely Doppler-based, and it is clear that duplex increases the value of the findings. In general, our findings show that ICA and MCA stenosis is higher in patients with ischemic stroke whose CRP is abnormal. This finding suggests

that abnormal CRP may be more associated with the narrowing of some arteries.

Availability of data and materials
The data used to support the findings of this study are available from the corresponding author upon request.

Statements and Declarations
The author declares that no conflict of interest is associated with this study.

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