Nitric Oxide and C-Reactive Protein Levels in Ischemic Stroke

İskemik İnmede Nitrik Oksit ve C-Reaktif Protein Düzeyleri

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Başvuru Tarihi: 06 Mart 2020 **Kabul Tarihi:** 19 Aralık 2020

ABSTRACT

Purpose: Nitric oxide(NO) is one of the important substances that are synthesized to keep the blood vessels dilated enough to provide adequate flow and to maintain cerebrovascular homeostasis. C-reactive protein(CRP) is a highly sensitive indicator of inflammation and tissue damage. High CRP concentrations are thought to have effects such as dysfunction of vascular endothelium and decreased NO release. The purpose of this study was to investigate the levels of CRP and NO and to see the correlation of these markers in ischemic stroke patients.

Material and Methods: Fifty ischemic stroke patients and 31 healthy control group were included in this study. Ischemic stroke was differentiated by computerized tomography scan. The patients blood samples were taken at admission to the emergency department, within 24 hours of stroke symptom onset, before any treatment was given. Serum CRP and NO levels were evaluated.

Results: The mean serum NO concentration of the patients (6.52±9.52 μmol/L) was significantly lower than control group (20.48±22.17 μmol/L) ($p<0.01$). Serum CRP levels in patients (13.47±18.58 mg/L) significantly higher than the control group (1.98±1.37 mg/L) ($p<0.01$). There was no significant correlation between NO and CRP levels ($p>0.05$).

Conclusion: Although we found decreased NO levels and increased CRP levels in the patients, there was no correlation between these two. The results of this study show NO's possible role in neuroprotection and increased levels of CRP may be associated with ischemic stroke. Further studies are necessary to assess the functional interactions between CRP and NO and their contribution to the pathophysiology of cerebral ischemia.

Key Words: Nitric Oxide, C-Reactive Protein, ischemic Stroke

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ÖZET

Amaç: Nitrik oksit (NO), kan damarlarını dilate tutarak yeterli akışı sağlayan ve serebrovasküler homeostazın korumasında önemli olabilecek maddelerden biridir. C-reaktif protein (CRP) iltihaplanma ve doku hasarının oldukça hassas bir göstergesidir. Yüksek CRP konsantrasyonlarının vasküler endotelyumun disfonksiyonu ve azalmış NO salınımı gibi etkileri olduğu düşünülmektedir. Bu çalışmanın amacı CRP ve NO düzeylerini araştırmak ve bu belirteçlerin iskemik inmeli hastalarda korelasyonunu görmekti.

Gereç ve Yöntem: Çalışmaya 50 iskemik inme hastası ve 31 sağlıklı kontrol grubu dahil edildi. İskemik inme bilgisayarlı tomografi taraması ile ayırt edildi. Acil servise başvuruda inme semptomlarının başlamasından 24 saat içinde, herhangi bir tedavi verilmeden önce hastaların kan örnekleri alındı. Serum CRP ve NO düzeyleri değerlendirildi.

Bulgular: Hastaların ortalama serum NO konsantrasyonu (6.52±9.52 μmol/L) kontrol grubundan (20.48±22.17 μmol/L) anlamlı olarak daha düşüktü ($p<0.01$). Serum CRP düzeyleri (13.47±18.58 mg/L) kontrol grubundan (1.98±1.37 mg/L) anlamlı olarak daha yüksekti ($p<0.01$). NO ve CRP düzeyleri arasında anlamlı korelasyon yoktu ($p>0.05$).

Sonuç: Hastalarda NO düzeyleri azalmış ve CRP düzeyleri artmış olsa da, bu ikisi arasında korelasyon tespit edilemedi. Bu çalışmanın sonuçları NO'in nöroproteksiyondaki olası rolünü göstermektedir ve artmış CRP seviyeleri iskemik inme ile ilişkili olabilir. CRP ve NO arasındaki fonksiyonel etkileşimleri ve serebral iskeminin patofizyolojisine katkılarını değerlendirmek için daha fazla çalışma gereklidir.

Anahtar Kelimeler: Nitrik Oksit, C-Reaktif Protein, iskemik İnme

INTRODUCTION

Stroke is a neurologic dysfunction in focal area of the brain due to interrupted or reduced blood supply. Ischemic stroke is most common (80%–85%) compared to hemorrhagic type (20%–15%) (1).

C-reactive protein (CRP), is a highly sensitive indicator of inflammation and tissue damage. Elevated CRP concentration has been associated with an increased risk of cerebrovascular and cardiovascular events. Many studies have also observed elevated CRP levels in the circulation of patients after acute ischemic stroke. A single measurement of CRP in serum may be a predictor of first or recurrent cerebrovascular events. Also many studies showed that, CRP assay may be important for assessing the acute inflammation and predicting the degree of long-term disability in ischemic stroke patients (2-4).

Nitric oxide (NO) is a principle vasodilator released by the endothelium, maintains cerebrovascular homeostasis and is an important biomarker of inflammation and oxidative stress. Physiological amounts of NO are neuroprotective, whereas higher concentrations are clearly neurotoxic (5). The CRP has also been associated with endothelial cell dysfunction and progression of atherosclerosis possibly by decreasing NO synthesis. Also it has been suggested that CRP may affect the NO pathway. High CRP concentrations are thought to have effects such as dysfunction of vascular endothelium and decreased NO release (6,7).

The data indicate that in the first 24 hours after ischemic injury, the post-stroke immune response occurs in a time-dependent fashion. Therefore, studies of CRP that have extended the time window beyond 24 hours would not accurately represent baseline inflammatory status. Consequental results were obtained in studies related to NO and CRP levels in ischemic stroke. So the purpose of this study was to investigate the levels of CRP and NO and to see the correlation of these markers in acute ischemic stroke in the early phase.

MATERIAL AND METHODS

50 patients with cerebrovascular infarction and healthy control group of 31 individuals

were included in this study. The study was approved by the ethical committee of the study hospital. Detailed anamnesis of each patient was taken, and neurological and systemic examinations were performed. All patients were examined by a qualified neurologist and ischemic stroke was differentiated by computerized tomography (CT) scan.

Patients with an anamnesis of major renal, hepatic, endocrinological disorders, skeletal disorders, cancerous diseases, cardiac diseases were excluded from this study. We also excluded the patients with recent infections and/or inflammatory events, with a hemorrhagic stroke and subarachnoid hemorrhage. Patients admitted to the hospital 24 hours after stroke symptoms onset were excluded from the study.

Infarct was confirmed on neuroimaging, blood samples for NO and CRP levels were taken within 24 hours of stroke symptom onset. The samples of patients were centrifuged, seperated and stored at –80 ° C for later analysis of NO. A commercial kit using sandwich ELISA method was used to determine serum NO levels (Human nitric oxide ELISA kit Andy Gene Biotechnology, China). Serum CRP levels were measured on Olympus AU 680 autoanalyzer (Beckman Coulter CRP Latex kit). Total cholesterol, LDL, HDL cholesterol and triglyceride levels were estimated by commercially available kits supplied by Beckman Coulter using an Olympus AU 2700 autoanalyzer.

All data were analysed using the statistical software package SPSS Statistics version 21. Kolmogorov Smirnov test was used for to see the normality of the distributions. The data were expressed as mean ± SD value. Mann-Whitney U test was used to compare means of variables. Correlations were measured with Spearman correlation analysis. Statistical significance level was taken as $p < 0.05$.

RESULTS

Patients (23 women, 27 men) with a mean age of 69.78 ± 12.42 and healthy subjects (15 women, 16 men) without any known disease and drug usage; with a mean age of 50.97 ± 15.15 were included in this study.

The mean serum NO concentrations of the patients and controls are shown in Figure 1.

CRP concentrations of the groups are shown in Figure 2.

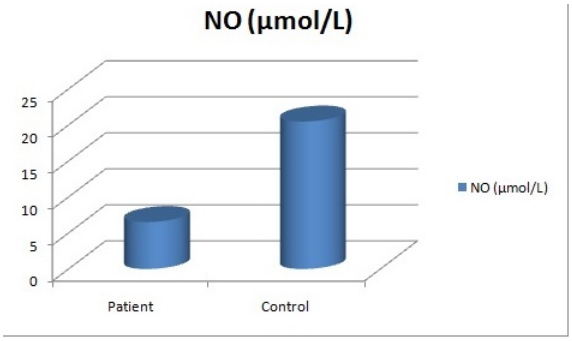

Figure 1. Mean Levels of Serum NO in patients and control group

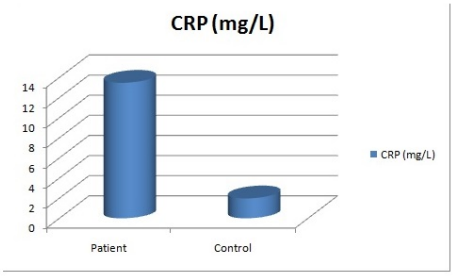

Figure 2. Mean Levels of Serum CRP in patients and control group

Clinical characteristics of stroke patients and controls were shown at Table 1. There were no significant differences between cholesterol, triglyceride and body mass indexes (BMI) of patients and control group levels.

The mean serum NO concentration of the patients was 6.52±9.52 µmol/L and the control group was 20.48±22.17 µmol/L and there was a statistically significant difference between them ($p < 0.01$).

Table 1. Clinical characteristics of stroke patients and controls

	Patients (n=50)	Controls (n=31)
Age	69.78 ± 12.42	50.97 ± 15.15
Male/female	27/23	16/15
Total cholesterol (mg/dl)	189.8±40.55	182.94±36.5
Triglycerides (mg/dl)	143.5±76.93	136.42±69.16
HDL cholesterol (mg/dl)	41.72±11.63	53.45±12.60
LDL cholesterol (mg/dl)	123.22±29.70	104.45±31.76
BMI (kg/m2)	24.2±1.9	24.1±1.7

Age, total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, body mass index levels are given as mean±SD.

In the comparison of serum CRP levels, the mean value in patients (13.47 ±18.58 mg/L) was significantly higher than the control group (1.98±1.37 mg/L) ($p < 0.01$).

There was no significant correlation between NO and CRP levels ($p=0.737$, $r=0.049$).

DISCUSSION

Cerebrovascular disease is one of the most common cause of death worldwide and the pathogenesis of stroke need to be further studied. Several studies in different populations have demonstrated that an elevated level of plasma CRP in healthy individuals is a strong predictor of future cerebrovascular and cardiovascular events. Also, the CRP levels have been reported to be elevated in the blood circulation of patients after acute ischemic stroke in some studies (8-12).

Elevated CRP levels secondary to brain damage through activation of the complement system was also detected in animal models of focal cerebral ischemia (13). Previous studies have assessed the value of CRP in the early phase of stroke as a prognostic factor for functional outcome. Most of these studies were small and tested the association between CRP and mortality rather than outcome (14,15). The largest study was carried out by Ladenvall et al. employing 600 patients and 600 controls in a Swedish population (16).

A study by Winbeck et al. documented raised CRP in 127 patients without thrombolysis with a first ischemic stroke no more than 12 h after the symptom onset. They also noticed a CRP increase between 12 and 24 h after symptom onset predicts an unfavorable outcome (17). Also, Kumar et al. found that the hs-CRP level is significantly higher in ischemic strokes and by its elevation within 72 h of symptom onset was a bad prognostic indicator. Elevated hs-CRP values were a risk factor in association with other risk factors such as diabetes/ hypertension (18). Anusha et al. found that CRP was elevated in 60 patients with stroke and patients with elevated CRP had increased risk of mortality. (19). Two prospective studies did not find an association between the CRP levels obtained within 6 or 12 h after symptom onset and death or dependency at follow-up (17,20). In the present study, our result was compatible with most of the other studies. CRP was measured after CT confirmation and within 24 h of onset of symptoms and we found that CRP was elevated in patients compared to control group significantly ($p<0.01$).

NO has a dual identity including neuroprotection and neurotoxicity during ischemia reperfusion (21). Increased NO metabolite concentration in cerebrospinal fluid has been associated with a greater brain injury and early neurological deterioration. However, endothelium-derived NO has been shown to be beneficial in experimental stroke, and it has been

suggested that administration of NO might be beneficial in acute stroke (22).

In this study, the serum levels of NO were found to be significantly lower in stroke patients in comparison with controls indicating oxidative stress ($p<0.01$). However, Rajeshwar et al. found that CRP and NO levels were significantly elevated in stroke patients in comparison with controls and they predict the incidence of ischemic stroke and CRP is an independent prognostic factor of poor outcome at 3 months (23). The significant increase in NO in patients studied within the first 24 h from stroke onset in comparison to controls is also reported by El Kossi and Zakhary (24), Also Castillo et al. and Aygül et al. reported higher NO levels in ischemic stroke patients (22,25). But Rashid et. al. assessed plasma NO levels in patients with acute stroke and their association with both severity and outcome and they found NO levels lower in ischemic stroke patients like us (26). Similarly, Cano et al. reported significantly decreased NO levels in thrombotic stroke patients compared with control subjects (27).

Although we found decreased NO levels and increased CRP levels in the patients, there was no significant correlation between these two ($p>0.05$).

The results of this study show NO's possible role in neuroprotection. Endothelial NO loss may be the central mechanism in the pathogenesis of endothelial dysfunction in ischemic stroke patients. Also increased levels of CRP may be biomarker for cerebrovascular infarction. Further studies are necessary to assess the functional interactions between CRP and NO and their contribution to the pathophysiology of cerebral ischemia.

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