

# Neopterin Levels in Patients with Cerebrovascular Disease

## Serebrovasküler Hastalıklarda Neopterin Düzeyi

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### Abstract

**Objective:** The recovery period in ischemic cerebrovascular disease is sometimes long and complicated. It is thought that a systemic inflammatory response plays an important role in this process. Therefore, promising studies have reported that neopterin and other cytokines serve as indicators of the inflammatory response. The present study investigated the effects of cerebrovascular diseases on plasma neopterin.

**Materials and Methods:** We studied 68 consecutive patients under 65 years of age with cerebrovascular disease; the mean age was 52.08±5.74 years (yrs) (ranging from 39 yrs to 64 yrs; 28 female and 40 male). We also studied 29 randomly selected age-matched healthy subjects (control); the mean age was 49.76±13.11 years (yrs) (ranging from 41 yrs to 62 yrs; 12 female and 17 male). Blood samples for assessing plasma levels of neopterin were usually taken within seven days after admission to the hospital and stored at -20°C until analysis. Serum neopterin levels in all the subjects were measured by Enzyme Immunoassay (EYA) using the BRAHMS method (Neopterin; Diagnostica GmbH, 16761 Berlin, Germany). Differences between the groups' means were analyzed with the Mann-Whitney U test.

**Results:** The mean values of neopterin levels in patients (mean neopterin levels in patients, 18.51 ± 11.56 nmol/L; vs. control, 12.26 ± 3.87 nmol/L [p=.001]) were significantly different when compared with the controls.

**Conclusion:** It has been suggested that the mortality and morbidity associated with cerebrovascular disease could be prevented by a reduction in the inflammatory response. We suggest that plasma neopterin levels were significantly increased. So, the levels of plasma neopterin may be useful monitoring of treatment and course of diseases.

**Keywords:** Ischemic cerebrovascular disease, Neopterin, Systemic inflammatory response

### Özet

**Amaç:** İskemik serebrovasküler hastalıklarda (SVH) iyileşme süreci bazen uzun ve problemlidir. Bu süreçte sistemik inflamatuvar yanıtın önemli rolü olduğu düşünülmektedir. Bu nedenle sistemik inflamatuvar yanıtın güvenilir göstergeleri olarak neopterin ve diğer sitokinler hakkında gittikçe artan umut verici çalışmalar yapılmaktadır. Bu çalışmada iskemik SVH' da neopterin düzeylerinin ne derece etkilendiklerini araştırdık.

**Gereç ve Yöntem:** Hasta ve kontrol grubundan uygun şartlarda venöz kan örnekleri alındı. Bu örneklerin serumları ayrıştırılıp neopterin düzeylerini ölçmek üzere -20oC'de saklanarak, neopterin değerleri Enzim İmmünoassay (EİA) yöntemiyle BRAHMS kitleri kullanılarak ölçüldü. (Neopterin; Diagnostica GmbH, 16761 Berlin, Almanya). Elde edilen değerler ortalama standart sapma olarak hesaplandı ve Mann-Whitney U testi kullanılarak karşılaştırıldı. İstatistiksel anlamlılık için p<0,05 değeri eşik alındı.

**Bulgular:** Çalışmaya 65 yaş altında 68 SVH'lı hasta ve 29 sağlıklı kontrol grubu alındı. Hasta yaş ortalaması 52.08 ± 5.74; 28 kadın, 40 erkek ve sağlıklı kontrol grubunun yaş ortalaması 49.76 ± 13.11; 12 kadın ve 17 erkek idi. SVH'lı hastaların serumunda ortalama neopterin düzeyi 18.51 ± 11.56 nmol/L; kontrollerde 12.26 ± 3.87 nmol/L (p=.001) idi. SVH'lı olgularda kontrol grubuna göre neopterin düzeyleri anlamlı derecede yüksek olarak saptandı.

**Sonuç:** Sonuç olarak; sistemik inflamatuvar yanıt kısmen önlenebilir-se hastanın mortalite ve morbiditesine neden olan olumsuz olaylar azaltılabilecektir. Bu amaçla tedaviye yönelik araştırmalar mevcuttur. SVH'lı hastaların takibinde neopterin düzeylerinin tespitinin yararlı olacağını düşünüyoruz.

**Anahtar Kelimeler:** İskemik serebrovasküler hastalık, Neopterin, Sistemik inflamatuvar cevap

## Introduction

**S**troke patients place a tremendous burden on health resources throughout the world [1]. In 25% to 40% of patients with ischemic stroke, neurological symptoms progress during the initial hours. Early clinical deterioration results in increased mortality and functional disability [2-5]. Clinical and experimental research over the last few years has shown that inflammatory mechanisms participate in stroke-induced brain damage [6-8]. Inflammatory reactions are mediated by cytokines, which are small glycoproteins expressed by many cell types in response to acute cerebral ischemia. Increased levels of cytokines such as interleukin (IL)-1, tumor necrosis factor-alpha (TNF- $\alpha$ ), and IL-6 [9], as well as adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) [10], have been observed after experimental brain ischemia. Also, several clinical studies have reported elevation of proinflammatory cytokines [11-13] and adhesion molecules [14] in the peripheral blood and cerebrospinal fluid (CSF) of patients with ischemic stroke. Independent of the stroke subtype, high IL-6 concentrations in CSF and plasma have been associated with a large infarct size, neurological deterioration, and poor clinical outcome [11, 12, 15, and 16].

In humans, neopterin is known as a sensitive marker for diseases associated with increased activity of the cellular immune system. Recent studies report that neopterin also exhibits distinct effects; for example, neopterin induces the expression of inducible nitric oxide synthase in rat vascular smooth muscle cells, activates translocation of nuclear factor-kappa B and affects cardiac function in the isolated perfused rat heart [17,18]. Neopterin may also induce oxidative stress causing apoptotic cell death, or superinduce apoptosis mediated by tumor necrosis factor-alpha [18,19]. Release of excitatory amino acids and oxygen free radicals, iron accumulation, and nitric oxide production or apoptosis have been suggested as mechanisms for clinical worsening [20,21]. Moreover, intraventricular injection of IL-1 and TNF- $\alpha$  enlarges infarct volume and brain edema after MCA occlusion in rats, whereas the injection of antibodies against IL-1 and TNF- $\alpha$  reduces brain injury [8,22].

One current strategy for treatment of stroke is based on the assumption that administration of pharmaceuticals may interfere with progressive tissue injury, primarily in ischemic penumbra regions. Recently, studies have been performed to evaluate the therapeutic potential of gene transfer to reduce ischemic brain injury while other studies have revealed that overexpression of an interleukin-1 receptor antagonist significantly reduced infarct size [22, 24]. Thus, multiple gene products such as anti-inflammatory peptides that reduce the inflammatory response, anti-oxidants that reduce damage produced by oxygen radicals, and anti-apoptosis agents that reduce neuronal death all potentially have therapeutic roles in protecting against ischemic brain injury [25].

In this study, we analyzed plasma neopterin levels in patients with acute cerebral ischemia.

## Materials and Methods

We studied 68 consecutive patients under 65 years of age with cerebral infarction; the mean age was  $52.08 \pm 5.74$  years (yrs) (ranging from 39 yrs to 64 yrs; 28 female and 40 male). Additionally, we included 29 randomly selected age-matched healthy subjects (control); the mean age was  $49.76 \pm 13.11$  years (yrs) (ranging from 41 yrs to 62 yrs; 12 female and 17 male). Both patients and control subjects were from the same region, Eastern Anatolia. The neurological diagnosis was confirmed by history, clinical findings, and computerized cerebral axial tomography. All other laboratory tests were performed in the clinical routine, including using the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) as markers of acute infection. Criteria for exclusion, for both the CVD patients and the controls, were acute and chronic inflammatory diseases, malignancy, trauma, surgery or acute vascular diseases that occurred within the four weeks prior to the study.

Blood samples for plasma levels of neopterin were usually taken within seven days after admission to the hospital and stored at  $-20^{\circ}\text{C}$  until analysis. Serum levels of neopterin were measured by Enzyme Immunoassay (EYA) using the BRAHMS method (Neopterin; Diagnostic GmbH, 16761 Berlin, Germany) in all the subjects. Data are expressed as the mean  $\pm$  standard deviation and were computed with SPSS (Statistical Package for the Social Sciences). Differences between the groups' means were analyzed with the Mann-Whitney-U test. Results were considered to be statistically and significantly different when the confidence limits exceeded 95% ( $P < 0.05$ ).

## Results

The mean values of neopterin levels in patients (mean neopterin levels in patients,  $18.51 \pm 11.56$  nmol/L; vs. control,  $12.26 \pm 3.87$  nmol/L ( $p = .001$ )) were significantly different when compared with the controls.

## Discussion

Measurement of neopterin concentrations is useful for monitoring cell-mediated (Th1-type) immune activation [26]. Moreover, the diagnostic value of neopterin has already been established in autoimmune diseases, rejection of transplanted organs, malignant diseases, as well as infectious diseases such as AIDS or hepatitis [26, 27]. Accumulating evidence has indicated that inflammatory-immunologic reactions are involved in the pathogenesis of cerebral ischemia [6-15]. We found significantly higher serum levels of neopterin in patients with acute ischemic stroke when compared with control subjects within seven days of the clinical onset, which is similar to results found in a previous study [28].

Clinical and experimental research over the last few years

has shown that inflammatory mechanisms participate in stroke-induced brain damage [6-8]. Also, proinflammatory cytokine-mediated cerebral damage participates in the mechanisms that lead to neurological worsening [11-14]. However, since the highest concentrations of proinflammatory cytokines are usually found in patients with large infarctions, there is debate over whether the release of proinflammatory cytokines after focal cerebral ischemia indicates a pathogenic step leading to tissue necrosis or if it simply reflects the amount of ischemic brain injury [10, 11]. Vila et al. [29] showed the direct correlation between IL-6 and the bulk of tissue loss and also found a positive association between IL-6 and the strength of the acute-phase response, which was recently described [10, 30] as a sensitive predictor of poor short-term clinical outcomes.

The current study has several limitations. Upon the subjects' admission to the hospital, we did not perform the appropriate serologies that might have detected the presence of asymptomatic infections. This omission may be relevant particularly because recent infections have been associated with an increased risk of impending stroke as well as the increased release of cytokines [31]. Therefore, it is theoretically possible that our measurement of neopterin was swayed by a history of recent infection. In the likely event that the misclassification of prior infection based on current status was equal between the case and control groups, the estimated association between prior infection and disease would be attenuated. To partially control for such an effect, we included in the analysis indirect markers of infections, such as body temperature and total leukocyte count upon admission.

Experiments have shown that neopterin is capable of enhancing the oxidative potential of reactive oxygen species (ROS) produced from immunocompetent cells [32]. In parallel, neopterin

was also found to inhibit the activity of xanthine oxidase and reduce nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase [33]. Therefore, neopterin may be a direct participant within the cascade of events leading to oxidative stress and could either induce oxidative stress resulting in apoptotic cell death, or superinduce TNF- $\alpha$ -mediated apoptosis [18, 19]. On the other hand, it is known human peripheral blood neutrophils that are activated (i.e., by adhesion on glass) generate ROS, or influence a number of phagocytosis-stimulating factors [18, 19]. Razumovitcha et al. [35] suggest that upon exposure to neopterin the neutrophils respond by generating singlet oxygen, hydroxyl radicals and nitric oxide by NADPH-independent pathways.

In conclusion, neurological worsening was associated with higher concentrations of proinflammatory cytokines in plasma and CSF, irrespective of the initial size, topography, or mechanism of the ischemic infarction [7-11, 16]. Cytokines have been implicated in several mechanisms that may potentiate ischemic brain injury [11-14, 36]. Thus, multiple gene products such as anti-inflammatory peptides that reduce the inflammatory response, anti-oxidants that reduce damage produced by oxygen radicals, and anti-apoptosis agents that reduce neuronal death have potential therapeutic roles in protection against ischemic brain injury [25]. Therefore, development of new neuroprotective therapies targeted to modulate cytokine-induced inflammation could be a promising way to prevent deterioration in acute ischemic stroke. In humans, neopterin serves as a sensitive marker for diseases associated with increased activity of the cellular immune system and may be useful to monitor treatment efficacy and disease progression in patients with acute cerebral ischemia. Forthcoming clinical trials with neuroprotective therapy will provide further information.

**Conflict interest statement** The authors declare that they have no conflict of interest to the publication of this article.

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