

ORIGINAL  
INVESTIGATION

## Investigation of Uric Acid and Nitric Oxide as Risk Factors for Acute Ischemic Stroke Patients

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## ABSTRACT

**Objective:** Cerebrovascular diseases are diseases with a multifactorial etiology, which is affected by genetic and environmental factors, with incidence rates increasing with age around the world. The primary objective of this study was to investigate uric acid and nitric oxide (NO) as risk factors for ischemic stroke patients and to examine the effects of these factors on a patient's prognosis at an early stage.

**Materials and Methods:** Forty patients who were admitted to the hospital because of ischemic stroke were included in the study.

**Results:** NO levels in the patient group were found to be 34.98  $\mu\text{mol/L}$ , which was significantly higher than that of the control group ( $p=0.0043$ ); however, uric acid levels in the patient group were found to be 6.54 mg/dl, which was also significantly higher than the control group ( $p=0.001$ ). In addition, it was shown that the relationship between the NO and uric acid measurements in the patient group was positively and statistically significant ( $r=0.397$ ,  $p<0.05$ ).

**Conclusion:** This study indicates that uric acid and NO play a role in the pathogenesis of ischemic stroke; however, they do not contribute to determining the prognosis. It was demonstrated that uric acid is an independent risk factor, when other risk factors are eliminated. When examining the risk factors for ischemic stroke patients, uric acid should be considered and should be among the treatment options.

**Keywords:** Uric acid, nitric oxide, ischemic stroke

### INTRODUCTION

Cerebrovascular diseases (CVDs) are a major cause of long-term disability and indeed rank first in the world among the serious illnesses that can lead to disability. Therefore, they constitute a considerable part of health expenditure in many countries (1). Ischemic stroke is a complex multifactorial disorder that is affected by genetic and environmental factors, and its incidence increases with age (2). Ischemic stroke, which is classed among the subtypes of stroke, is seen at a rate of 70–80% (3, 4). Investigations into measures to prevent CVDs are leading research subjects at the present time. Preventive treatment depends on the exact determination of the risk factors for CVD and how they can be eliminated.

Free radicals have been neutralized by catalase, superoxide dismutase, glutathione peroxidase, reduced glutathione, and antioxidants such as vitamins C and E, to maintain metabolic functions properly under normal physiological conditions, thereby keeping a fine balance between them. However, a large amount of free radicals are produced due to the cleaning capacity of antioxidants, since oxygen supplies return to the tissue at the onset of ischemia and especially during post-ischemic reperfusion. This causes intracellular oxidative stress. Therefore, intracellular proteins, lipids, and nucleic acids are damaged. Then, the blood–brain barrier is disrupted, vasogenic edema occurs, and leukocytes promote inflammation by passing to ischemic tissue, thus resulting in an increase in the morbidity and mortality rates (5).

Uric acid is a metabolically inert end product of adenosine and guanosine-based purine metabolism. Hyperuricemia is associated with obesity, hyperlipidemia, hypertension, and atherosclerosis. Epidemiological studies show that hyperuricemia is linked with CVD (6). It is known that uric acid acts as an antioxidant in the early stages of atherosclerosis and is the most powerful determinant of plasma antioxidant capacity. When serum uric acid levels rise above 5–6 mg/dL in the late stages of atherosclerosis, their antioxidant properties turn into a pro-oxidant structure. Indeed, this may be attributed to many factors, such as the stage of atherosclerosis, acidity, an oxidant environment, and the reduction of other antioxidants (7, 8). Nitric oxide (NO) was first defined as an endothelium-derived relaxing factor. As endogenously produced NO affects the host defense and immunity, it plays an important role in the regulation of many physiological processes, including vascular tone and neuronal communication. Also, NO reduces platelet adhesion and aggregation by increasing the soluble guanylate cyclase activity in platelets (9).

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Today, a decrease in NO bioavailability against hemodynamic or pharmacological stimulations is regarded as one of the earliest signs of atherosclerosis. The dysregulation of NO biosynthesis is related to the development of endothelial dysfunction and subsequent vascular events (10, 11).

The aim of this study was to investigate uric acid and NO as risk factors in patients with ischemic stroke and examine their impact on patient prognosis in the early stages.

## MATERIALS and METHODS

Forty patients aged over 50 years, who were hospitalized with an early diagnosis of acute ischemic stroke in Erciyes University Medical Faculty's Neurology Clinic between January 2011 and December 2011, were included in this study. Ethical approval was obtained for this study. The diagnosis of ischemic stroke was confirmed with imaging and neurological examination of the patients. In addition, 40 healthy volunteers without neurological disease were included in the study as the control group. The exclusion criteria were determined as follows: patients using diuretics and lipid-lowering medicines, those with gout, patients with poor thyroid, kidney or liver function tests, cancer patients, patients with prior CVD, patients who smoked and drank alcohol, and patients with atrial fibrillation and heart valve disease.

Patients' demographic characteristics (age and gender) in the course of their first admission to the hospital, risk factors, the clinical pictures on admission to the hospital (according to the NIH Stroke Scale), and laboratory and neurological findings were evaluated. From all the patients and volunteers, 10 mL of blood samples were collected. The postprandial serum uric acid levels were measured in mg/dL using a spectrophotometric method at the central laboratory of Erciyes University Faculty of Medicine. The uric acid level above 5 mg/dL was considered as hyperuricemia. The blood glucose and cholesterol levels of the patients were also measured with the same device. The NO levels of the same groups were measured by the ELISA method using a human serum kit at Erciyes University Faculty of Medicine Biochemistry Research Lab.

### Statistical analysis

NCSS (Number Cruncher Statistical System) 2007 & PASS (Power Analysis and Sample Size) and 2008 Statistical Software (Utah, USA) programs were used to analyze the data. Descriptive statistical methods (mean, standard deviation, frequency, and ratio), Student t-test, and chi-square test were employed to analyze and compare the data. Pearson correlation analysis was used to evaluate the relationships between parameters. A p-value less than 0.05 ( $p < 0.05$ ) was considered to be statistically significant.

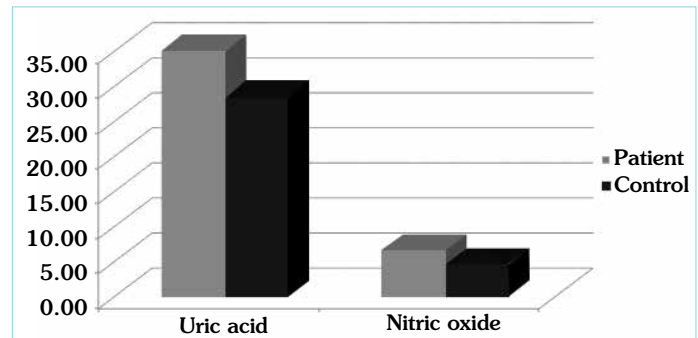
## RESULTS

A total of 80 patients were included in this study. Patients' ages ranged from 50 to 86 years old. The average age of the patients was found to be  $64.45 \pm 10.83$  years old; 55% ( $n=44$ ) of the participants were female and 45% ( $n=36$ ) were male. There was no difference between age and gender. The NO values of the patients were significantly higher than those of the control group ( $p < 0.01$ ) (Table 1). The uric acid values of the patients were significantly higher than those of the control group ( $p < 0.01$ ) (Figure 1).

**Table 1.** NO and uric acid values of the patients and controls

	Patients (n=40)	Controls (n=40)	p <sup>a</sup>
Uric acid	34.98±19.17	28.38±7.70	0.043*
Nitric oxide	6.54±2.00	4.58±1.39	0.001**

\*Student t-test; \* $p < 0.05$ ; \*\* $p < 0.01$

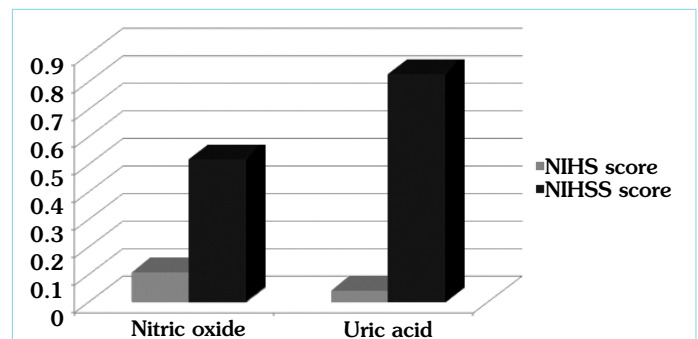


**Figure 1.** NO and uric acid values of the patients and controls

**Table 2.** Correlation between NO and uric acid

		Nitric oxide
Uric acid	r	0.397
Uric acid	p	0.011*

r: pearson correlation coefficient; \* $p < 0.05$



**Figure 2.** Correlation between NO, uric acid and the NIHSS scores

In addition, a positive correlation was found between NO and the uric acid values of the patients. In other words, uric acid levels rose as NO levels increased. This correlation was statistically significant ( $r=0.397$ ,  $p < 0.05$ ) (Table 2).

Our study revealed that there was no statistically significant correlation between NO and uric acid and NIHSS scores ( $p > 0.05$ ) ( $p > 0.05$ ) (Figure 2).

There was no statistically significant difference between NO and uric acid measurements of the patient group by sex ( $p > 0.05$ ). However, only the NO measurements of the women in the control group were found to be significantly lower than those in the men ( $p < 0.05$ ).

There was no statistically significant relationship between age and NO and uric acid in the patient group ( $p > 0.05$ ). While there was

**Table 3.** Relationship between age and NO and uric acid in the groups

		Age	Age
		r	p
Patient	Nitric oxide	-0.056	0.730
	Uric acid	-0.074	0.651
Control	Nitric oxide	0.369	0.019*
	Uric acid	-0.023	0.886

r: Pearson correlation coefficient; \*p<0.05

no significant relationship between age and uric acid in the control group, a positive significant correlation was found between age and NO ( $r=0.369$ ;  $p<0.05$ ) (Table 3).

## DISCUSSION

The data of this study suggest that uric acid can be considered as an independent risk factor for ischemic stroke when other risk factors are eliminated. We also detected hyperuricemia in 72.5% of patients over 50 years of age, but there was no gender difference among our patients in terms of the elevation of uric acid. Despite these data, however, no significant relationship was found between the impact of uric acid elevation and NIHSS on early prognosis in this study.

Our study indicates that the elevation of uric acid is an independent risk factor. Previous studies reported that a high level of uric acid in patients who had suffered an ischemic stroke was an independent risk factor for the development of new vascular events (12, 13). Weir et al. (6) examined the relationship between prognosis after 90 days and uric acid levels measured at the first admission to hospital in ischemic and hemorrhagic stroke patients, and found that hemorrhagic stroke, advancing age, high glucose, and high levels of uric acid adversely affected the prognosis. The results of multiple logistic regression analysis suggest that high levels of uric acid are associated with poor prognosis independently of other prognostic factors in patients with acute ischemic stroke, but no causal connection between hemorrhagic stroke and uric acid has been established (6).

Clinical studies have shown that hyperuricemia is associated with endothelial dysfunction, and that an excess of uric acid impairs endothelial function (14, 15). Patients with hypertension and hyperuricemia develop 3–5 times more coronary artery disease or cerebrovascular disease than patients with normal levels of uric acid (16). Uric acid-lowering therapy is thought to reduce ischemic injury. It has been reported that a rise in uric acid level per one mg/dl increased the vascular disease risk, including ischemic stroke, at a rate of 8–13% (17).

A wealth of data show that uric acid is a pro-oxidant agent. Uric acid leads to lipid peroxidation by increasing oxygen radicals in the circulation. It also causes vascular endothelial dysfunction, the adhesion of granulocytes to the endothelium, crystallization in atherosclerotic plaques, increased platelet adhesion, and macrophage infiltration in the vascular endothelium, and can also trigger atherosclerosis and induce ischemic stroke (18).

NO was initially defined as an endothelium-derived relaxing factor. It fulfills important regulatory roles in the cardiovascular system. In some cases, NO binds superoxides and other reactive oxygen species in the environment and exhibits antioxidant properties. When considered from this point of view, an increased NO level during the inflammatory processes is probably considered as a protective antioxidant property (19). Hence, a significant elevation of NO in our study can be interpreted as an antioxidant activity response against endothelial damage.

Our study showed that there was no statistically significant correlation between NO and uric acid and NIHSS. It was found, however, that an elevation of uric acid in the patient group was positively associated with high levels of NO. It has been shown that there is a sharp decline in serum antioxidants, apart from NO, in ischemic stroke patients, and that patients with a low level of antioxidants during the stroke have a worse prognosis, indicating that NO release was inhibited, as was found in a study with rats exposed to experimental hyperuricemia that were observed to develop hypertension (20, 21).

NO is also inactivated by free radical superoxides. Thus, enzymes such as superoxide dismutase that eliminate superoxides can extend the life of NO. Peroxynitrite (ONOO<sup>-</sup>) forms by the reaction of NO with superoxides. This substance is quite a powerful agent that can lead to tissue damage, and that has cytotoxic and oxidative properties and is responsible for the inflammatory effects of NO (22). A significantly high level of NO in our study supports the evidence that there are degradation products, which thus show the inflammatory effects of NO.

A significant relationship was found between NO and uric acid measurements (as the NO values increased, a rise in uric acid values was shown). We suggest that this leads to an increase in anti-inflammatory agents, i.e., NO, by combining uric acid with proinflammatory ONOO<sup>-</sup> to limit the existing inflammation.

The studies carried out so far indicate that surplus free oxygen radicals cause endothelial dysfunction by disrupting NO synthesis and increasing its degradation (23). Consistent with the literature, our study shows that a rise in uric acid is positively related to an increased NO. This can be regarded as a response to an inflammatory process that has occurred. The clarification of the etiopathogenesis of stroke is of critical importance for clinical research, because methods for preventing CVD and CVD treatment strategies can be determined based on the findings obtained.

## CONCLUSION

Our data suggest that uric acid and NO may play a role in the pathogenesis of acute ischemic stroke, but they do not make any contribution to the determination of prognosis. We assume that a rise in uric acid is an independent risk factor for the development of CVD events. Uric acid-lowering treatments should be administered for prophylaxis. Further studies involving more individuals are needed to more clearly demonstrate the data in this study.

**Ethics Committee Approval:** Ethics committee approval was received for this study from Kayseri Training and Research Hospital.

**Informed Consent:** Written informed consent was obtained from patients who participated in this study.

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Conceived and designed the experiments: ED, MG. Performed the experiments: ED. Analyzed the data: ED, ŞD. Wrote the paper: MG. All authors have read and approved the final manuscript.

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