The relationship between blood nitric oxide levels and brain infarct volume in patients with ischemic stroke

Comparison nitric oxide levels and ischemic volume in stroke

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Abstract

Aim: Cerebrovascular disease (CVD) is the third leading cause of morbidity and mortality. Most risk factors disrupt the contraction and enlargement of the cerebrovascular endothelium. One of the main players in the maintenance of cerebrovascular homeostasis is nitric oxide (NO). In this study, we aimed to investigate the level of NO in patients with ischemic stroke and to determine the role of NO by comparing the infarct volumes of patients’ brains as measured by computed tomography (CT) with the levels of NO.

Material and Methods: This is a prospective observational study. Blood samples and brain computed tomography images were taken at 0 and 24 hours from the ischemic stroke patients. Serum NO levels and the volumes of ischemic brain lesions were measured using CT imaging.

Results: In our study, we had 60 patients who were diagnosed with acute ischemic stroke and 37 healthy individuals. Mean NO levels in stroke patients were significantly lower compared with the control group. Patients were classified into 3 groups as 0-7, 8-14, 15≤ according to their NIHSS scores to compare their NO levels and infarct volumes. When the infarct volume of the group, which had an NIHSS score of 15≤ at 0 hours was compared with the other groups, it was found to be significantly higher (p<0.001). NO levels in the same group at 0 hours were significantly lower than in the other groups.

Discussion: The relationship between serum NO levels and brain infarct volume in ischemic stroke patients was clearly demonstrated in our study.

Keywords
Cerebrovascular Disease; Ischemic Stroke; Nitric Oxide; Infarct Volume

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Introduction

Stoke is a clinical case occurring after sudden cerebral functional failure due to pathology of the vessels in the brain, and it is the third leading cause of death following cardiovascular diseases and cancer [1]. The most common type of stroke is ischemic stroke [2]. Ischemic stroke is caused by several risk factors such as herofamilial predisposition, hypertension, diabetes mellitus, obesity, and elevated blood lipids, all of which might be independently associated with each other [3]. These risk factors may cause failure in cerebrovascular endothelin function, which plays a critical role in the regulation of cerebral blood flow [4]. Microvascular endothelial cells in the brain can produce and release many vasoactive substances, including nitric oxide (NO) and endothelin-1 that play a major role in the maintenance of cerebrovascular homeostasis [5,6].

NO is an inorganic gas that plays important roles in the control of cerebral blood flow, thrombogenesis, and the modulation of neuronal activity [7,8]. NO is produced in the endothelial cells, glial cells, neurons and macrophages by 3 different isoforms of the enzyme nitric oxide synthase (NOS) [9]. The identified functions of NO in the vascular system are regulation of vasomotor tone, inhibition of thrombocyte adhesion and aggregation, suppression of cell proliferation and platelet activation, and the modulation of myocardial contraction [10].

Endothelial NO derived from endothelial nitric oxide synthase (eNOS) is the most important determinant of basal vascular tone, which regulates systemic circulation. NO is also involved in the regulation of local circulation of some organs (e.g., in the heart and brain) [11,12]. Studies have shown that both inactivation of eNOS genes by neural nitric oxide synthase (nNOS) inhibitors and eNOS deficiency cause hypertension in rats [11].

Risk factors predisposing to atherosclerosis are associated with a reduction in bioactive NO levels, and abnormal endothelial function. This depends on whether NO is truly deficient or inactivated by entering the reaction with oxygen-derived radicals. In addition, platelets also contribute to the control of platelet activation by synthesizing NO [13,14]. A growing number of studies were conducted on the relationship between stroke and NO levels in order to understand whether NO production and its levels are important in terms of the diagnosis, prognosis and prevention of cerebrovascular diseases. Therefore, this study aims to investigate the relationship between blood NO levels and infarct volumes of the brain measured by computed tomography in patients with ischemic stroke.

Material and Methods

Study design and patients

This is a prospective observational study conducted between November 2017 and January 2018 on ischemic stroke patients who were admitted to the emergency room and were diagnosed with ischemic stroke. We received ethical approval from Clinical Research Ethics Committee of Izmir Bozyaka Training and Research Hospital (No:8.11.2017/4). The study population consisted of 60 patients with a first episode of hemispheric ischemic stroke within 24-hour after the onset of symptoms. Thirty-seven healthy subjects served as controls. Systemic and neurological examinations were performed following a detailed anamnesis of each patient.

Assessments and analyses

The severity of the neurological deficit was determined by the National Institute of Health Stroke Scale (NIHSS) score. The NIHSS is widely used to assess the severity of acute ischemic stroke. For a more detailed study of the patients, we divided them into 5 groups according to the NIHSS score. Patients were grouped according to the NIHSS scoring system as mild (0-7), moderate (7-14), and severe (greater than 15, 15±).

Routine blood tests and ECG were also done. Cerebral infarct location was detected using CT. The aforementioned assessments and tests were performed again together with neurological examination 24 hours after the first admission. Venous blood samples (8-mL vacuum and gel separator tubes, BD Vacutainer SST™ II Advance) were collected and centrifuged for 10 minutes at 3,000 rpm after blood clotted. The sera were decanted and stored at -80 °C until the ELISA assay. Serum NO levels were determined using a commercial ELISA kit (Andy Gene Biotechnology, China) according to the manufacturer’s instructions.

CT was used to find and measure the ischemic area of the brain at the time of the first admission and 24 hours after the first admission. The volume of the ischemic area of the brain was calculated according to the Cavalieri method by multiplying the surface area of the lesion with the imaging cross-sectional thickness (5mm) (Figure 1) [15].

Statistical analysis

All data were evaluated using the SPSS version 22.0 package program. Data were expressed as mean ± SD (Standard Deviation) values in the tables. The data were evaluated using Shapiro-Wilk test for normal distribution. The Wilcoxon Sign Test was conducted for dependent variables and the Kruskal-Wallis Test was done for more than two independent groups when assumptions of normal distribution and/or homogeneity of variance were violated. The Mann-Whitney U test was performed between two independent groups that did not meet the normal distribution when necessary. In all patients, Spearman's correlation analysis was used to examine correlations between NO levels and infarct volumes. Since the data between the groups separated according to the NIHSS score were not normally distributed, Logarithmic transformation was performed followed by one-way ANOVA test on these data. Duncan's Post-Hoc analysis was performed to determine which groups were different because the relationship between infarct volume and NO levels was significant among these groups. The Mann-Whitney U test was performed because the data were not normally distributed when comparing NO levels in the control group and the patient group at 0 hours. The effects of group and gender on NO levels in the NIHSS groups were evaluated by a two-way analysis of variance. Among the NIHSS score groups, a two-way ANOVA test was used for repeated measurements to compare NO levels at 0 and 24 hours. Statistical significance level was taken p < 0.05 in all tests.

Results

Baseline characteristics of patients with stroke are shown in Table 1; 84.4% of patients were diagnosed with at least one comorbid disease, while 16.6% of them had no comorbid disease. For control subjects (n=37), the mean age of males (n=20) and females (n=17) was 51.08 ± 8.37 (Table 1).

Table 1; 84.4% of patients were diagnosed with at least one comorbid disease, while 16.6% of them had no comorbid disease. For control subjects (n=37), the mean age of males (n=20) and females (n=17) was 51.08 ± 8.37 (Table 1).
The mean NO level in the control group was 37.965 ± 7.269 μmol/L (male / female 39.315 ± 7.223 / 36.376 ± 6.824 μmol/L). In the patient group, it was 21.184 ± 7.2847 at 0 hours and 25.955 ± 9.372 μmol/L at 24 hours. According to gender, the male/female ratio at 0 hours was 21.56 ± 6.860/20.715 ± 7.646 μmol/L. At the 24th hour, it was 26.16 ± 8.814/25.701 ± 9.850 μmol/L. We found that gender had no effect on NO levels (p>0.05). The mean NO levels for the entire patient group at 0 and 24 hours were significantly lower when compared to the control group (p<0.001). We also found that the mean NO level in the control group significantly changed in the follow-up period as compared to the patient group at 24 hours.

Table 1. Baseline characteristics of individuals in the study

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients with stroke</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (male/female)</td>
<td>69.38 ± 12.48 (68.82 / 70.63)</td>
<td>51.081 ± 8.367 (51.39/52.33)</td>
</tr>
<tr>
<td>Gender (male/female) (%)</td>
<td>53/27 (59%-45%)</td>
<td>20/17 (54.1%-45.9%)</td>
</tr>
<tr>
<td>Diabetes (no/yes) (%)</td>
<td>36/24 (40%)</td>
<td></td>
</tr>
<tr>
<td>Hypertension (no/yes) (%)</td>
<td>19/41 (68%)</td>
<td></td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>45/15 (25%)</td>
<td></td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>44/16 (26.6%)</td>
<td></td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>38/22 (36.6%)</td>
<td></td>
</tr>
<tr>
<td>Echo (pathologic/normal)*</td>
<td>36/24 (60%)</td>
<td></td>
</tr>
<tr>
<td>Carotid artery stenosis or plaque</td>
<td>40/20 (66.6%)</td>
<td></td>
</tr>
<tr>
<td>Vertebral artery stenosis or plaque</td>
<td>10/50 (16.6%)</td>
<td></td>
</tr>
<tr>
<td>ECG (normal / AF)</td>
<td>48/12 (80%-20%)</td>
<td></td>
</tr>
</tbody>
</table>

* Pathologic findings are left ventricular hypertrophy, valve insufficiency, left atrial enlargement, and thrombus. ECG: Electrocardiogram, AF: Atrial fibrillation

Table 2. According to NIHSS scores, NO levels and brain infarct volumes at 0 and 24 hour

<table>
<thead>
<tr>
<th>0-hour NIHSS score</th>
<th>24-hour NIHSS score</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-7 (n=32)</td>
<td>22.366 ± 6.857</td>
</tr>
<tr>
<td>8-14 (n=20)</td>
<td>21.957 ± 7.0798</td>
</tr>
<tr>
<td>15≤ (n=8)</td>
<td>14.472 ± 2.984 *</td>
</tr>
</tbody>
</table>

*p < 0.001 NIHSS 15 ≤ group when compared with NIHSS 0-7 and 8-14 groups (at 0 hours).
*p < 0.001 NIHSS 15 ≤ group when compared with NIHSS 0-7 and 8-14 groups (at 24 hours).
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*p < 0.001 NIHSS 15 ≤ group when compared with NIHSS 0-7 and 8-14 groups (at 24 hours).

Table 3. NO levels and brain infarct volumes of patients in decreased, increased and unchanged NIHSS score groups (at 24 hours)

<table>
<thead>
<tr>
<th>0-hour NO levels μmol/L</th>
<th>24-hour NO levels μmol/L</th>
<th>Brain infarct volumes (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unchanged NIHSS score group (n=35)</td>
<td>0.929 ± 6.891</td>
<td>22.485 ± 2.728</td>
</tr>
<tr>
<td>Decreased NIHSS score group (n=18)</td>
<td>26.466 ± 9.067</td>
<td>26.444 ± 10.851</td>
</tr>
<tr>
<td>Increased NIHSS score group (n=7)</td>
<td>15.188 ± 23.056</td>
<td>14.307 ± 16.740</td>
</tr>
</tbody>
</table>

*p < 0.001 Increased NIHSS score group when compared with the decreased and unchanged NIHSS score groups.
*p < 0.05 Increased NIHSS score group when compared with the decreased and unchanged NIHSS score groups.
*p < 0.05 NO levels of Increased NIHSS score group (at 24 hours) when compared with the NO levels of unchanged NIHSS score group (at 0 hours).

The mean NO level in the control group was 37.965 ± 7.269 μmol/L (male / female 39.315 ± 7.223 / 36.376 ± 6.824 μmol/L). In the patient group, it was 21.184 ± 7.2847 at 0 hours and 25.955 ± 9.372 μmol/L at 24 hours. According to gender, the male/female ratio at 0 hours was 21.56 ± 6.860/20.715 ± 7.646 μmol/L. At the 24th hour, it was 26.16 ± 8.814/25.701 ± 9.850 μmol/L. We found that gender had no effect on NO levels (p>0.05). The mean NO levels for the entire patient group at 0 and 24 hours were significantly lower when compared to the control group (p<0.001). We also found that the mean NO level in the control group significantly changed in the follow-up period as compared to the patient group at 24 hours. The mean NIHSS score of the patients at the time of admission (0 hours) was calculated as 8.016 ± 4.335. NIHSS score was 0-7 in 33 patients (53.3%), NIHSS score was 8-14 in 19 patients (33.3%) and NIHSS score was 15 and over in 8 patients (13.3%). Additionally, the mean NIHSS score of the patients at 24 hours was calculated as 7.6 ± 4.603. NIHSS score at 24 hours was between 0-7 in 37 patients (61.6%), between 8-14 in 16 patients (26.6%), and 15 and over in 7 patients (11.6%).
other score groups (p<0.001), although a significant increase
was observed in infarct volume of patients NIHSS score 15≤
(p<0.001). Likewise, mean NO levels of the patients with NIHSS
score of 15≤ at 24 hours were significantly lower than in other
groups at 24 hours (p<0.001). In the same manner, there was a
significant increase in infarct volume of patients’ NIHSS score
15≤ at 24 hour (p<0.001) (Table 2).

Patients were divided into 3 groups according to their altered
NIHSS scores when comparing the difference between 0 and
24 hours, as increased NIHSS score, decreased NIHSS score,
and unchanged NIHSS score. NIHSS scores were increased in 7
patients, decreased in 18 of them, and no changes were found
in 35 of them. It was found that the NO levels measured at 24
hours in patients with increased NIHSS score group were not
statistically significant, but were lower than the NO levels at
0 hours (p>0.05). In addition, it was observed that the mean
infarct volume was significantly higher in the increased NIHSS
score group compared to the other groups (p<0.001). Likewise,
in the group with increased NIHSS score, it was observed
that NO levels at 24 hours were lower than the 0-hour levels,
although it is not statistically significant, while the opposite
situation was also observed in the group with a decreased
NIHSS score (Table 3).

Regarding the results of comparison between altered NIHSS
score groups in Table 3, we further analyzed the relationship
among these groups, and used Spearman’s correlation analysis
for this purpose. We found statistically significant negative
correlations between both infarct volume-NO levels and NIHSS
scores-NO levels at 0 and 24 hours (p<0.01 and p<0.001)
(Figure 2).

Discussion

Studies have shown that early diagnosis and treatment of
patients who come to emergency services with CVD can reduce
the effects of this disease on mortality and morbidity. Thus,
for use in the early diagnosis and treatment of patients with
ischemic stroke, studies with many molecules that are effective
in pathogenesis are being made. Chemerin, basic fibroblast
growth factor (bFGF), Adropine, Pentraxin 3 and NO are some
of them [14,16].

One of the most important factors affecting the pathogenesis
of ischemic stroke is the deterioration of endothelial function.
In patients exposed to vascular risk factors, endothelium-
dependent relaxation dysfunction is detected prior to
morphological changes in the cell wall [17,18]. According to
a study, such as hypertension, hyperlipidemia, smoking and
diabetes, abnormal endothelial functions have been shown to
be associated with a decrease in NO levels [19].

NO is a potent vasodilator synthesized by the nitric oxide
synthase enzyme from L-arginine. NO inhibits platelet
aggregation, leukocyte chemotaxis and their adhesion, as
well as downregulates chemokine expression, reduces smooth
muscle cell proliferation, migration and LDL oxidation, and
thus it has anti-atherogenic and anti-thromboembolic effects
[11,19]. In studies conducted by Drake and colleagues, it has
been shown that the most important signaling molecule that
plays a role in the autoregulation of cerebral blood flow and in
cerebrovascular endothelium is endothelial NO [11].

In general, according to some studies, loss of endothelial NO is
considered to be the central mechanism in the pathogenesis of
dimethylarginine, endothelial nitric oxide synthase and nitric
oxide as a risk factor for early-onset ischemic stroke cases
in Eastern Anatolia Region, (PhD Thesis), Erzurum, 2014.). In
another study, the NO levels in 81 patients with acute
ischemic stroke were 58.46 ± 1.92 μmol and were 61.22 ± 0.95
μmol in 50 healthy controls (Bengü Ş. Evaluation of asymmetric
dimethylarginine, endothelial nitric oxide synthase and nitric
oxide in cerebrospinal fluid and in serum were higher in stroke
patients than in the control group [23].

In our study, similar to other studies in the literature, mean NO
levels in stroke patients were significantly lower (p<0.001). In
addition, although the NO levels of patients measured at 24
hours were significantly increased compared to the 0-hour
level, they were significantly lower than in the control group
(p<0.001).

The infarct volumes were significantly higher in the group of
NIHSS 15≤ at 0 hours when compared with the other groups
(p<0.001). NO levels at 0 hours were significantly lower in the
group with NIHSS score 15 and over compared with the groups
of NIHSS score 0-7 and 8-14 (p<0.001).

According to the investigation among these groups, NO levels
at 0 hours were significantly lower in the group of NIHSS score
15 and over when compared with the other groups, additionally,
NO levels for the same group at the 24 hours were also lower
(p<0.001). When we compared NO levels at 0 hours and 24
hours, the increase in NO levels for the NIHSS score 0-7 and
8-14 groups was significant (p<0.001). Nevertheless, there was

According to these studies, it has been demonstrated that NO
is an important mediator in the regulation of cerebral blood
flow [10,21].

In a study by Rashid and colleagues, plasma levels of NO in 38
controls, 228 ischemic stroke, and 49 hemorrhagic stroke
patients were examined, and as a result, levels of NO in control
group was 64.0 ± 36.3 μmol/L, 49.9 ± 26.1 μmol/L in the group
of ischemic stroke and 41.7 ± 19.5 μmol/L in the group of
hemorrhagic stroke [22]. Nandhagopal found that serum NO
levels were lower in stroke patients (40.9 ± 5.9 μmol / L) than in
the control group (59.9 ± 7.3 μmol / L) in a study of 40 stroke
patients (Nandhagopal R, Krishnamoorthy SG, Vengamma B.
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NO levels for the same group at the 24 hours were also lower
(p<0.001). When we compared NO levels at 0 hours and 24
hours, the increase in NO levels for the NIHSS score 0-7 and
8-14 groups was significant (p<0.001). Nevertheless, there was
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no change in the levels of NO in the group of NIHSS score 15 and over. According to these results, it can be said that the infarct volume may be lowered due to the protective effect of NO that occurs as a result of its increase.

To examine our data from another perspective, patients were divided into 3 groups according to the NIHSS score at 24 hours as increased, decreased and unchanged. In patients with an increased NIHSS score, NO levels decreased at 24 hours compared to 0 hours, and the infarct volumes were significantly higher than in the other groups (p<0.001). NO levels of the patients in the groups of decreased and unchanged NIHSS score at 24 hours were increased compared to 0 hours. These results also support the association between NO levels and infarct volume.

Conclusion

Our study investigates the relationship between NO levels and infarct volume in stroke patients. In many experimental animal studies, NO activity and eNOS are associated with such disease models and their protective effects are known. This study highlights the protective effects of NO in ischemic stroke through demonstrating the significant relationship between infarct volumes and NO. However, the molecular mechanisms underlying this relationship need to be studied with more detailed animal studies, and should be strengthened by clinical studies involving even more patients.

Scientific Responsibility Statement

The authors declare that they are responsible for the article’s scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

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References