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Do Plasma Nitric Oxide Levels Have an Impact on Unprovoked Migraine Attacks?

Plazma Nitrik Oksit Düzeylerinin Provoke Edilmemiş Migren Ataklarında Etkisi Var mı?

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Abstract

Objective: Provocation studies on the role of nitric oxide (NO) in the pathophysiology of migraine have long been conducted and provide important information. Although there are many provocation studies in the literature, there are fewer publications on the role of NO in spontaneous migraine attacks. In this study, we aimed to compare the serum NO levels during unprovoked attacks in patients with migraine using the values in the interictal period and the results of the control group.

Materials and Methods: Thirty migraine patients were evaluated for plasma NO levels during the natural course of unprovoked attacks and in the interictal period. They were also compared with the results of 25 healthy controls.

Results: The mean plasma NO levels were 20.99 ± 4.93 µmol/l during migraine attacks, 25.97 ± 9.83 µmol/l between attacks and 23.22 ± 7.57 µmol/l in the control group. According to these results, the mean plasma NO levels were significantly lower in migraineurs during attacks compared to the non-attack period (P = 0.025), but there was no significant difference with controls (P = 0.212 and P = 0.247, respectively).

Conclusion: To evaluate whether NO has an effect on the natural history of migraine attacks, more spontaneous attack studies involving methods other than measuring serum levels are needed.

Keywords: Migraine, nitric oxide, spontaneous attack

Öz

Amaç: Migren patofizyolojisinde nitrik oksitin (NO) rolü ile ilgili provokasyon çalışmaları uzun zamandır yapılmakta ve önemli bilgiler vermektedir. Literatürde çok sayıda provokasyon çalışması olmasına rağmen spontan migren ataklarında NO'nun rolü ile ilgili daha az sayıda yayın mevcuttur. Bu çalışmada migren hastalarında provoke edilmemiş ataklar sırasındaki serum NO değerleri ile interiktal dönemdeki değerler ve kontrol grubunun sonuçlarının karşılaştırılması amaçlanmıştır.

Gereç ve Yöntem: Otuz migrenli hastanın provoke edilmemiş ataklarının doğal seyrinde ve ataksız dönemlerinde plazma NO düzeylerini değerlendirildi. Ayrıca bunlar 25 sağlıklı kontrolün sonuçlarıyla karşılaştırıldı.

Bulgular: Ortalama plazma NO seviyeleri migren atakları sırasında 20,99 ± 4,93 µmol/l, ataklar arasında 25,97 ± 9,83 µmol/l ve kontrol grubunda 23,22 ± 7,57 µmol/l idi. Bu sonuçlara göre migrenlilerde ataklar sırasında ortalama plazma NO düzeyleri ataksız döneme göre anlamlı olarak daha düşüktü (P = 0,025), ancak kontrollerle anlamlı bir fark yoktu (sırasıyla; P = 0,212 ve P = 0,247).

Sonuç: NO'nun migren ataklarının doğal seyrinde bir etkisinin olup olmadığını değerlendirmek için daha fazla sayıda ve serum düzeylerinin ölçülmesinden farklı yöntemlerle yapılan spontan atak çalışmalarına gerek vardır.

Anahtar Kelimeler: Migren, nitrik oksit, spontan atak

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Introduction

Migraine is one of the most common primary headache syndromes in the general population. It is a public health problem that causes a serious loss productive time due to headache among young adults (1).

Despite intensive research in recent years, the pathophysiology of migraine remains unclear. It has been posited that vascular and/or neurogenic factors play a role in its formation. During a migraine attack, cortical depression, neurogenic inflammation, and changes in vascular reactivity occur (1). It is believed that the trigeminovascular system plays an important role in the initiation and continuation of the attack process (2).

Nitric oxide (NO) is an important intercellular messenger that plays a role in many physiological functions in the body and the pathophysiology of various diseases (3). Experimental studies suggest that NO causes vasodilation in cerebral and extracerebral circulation in the pathophysiology of migraine, that it is an important molecule in pain pathways, and is produced and released during cortical spreading depression (4). It is thought that increased NO levels during migraine attacks increase blood flow with a vasodilator effect and play a role in causing headaches. In addition, it has been suggested that NO may increase the release of calcitonin gene-related peptide (CGRP) in the trigeminal nerve terminals and trigger migraine attacks. It may also contribute to the aggravation of pain by increasing the sensitivity of the trigeminal nerve and facilitating the transmission of pain signals (4).

Cyclic guanosine monophosphate (cGMP)-dependent pathways are thought to play a role in the pathophysiology of migraine. It is known that NO molecules also increase the cGMP levels in the smooth muscle cells of intracranial arteries (5). With the activation of these pathways, perivascular trigeminal afferents are activated (5). In migraine provocation studies, individuals were given glyceryl trinitrate, an NO donor, and it was shown that the patients with migraine developed more severe headaches than the healthy controls (5,6). Accordingly, provocation studies indicate that the role of NO is important in the development of migraines (7).

Although there are many provocation studies in the literature, there are fewer studies on the role of NO in spontaneous migraine attacks (8,9). In this study, the serum NO levels during unprovoked attacks in patients with migraine were compared with those in the interictal period and those in the control group. In addition, whether the serum NO levels exhibited a difference that could be associated with the frequency of headache attacks in the past 3 months was investigated, as well as the severity of the headaches, the provoking factors, and the accompanying clinical findings.

Materials and Methods

Thirty patients between the ages of 18 and 50 who were admitted to the Neurology outpatient clinics of the SANKO University Sani Konukoglu Application and Research Hospital and the Dr. Ersin Arslan Training and Research Hospital, who had been diagnosed as having migraine according to the International Classification of Headache Disorders-III (10) were included in this study. Patients who experienced chronic migraine and medication overuse headaches were not included in the patient group. Twenty-five age- and sex-matched patients without primary headaches were included in the study as the control group.

When forming the patient and control groups, patients with hypertension, renal dysfunction, signs of active infection, and endocrinological and rheumatological diseases were excluded.

The socio-demographic and medical information of the patients, including drugs used in the past month, severity, duration, frequency, time of onset of headache attacks, symptoms accompanying attacks, sensitivity to noise and light (phonophobia and photophobia) during attacks, nausea and vomiting, the use of analgesics, response to analgesics, attack-provoking factors, loss of productive time at work/household work and in social life due to headache, and neurological examination results, was recorded. The sociodemographic and medical information of the controls was recorded.

Venous blood samples of 10–15 ml were collected from the patients with migraine twice, during the attack and interictal periods, and at any point from the control group. The blood samples collected during attacks were collected before the patient took any medication for the attack. All blood samples were collected in the outpatient clinics. Since the blood samples were collected during the first attack in the patient group, a fixed time for blood sample collection could not be determined and samples were collected at any time when the patients presented with an attack. However, all blood samples were collected during daytime working hours. The blood samples collected during the attack-free (interictal) period were collected at least 10 days after the attack. The samples were collected into yellow-capped tubes and centrifuged at 3,500 rpm for 5 min, and their serum parts were aliquoted and thereafter stored at -80 °C until the day of the study. Prior to starting the analysis, frozen samples were gradually thawed at room temperature, avoiding re-thawing–freezing processes. All samples and kits used were brought to room temperature (18 °C–26 °C) before starting the study. The researchers studying the serum levels were blinded to the clinical information associated with the groups.

Nitric Oxide Measurement

The NO levels were measured colorimetrically using the commercial Elabscience®, E-BC-K035-M kit (Houston, TX, USA). NO is readily oxidized to NO_2 (*in vivo* or in an aqueous solution), which forms a reddish azo compound using a color developer, and the concentration of the azo compound is linearly related to the NO concentration. Here, the NO concentration was calculated indirectly by measuring the optical density value at 550 nm and obtaining a result in µmol/l. Then, the clinical information and laboratory findings were matched and evaluated using statistical tests.

The study was carried out following the Principles of the Declaration of Helsinki and approval was obtained from the SANKO University Clinical Research Ethics Committee before conducting the research (date: 03.10.2019, number: 2019/13-11). All volunteers provided signed informed consent to participate in the study.

The CGRP and adrenomedullin levels were also measured across the participants, but these data are discussed in a different article.

Statistical Analysis

The conformity of the continuous variables to normal distribution was evaluated using the Shapiro–Wilk test. A

Student's t-test (for normally distributed data) and a Mann–Whitney U test (for non-normally distributed data) were used to compare the numerical variables across the two groups. A dependent sample t-test (for normally distributed data) and a Wilcoxon test (for non-normally distributed data) were conducted for intra-group comparisons. A chi-square test was applied to investigate the relationships between categorical variables. The statistical analysis was performed using the SPSS (v.24.0) software package for Windows and a *P* value of <0.05 was considered statistically significant.

Table 1. Age, gender, and serum NO levels of the patient and control groups

	Patient (n = 30)	Control (n = 25)	*P*
Gender (M/F)	4/26	4/21	0.780
Age (min–max) Median [25%–75%]	18–41 25 [23–32]	22–40 25 [23–28]	0.628
NO during an attack (µmol/l) (mean ± SD)	20.99 ± 4.93	23.22 ± 7.57	0.212
NO during an attack-free (µmol/l) (mean ± SD)	25.97 ± 9.83	23.22 ± 7.57	0.247
NO within groups (µmol/L) (during attack/ attack-free)	*P* = 0.025*	-	-

* *P* < 0.05 indicates a significant difference; inter-group comparisons used a Student's t-test, intra-group comparisons used a dependent sample t-test. NO: Nitric oxide, SD: Standard deviation, M: Male, F: Female

Table 2. Clinical features associated with attacks

	n	%
Loss productive time due to headache		
No	2	6.7
Less than one day per month	6	20.0
More than one day a month	12	73.3
Duration of attacks		
4–24 hours	16	53.3
25–72 hours	12	40.0
>72 hours	2	6.6
Frequency of headache attacks in the past 3 months		
At least 1 per week	13	43.3
Less than once a week	12	56.7
Provoking factors		
Psychological stress	26	86.7%
Changes in sleep patterns	25	83.3%
Physical stress	21	70%
Symptoms accompanying headache		
Distractibility	25	83.3%
Mood change	24	80%
Sweating/chills	21	70%

n: Number

Results

Thirty patients with migraine (26 of whom were women) aged 23–32, and 25 healthy controls (21 of whom were women) aged 23–28 were included in the study (Table 1).

Headache was present for more than 5 years in 40% of the patients. Among the patients with migraine, 93.3% reported having experienced at least one day of headache-related loss of work power in the past 3 months, while 83.3% had had at least one attack per month in the past 3 months; the duration of the attacks was less than 24 hours in 53.3% and lasted 2–3 days in 40%. Moreover, 83.3% reported their headaches as having been severe or very severe (Table 2), while 80% always experienced phonophobia during their attacks, and 83.3% always experienced photophobia. Nausea always accompanied the attacks in 66.7% and vomiting in 13.3%.

Four (13.3%) of the patients were receiving prophylactic treatment, with two patients using amitriptyline, one using a calcium channel blocker, and one using topiramate.

Among the patients with migraine, 66.7% stated that they do not use analgesics for headache or used analgesics once a week or more rarely. Among those who used analgesics for headache, with 46.7% reporting they sometimes had an analgesic response and 20% stating that they did not see any benefit.

The most frequently described provoking factors were psychological stress, changes in sleep patterns, and fatigue (86.7%, 83.3%, and 70%, respectively) (Table 2). Distraction, mood changes, and sweating/chills were the most common symptoms accompanying the headache attacks (83.3%, 80%, and 70%, respectively) (Table 2).

In the patients with migraine, the serum NO level during the attack was statistically significantly lower than during the interictal period (*P* = 0.025). There was no statistical difference in terms of the serum NO levels between the control and migraine groups in either the attack periods or the attack-free periods (Table 1).

There was no significant relationship between any of the clinical features of migraine attacks and NO levels in the attack or the attack-free period.

Discussion

In this study, the serum NO levels measured during an attack were lower than in the attack-free period. Provocation studies on the role of NO in the pathophysiology of migraine have long been conducted and provide important information in this regard. It was found that the pathways comprising NO synthesis were activated in experimental headache models (e.g., nitroglycerin-induced headache) (4). NO is one of the most important substances that has a vasodilator effect produced by the endothelium (3). Experimental studies and hypotheses have been based on the indication that NO plays a role by acting on pain pathways, causing vasodilation, or stimulating the release of CGRP (11).

However, the measurement of serum NO levels in spontaneous migraine attacks has been studied less frequently. The NO levels from peripheral venous blood samples differ between studies. While no difference was found in some studies, higher NO levels were observed among patients with migraine in others (12,13,14,15). With the belief that measurement from the jugular vein may be more valuable, in a study in which NO measurement

was performed from the internal jugular vein in spontaneous migraine attacks without aura, the NO level was found to be high during the attack, while a decrease was observed in the follow-up measurements. In the same study, simultaneous peripheral venous blood levels were also examined, and a slight increase was observed during the first hour of the attack, but the results were lower in the peripheral blood compared to the jugular vein (9). Due to the invasive procedure of sampling from the jugular vein, these studies are difficult to conduct, and similar studies cannot be found in the literature.

While the serum NO levels were high during migraine attacks in one study, no significant difference was observed between the serum NO levels in the attack-free period and those of the healthy control group (12). However, in another study, while there was no difference between the ictal and interictal periods, higher NO levels were observed in patients with migraine when compared to the control group (13). Conflicting results were also obtained in studies regarding the NO level. One study reported that the NO level was higher in the interictal period in patients with migraine compared to the control group (14), while another reported no difference (15). In the present study, no significant difference was observed between the serum NO levels of the migraine group in the ictal or interictal period or the serum NO levels of the control group. The conflicting results among studies may be due to the use of different methods, the heterogeneous clinical characteristics among patients with migraine, collecting of blood samples at different times during the attack or collecting the attack-free period and the different effects of factors triggering migraine attacks on NO.

In the existing literature, the results of studies on the relationship between serum NO levels and the duration and frequency of attacks are also contradictory. In the present study, no relationship was found between the duration and frequency of headache attacks and NO level. The findings of some of the existing studies are consistent with those of the present study (13,15,16).

Study Limitations

Our patients reported that the most common symptoms accompanying a headache were being distracted, mood changes, and sweating/chills. No significant relationship involving the NO levels was detected in the attack and non-attack periods, and these results could suggest that NO may not be associated with these findings during the natural course of a migraine. However, the clinical findings of migraine attacks can vary between patients and even between attacks in the same patient, which makes it difficult to investigate them. To the best of our knowledge, no study in the current literature evaluated the relationship between NO level and the accompanying findings during spontaneous attacks. The results of a relatively small number of patients were evaluated in this study. We believe that more detailed studies are needed on this subject.

Conclusion

More spontaneous attack studies involving methods other than measuring serum levels are needed to evaluate whether NO affects the natural history of migraine attacks.

Ethics

Ethics Committee Approval: The study was carried out following the Principles of the Declaration of Helsinki and approval was obtained from the SANKO University Clinical Research Ethics Committee before conducting the research (date: 03.10.2019, number: 2019/13-11).

Informed Consent: All volunteers provided signed informed consent to participate in the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Y.E.F., E.K.C., A.N., A.M.N., Design: Y.E.F., S.K.A., E.K.C., A.N., M.B.Ç., A.M.N., Data Collection or Processing: Y.E.F., E.K.C., A.N., A.M.N., Analysis or Interpretation: Y.E.F., S.K.A., E.K.C., A.N., M.B.Ç., A.M.N., Literature Search: Y.E.F., S.K.A., E.K.C., A.N., M.B.Ç., A.M.N., Writing: Y.E.F., S.K.A., E.K.C., A.N., M.B.Ç., A.M.N.

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