Elevated plasma total nitrite levels may be related to migraine attacks

Migren atakları plazma total nitrit seviyesinde yükselme ile ilişkili olabilir

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Abstract
Both vascular and neuronal mechanisms are the main foci of investigation in defining the pathophysiology of migraine attacks. This study was designed to evaluate the possible role of nitric oxide (NO) in migraine patients in the natural course of unprovoked attacks and attack-free periods. The mean plasma nitrite levels of 26 migraine patients during attacks and attack-free periods were compared within group and with those of 26 healthy controls. Plasma total nitrite levels were measured by Greiss reaction. Mean plasma total nitrite levels of migraine patients during attacks and attack-free periods and of controls were 36.5±7.5 µmol/l, 27.81±4.8 µmol/l and 25.19±4.1 µmol/l, respectively. These results demonstrated that total nitrite levels during attacks were significantly higher in migraineurs than both during attack-free periods and those of controls (P=0.001 and P=0.001, respectively). No significant difference was observed between migraineurs during attack-free periods and controls in this regard (P=0.534). Based on these results, we suggest that NO pathway may play a key role in the pathogenesis of migraine attacks.
Keywords: Etiopathogenesis; migraine; nitric oxide; serum total nitrite levels

Özet
Bu çalışma migren hastalarında provoke edilmemiş atak döneminde ve ataksız dönemde plazma nitrik oksit düzeyinin olası rolünü değerlendirmek üzere tasarlandı. Yirmi altı migren hastasında ataklı ve ataksız dönmede ortalama plazma nitrit seviyesi birbirleri ile ve sağlıklı kontrol grubunun ortalama plazma nitrit seviyesi ile karşılaştırıldı. Plazma total nitrit seviyesi Greiss reaksiyonu ile ölçüldü. Migren atak dönemi, ataksız dönem ve sağlıklı kontrol grubunda ortalama plazma nitrit seviyeleri sırasıyla; 36.5±7.5 µmol/l, 27.81±4.8 µmol/l ve 25.19±4.1 µmol/l olarak ölçüldü. Bu sonuçlara göre total plazma nitrit seviyesi migrenli hastalarda atak döneminde ataksız döneme ve sağlıklı kontrol grubuna göre anlamlı düzeyde yüksekti (sırasıyla; P=0.001, P=0.001). Plazma total nitrit düzeyleri açısından migren hastalarının ataksız dönemi ile sağlıklı kontrol grubu arasında anlamlı bir fark tespit edilmedi (P=0.534). Bu sonuçlara göre NO yolağı migren atak patogenezinde önemli bir rol oynayabilir.
Anahtar kelimeler: Etyopatogenez; migren; nitrik oksit; serum total nitrit seviyesi

Introduction

Both vascular and neuronal mechanisms are the main foci of investigation in defining the pathophysiology of migraine attacks. Previous studies revealed that nitric oxide (NO) might have an important role in the pathogenesis of migraine. A number of studies have agreed that NO donors might precipitate migraine attacks and inhibitors might block this effect in migraineurs (1-3).

NO is an endogenous molecule released primarily from endothelial cells. NO, which is a potent anti-aggregant and causes relaxation of smooth muscle, has an important role in the control of vascular tonus and regulation of systemic blood pressure. It is also involved in pain modulation, since NO synthase (NOS) inhibition causes analgesia in headache via various ways (4,5). Existing data in means of NO involvement in migraine have been mostly obtained from studies that were conducted in NO induced headache attacks in healthy subjects and in primary headache cases. However, findings in provoked attacks may not tell us the full story about the role of NO pathways in the natural course of migraine.

We measured the plasma nitrite levels in migraine cases with and without attacks to determine whether they differed from controls in terms of plasma nitrite levels, in attacks or attack-free periods.

Materials and Methods

A total of 26 migraine patients with or without aura were enrolled into the study group; control group consisted of 26 healthy subjects. Migraine was diagnosed using the International Headache Society (IHS) criteria (6). Twenty-nine patients were asked to participate in the study, 3 of them did not give

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consent. The study was approved by the Local Ethics Committee of the Institution (Voucher no: 06/06/2005/11) and written informed consents were obtained from all subjects before enrollment. The eligibility criteria for the migraine patients were diagnosis of migraine (according to IHS criteria), being between 18-50 years of age, accepting to provide a written informed consent and absence of any of the exclusion criteria. The exclusion criteria included presence of hypertension, renal dysfunction, any endocrinological and rheumatologic disease, symptoms of an acute systemic infection, presence of chronic migraine or drug overuse headache, and pregnancy for female subjects.

In healthy volunteers between the ages of 18-50 years who agreed to take part in the study, only the absence of exclusion criteria was checked. A detailed medical history (including symptoms, age, medical history, family history, characteristics of the headache, treatment regimen, symptoms accompanying the headache etc.) was obtained from all migraine and control subjects. Blood pressure was measured and physical and neurological examinations were performed. The socio-demographic and medical information of both patients and control subjects were recorded on a preformed form.

Two samples were obtained from each migraine patient: one during an attack (while the headache is present but prior to use of any medication) and one during attack-free period (without any symptoms for at least 48 hours). Only one sample was taken from control subjects. Ten ml of blood sample was taken from the cubital vein into a tube containing aprotinin (0.6 TIU/ml). The tubes were shaken gently and allowed to stand for 15 minutes, then centrifuged at 1600 *g* for 10 minutes. The plasma samples were stored at -20°C until the biochemical laboratory evaluation.

Total nitrite reacts with molecular oxygen and accumulates in plasma in the form of nitrite and nitrate ions. Total nitrite, the stable oxidation product of nitrite (NO_2) and nitrate (NO_3), can be readily measured in biological fluids and used as the indicators of NO products both in vivo and in vitro (7). Total nitrite was assayed by Greiss reaction after incubation of the supernatant with *E. coli* nitrate reductase that converts NO_3 to NO_2. Greiss reagent (1% sulfanilamide and 1% naphthyl ethylenediamide dehydrochloride in 2.5% H_3PO_4) was added to 1 ml of supernatant and the absorbance was recorded at 545 nm after incubation for 30 minutes (Shimadzu UV mini-1240 Kyoto, Japan). The absorbances were analyzed using the standard curve constructed using $NaNO_2$ which is a reduction product of $NaNO_3$. The reaction was linear between 0.25-100 µmol/l (8).

The assays were confirmed in two ways. The inter- and intra-assay variations were 7.52% and 4.61%, respectively. In order to control conversion of nitrate to nitrite, a certain amount of nitrate was added to control plasma samples and they were deproteinized as described above. The recovery of nitrate was found to be 98.3±7.09%.

The procedure was the same in both migraine and control cases and the laboratory team that made the assessment was blinded to the groups.

The socio-demographic and clinical characteristics of the patients and controls were presented in simple distribution. The comparison of these parameters was performed using Chi-Square and Fisher's Exact test. Student's t test was used to compare quantitative variables (i.e., age). Comparison of two-group variables and plasma NO levels was evaluated by Mann-Whitney U test. Multiple comparisons were performed using Kruskall Wallis variance of analysis (ANOVA). Spearman test was used for the analysis of correlation between laboratory values and plasma NO levels. Significance level was set at $P<0.05$. All statistical calculations were made using SPSS for Windows version 20.0 software program (SPSS Inc. Chicago, IL, USA).

Results

The migraine group consisted of 22 female (84.6%) and 4 male (15.6%) patients and the control group consisted of 17 female (65.4%) and 9 male (34.6%) subjects. The mean age was 28.65±5.87 (19-47) years and 28.12±5.89 (18-48) years in the migraine group and the control group, respectively. Both groups were similar regarding age and gender ($P=0.472$ and $P=0.349$, respectively).

In the migraine group, 11 cases had migraine with aura (42.3%) and 15 had migraine without aura (57.7%). When the incidence of the attacks within the last 3 months was questioned, the data was as follows: 7 patients had 3 to 4 attacks per month (26.9%), 14 had 1 to 2 attacks per month (55.8%) and 5 had 2 to 3 attacks per 1 or 2 months (19.2%). The most commonly experienced accompanying symptom during attacks was photophobia (84.6%). Other common symptoms were nausea (61.5%) and vomiting (61.5%). Mean plasma nitrite levels of migraine patients did not show any significant difference by any demographic variables or associated clinical features ($P>0.05$, for all parameters).

Table 1. Comparison of plasma total nitrite levels of migraine patients in attack and attack-free periods and control group

		Total nitrite (µmol/l)
Migraine group n=26	Attack	36.5±7.5
	Attack-free	27.81±4.8
Control group n=26		25.19±4.1

Mean plasma NO levels were significantly higher in migraineurs during attacks (36.5±7.5 μmol/l) compared to both attack-free periods (27.81±4.8 μmol/l) and healthy controls (25.19±4.1 μmol/l) (P=0.001 and P=0.001, respectively) (Table 1). Plasma nitrite levels during attack-free periods in migraineurs were found to be similar to the plasma nitrite levels of the controls (P>0.05). There were no statistically significant differences between patients with migraine with and without aura based on plasma nitrite levels either in attack or attack free periods (P>0.05, P>0.05 respectively) (Table 2).

Table 2. Comparison of plasma total nitrite levels of migraine patients with or without aura in attack and attack-free periods

		With aura n=11	Without aura n=15	P value
Total nitrite (μmol/l)	Attack	17.1±2.8	19.4±3.0	0.433
	Attack-free	11.45±1.8	16.36±3.3	0.087

Discussion

Migraine is a common, chronic and disabling neurovascular disorder. Although the pathogenesis of migraine is still unclear, different vascular, neurological, and neuroinflammatory mechanisms have been suggested (9). NO is one of the molecule that takes a part in the pathophysiology of migraine. NO is involved in the regulation of the cerebral vessels tone. The design of the present study gave us an opportunity to compare the attack period plasma total nitrite levels of migraine cases, both with the attack-free period of the same group and with the control group. We found that plasma total nitrite levels elevated during migraine attacks and returned to low levels close to the control cases during the attack-free periods.

It is well known that migraine attacks may be provoked with the administration of NO donors. Infusion of NO donors may cause abrupt but nonspecific headaches. Apart from immediate headaches, more specific headaches, usually with associated features like nausea/vomiting and photophobia/phonophobia, following a few hours after the administration may also be initiated by NO donors (10,11). Migraine subjects are more susceptible to NO donors and they experience more severe headaches than healthy controls and tension type headache cases and the majority of migraineurs report that typical migraine without aura occurs within 3-6 hours following the administration (1,12). Administration of high doses of NO donors may initiate migraine attacks even in healthy subjects (13). It was also reported that administration of NOS inhibitor (L-NG methyl arginine hydrochloride) was successful in the treatment of headache and provided a decrease in accompanying symptoms such as photophobia and phonophobia (2).

The mechanisms that are involved in delayed headaches have not yet been understood. Trigeminovascular system activation seems to be the key point for the initiation of migraine attacks. Therefore, NO may be involved in the initiation of a series of alterations, such as changes in the functions of ion channels, or calcitonin gene-related peptide (CGRP) or glutamate release that may result in migraine type headaches (14,15). CGRP is a significant molecule in this respect. Increased plasma CGRP level was reported previously in GTN-induced migraine attacks, supporting the concept that NO may be involved in basic mechanisms in migraine attacks. On the other hand, although CGRP antagonists may be helpful in migraine attacks, they do not relieve GTN-induced migraine attacks (16). The most important effect of NO is the activation of the soluble guanylate cyclase. This enzyme causes the synthesis of cyclic guanosine monophosphate (cGMP) and NO-cGMP pathway in smooth vascular muscles causes vascular dilation and relaxation (10). It is suggested that NO could be an important mediator in the initiation or the propagation of a neurogenic cranial vessel inflammatory response that might eventually result in a migraine attack (17). Therefore NO may be involved in various mechanisms in migraine, some of which have not yet been discovered.

In the last twenty years, substantial information has been collected about the involvement of NO in the pathophysiology of migraine. However, current data cannot answer the question how endogenous NO alterations affect the natural course of migraine attacks. We do not even know which circumstances may induce the alterations of plasma NO levels in migraine cases; therefore, it is still not easy to translate previous data to clinical practice. Moreover, in contradiction to strong clues on involvement of NO in migraine attacks, new drug studies on inhibition of NO pathways have mostly failed to be an option for migraine treatment so far.

Our results supported that elevation of plasma NO levels might had an important role in migraine attacks. One of the limitations of the present study was the relatively small number of patients included in it. However, we believe that studying plasma nitrite levels in both attack-free and unprovoked attack periods in the same migraine group by comparing with controls is the strength of our study. We could not find a significant relationship between plasma nitrite levels of migraine cases and associated clinical or demographic features. So we could not make any comments on the patients' variables in relation to demographic or clinical data that might explain the involvement of NO pathways in the natural course of migraine. NO may be involved in some other primary headache types (18-20), but probably not in the same manner as in migraine attacks (20). On the other hand, it is not clear yet whether NO involvement in primary headaches is the cause or the consequence. Further studies regarding mediators involved in the modulation of pain in

various headache states and even in clinically distant painful diseases would help to clarify the mechanisms of pain and would help expand our point of view.

References

1. Olesen J, Iversen HK, Thomsen LL. Nitric oxide supersensitivity: a possible moleculer mechanism of migraine pain. Neuroreport 1993;4(8):1027-30.
2. Lassen LH, Cristiansen I, Olesen J, Ashina M, Uldrich V. Nitric oxide synthase inhibition in the treatment of migrain attacks. Cephalalgia 1998;18(1):27-9.
3. Afridi SK, Kaube H, Goadsby PJ. Glyceryl trinitrate triggers premonitory symptoms in migraineurs. Pain 2004;110(3);675–80.
4. Akerman S, Williamson DJ, Kaube H, Goadsby PJ. Nitric oxide synthse inhibitor can antagonize neurojenic and calcitonin gene-releated peptide dilation of dural meningeal vessels. Br J Pharmacol 2002;137(1):62-8.
5. De Alba J, Clayton NM, Collins SD, Colthup P, Chessell I, Knowles RG. GW274150, a novel and highly selective inhibitor of the inducible isoform of nitric oxide synthase (iNOS), shows analgesic effects in rat models of inflammatory and neuropathic pain. Pain 2006;120(1-2):170–81.
6. Headache classification subcommittee of the international headache society. The international classification of headache disorders. 2 nd edition. Cephalagia 2004;24:24-37.
7. Rhodes PM, Leone AM, Francis PL, Struthers AD, Moncada S. The L-arginine:nitric oxide pathway is the major source of plasma nitrite in fasted humans. Biochem Biophys Res Commun 1995;209(2):590-6.
8. Henderson DE, Mello JA. Physicochemical studies of biologically active peptides by low-temperature reversed-phase high-performance liquid chromatography. J Chromatogr 1990;19(499):79-88.
9. Silva FA, Rueda-Clausen CF, Silva SY, Zarruk JG, Guzmán JC, Morillo CA, et al. Endothelial function in patients with migraine during the interictal period. Headache 2007;47(1):45–51.
10. Olesen J, Thomsen LL, Iversen H. Nitric oxide is a key molecule in migraine and other vascular headaches. Trends Pharmacol Sci 1994;15(5):149–53.
11. Sances G, Tassorelli C, Pucci E, Ghiotto N, Sandrini G, Nappi G. Reliability of the nitroglycerin provocative test in the diagnosis of neurovascular headaches. Cephalalgia 2004;24(2):110–9.
12. Thomsen LL, Olesen J. A pivotal role of nitric oxide in migraine pain. Ann N Y Acad Sci 1997;19(835):363–72.
13. Christiansen I, Iversen HK, Olesen J. Headache characteristics during the development of tolerance to nitrates: pathophysiological implications. Cephalalgia 2000;20(5):437–44.
14. Capuano A, De Corato A, Lisi L, Tringali G, Navarra P, Dello Russo C. Proinflammatory-activated trigeminal satellite cells promote neuronal sensitization: relevance for migraine pathology. Mol Pain 2009;5:43.
15. Bagdy G, Riba P, Kecskeméti V, Chase D, Juhász G. Headache-type adverse effects of NO donors: vasodilation and beyond. Br J Pharmacol 2010;160(1):20-35.
16. Tvedskov JF, Tfelt-Hansen P, Petersen KA, Jensen LT, Olesen J. CGRP receptor antagonist olcegepant (BIBN4096BS) does not prevent glyceryl trinitrate-induced migraine. Cephalalgia 2010;30(11):1346-53.
17. Napoli R, Guardasole V, Zarra E, Matarazzo M, D'Anna C, Saccà F, et al. Vascular smooth muscle cell dysfunction in patients with migraine. Neurology 2009;72(24):2111-4.
18. Messlinger K, Lennerz JK, Eberhardt M, Fischer MJ. CGRP and NO in the trigeminal system: mechanisms and role in headache generation. Headache 2012;52(9):1411-27.
19. Steinberg A, Nilsson Remahl AI. Role of nitric oxide in cluster headache. Curr Pain Headache Rep 2012;16(2):185-90.
20. Neyal M, Yimenicioglu F, Aydeniz A, Taskin A, Saglam S, Cekmen M, et al. Plasma nitrite levels, total antioxidant status, total oxidant status, and oxidative stress index in patients with tension-type headache and fibromyalgia. Clin Neurol Neurosurg 2013;115(6):736-40.

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