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Changes in plasma nitric oxide levels during migraine initial and attack periods in migraine patients

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The most important primary headaches are migraine. Migraine has a prevalence of 10% in the general population and its societal costs are high. The precise mechanisms underlying the pathophysiology of migraine are still elusive. Nitric oxide (NO) is believed to play a key role in migraine pathogenesis. In this study, we compared migraine patients with healthy controls by measuring plasma nitrite levels in order to investigate the effects of nitric oxide in migraine patients during headache free and attacks period. A total of 26 patients with migraine in headache free period and 24 patients with migraine in attack period participated in our study. Control group consisted of 24 healthy subjects. Plasma NO levels were measured spectrophotometrically. Plasma nitrite levels were found significantly higher than controls during headache free period ($p < 0.05$). By contrast, it was found significantly decreased in attack periods compared to headache free period. No significant change in plasma nitrite levels in migraine attacks period compared to controls was observed ($p > 0.05$). Plasma nitrite levels were not altered significantly after an attack in the patients with migraine. These results suggested that increased level of plasma NO during headache free period may play an important role in migraine pathogenesis.

Key words: Headache, migraine, nitric oxide, oxidative stress.

INTRODUCTION

Migraine is a common, chronic and disabling neurovascular disorder that is characterized by severe headache attacks with photophobia, nausea, vomiting, autonomic symptoms, and in some patients with aura involving neurological symptoms (Uzar et al., 2011). Migraine is the most prevalent neurological disorder, with an estimated 43 million sufferers in Europe and slightly smaller numbers in the USA (Andlin-Sobocki et al., 2005; Lipton et al., 2007). It is a very painful condition that often leads to absenteeism from work and more often to considerably decreased efficiency at work. The estimated cost of migraine in Europe is €27,000 million per year which, among the neurological disorders, ranks third after dementia and stroke (Andlin-Sobocki et al., 2005). Migraine is considered to be a disorder of neurovascular transmission without structural lesions (Olesen et al., 1993).

The molecular mechanisms of migraine have not been fully clarified yet. It has been hypothesized that increased oxidative and nitrosative stress may together take place in patients with migraine, especially during attacks (Yilmaz et al., 2007). There are many theories about the pathophysiology of migraine attacks. Most of these involve hereditary (Alexandrea, 1998; Lance and Goodsby,

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1998). Biological states that may cause increases in free fatty acids and blood lipids increased platelet aggregation, decreased serotonin levels and increased prostaglandin levels (Bic et al., 1998). Such changes can cause the vasodilatation that precedes migraine headache (Alexandrea, 1998; Bic, 1998).

Before the pain attack, cerebrovascular spasm induced by factors such as menstruation, lack of sleep, skipped meals, allergic reaction, and physical or mental stress, among others initially causes ischaemia which, in turn, produces prodromal symptoms such as nausea, photophobia or phonophobia (Alexandrea, 1998; Lance and Goodsby, 1998; Sadovsky, 1998).

Nitric oxide (NO) is a gaseous mediator synthesized mainly in the endothelium that exerts many important regulatory functions on the vessel wall and platelet (Moncada et al., 1991). NO can play a modulatory role on biological processes such as vasodilatation in migraine attacks (Bellantonio et al., 1998; Olesen, 1993). It has also been reported that NO can be released from endothelial cells, neurons, macrophages, and platelets. Because human platelets contain an L-arginine/NO pathway which may be associated with platelet aggregation (Radomski et al., 1990), NO may play a role in the pathogenesis of migraine and other vascular headaches. The final products of NO *in vivo* are nitrite (NO^{-2}) and nitrate (NO^{-3}), but the relative proportions of nitrite and nitrate are variable (Moncada et al., 1991). The best index of overall NO production, therefore, is the total concentration of both nitrate and nitrite (total nitrate/nitrite).

In our study, we compared migraine patients with healthy controls by measuring plasma nitrite levels in order to investigate the effects of nitric oxide in migraine patients during headache free and attacks period.

MATERIAL AND METHODS

Patients

In our hospital-based case-control study, 26 patients with migraine in headache free period and 24 patients with migraine in attack period participated. 24 controls without migraine diseases were recruited. The diagnosis of headache was made in accordance with the International Headache Society (IHS) criteria (Olesen, 2008). The migraine patients in headache free period group consisted of 26 subjects. The mean age was 33.3 ± 6.3 years [mean ± standard deviation (SD)]. The migraine patients in attack period group consisted of 24 subjects. The mean age was 31.4 ± 4.9 years (mean ± SD). The 24 normal controls without migraine diseases were recruited; the mean age was 30.1 ± 4.1 years (mean ± SD). Written informed consent was obtained from all subjects.

The study was carried out in the neurology clinic of Firat University Research and Application Hospital. Patient and healthy volunteers were recruited from the Department of Neurology, University of Firat. Furthermore, this study was approved by the Ethics Committee of the Medical Faculty of the same university. Medical, neurologic, and psychiatric evaluations were made on all of the patients. Patients having these characteristic were excluded; first-axis psychiatric disorder now or in the past, history of alcohol and cigarette consumption, history of any kind of medicine consumption within the last 2 weeks prior to the study (except simple analgesic), neurologic disorder and/or symptom in clinic and history, history of head trauma, history of cardiovascular, renal and endocrinologic disorder and existing medical disorder.

Measurement of plasma nitrite

Venous blood samples were taken from the antecubital vein with suitable vacutainers with ethylenediaminetetraacetic acid (EDTA) as anticoagulant. The basal venous blood was obtained from all the participants in this study on the morning after 12 h of overnight fasting. In all cases, blood samples were taken according to the principles of the Helsinki declaration. The blood samples were drawn from the patients. These samples were centrifuged at +4°C at 3,000 rpm for 10 min, and after separating plasma they were kept at -20°C until analysis. Among all participants, information on demographic characteristics and risk factors was collected using a structured questionnaire. NO measurement is very difficult in biological specimens because it is easily oxidized to nitrite (NO_2) and subsequently to nitrate (NO_3) which serve as index parameters of NO production. Samples were initially deproteinized with NaOH and $ZnSO_4$. Total nitrite (NO_2 to NO_3) was measured by spectrophotometer at 545 nm after conversion of NO_2 to NO_3 by assay reactive. A standard curve was established by a set of serial dilutions of sodium nitrite. Results were expressed as µmol/L per plasma (Lyall et al., 1995).

Statistical analysis

The statistical package for the Social Sciences (SPSS) 15.0 (SPSS Inc, Chicago, IL, USA) was used for statistical analysis. The plasma NO levels were evaluated statistically by Mann-Whitney U test. Results were given as mean ± SD, and $p < 0.05$ was accepted to indicate significant levels.

RESULTS

In control group, plasma nitrite levels were found to be 85.8 ± 3.2 µmol/L. In migraine patients, plasma nitrite level was detected to be 95.9 ± 21.2 µmol/L during headache free period and 85.2 ± 2.7 µmol/L during attacks. According to our results, slightly higher levels of nitrite in plasma of migraineurs during headache free period were found than in control subject. A statistically significant difference was observed in plasma nitrite level between migraine patients and control groups ($p = 0.004$). The plasma nitrite values of the patients and control groups are shown in Table 1.

When the patients were grouped as migraineurs with in headache free period and in attack period, plasma nitrite levels were significantly decreased in attacks periods compared to headache free period ($p < 0.05$). No significant change in plasma nitrite levels in migraine attacks period compared to controls was observed ($p > 0.05$). The plasma NO values in the attack period and initial period in migraine patients are shown in Table 2 and Figure 1.

Table 1. The values of plasma nitric oxide in migraine patients and control groups.

Variable	Control group (n=24)	Patient (n=26)	Total (n=40)	p	95% CI
Age (years)	30.1±4.1	33.3±6.3	31.6±5.2	0.287	0.488-0.283
Nitric oxide	85.8±3.2	95.9±21.2	89.29±13.3	0.004*	23.090-2.76

*p < 0.05 as significant level. CI = confidence interval.

Table 2. The values of plasma nitric oxide in the attack period and initial period in migraine patients.

Variable	Control group (n=24)	Patient (n=26)	Migraine during attack (n=24)
Age (years)	30.1±4.1	33.3±6.3	31.4±4.9
Nitric oxide	85.8±3.2[a]	95.9±21.2[b]	85.2±2.7[a]

[a,b]p < 0.05 as significant level.

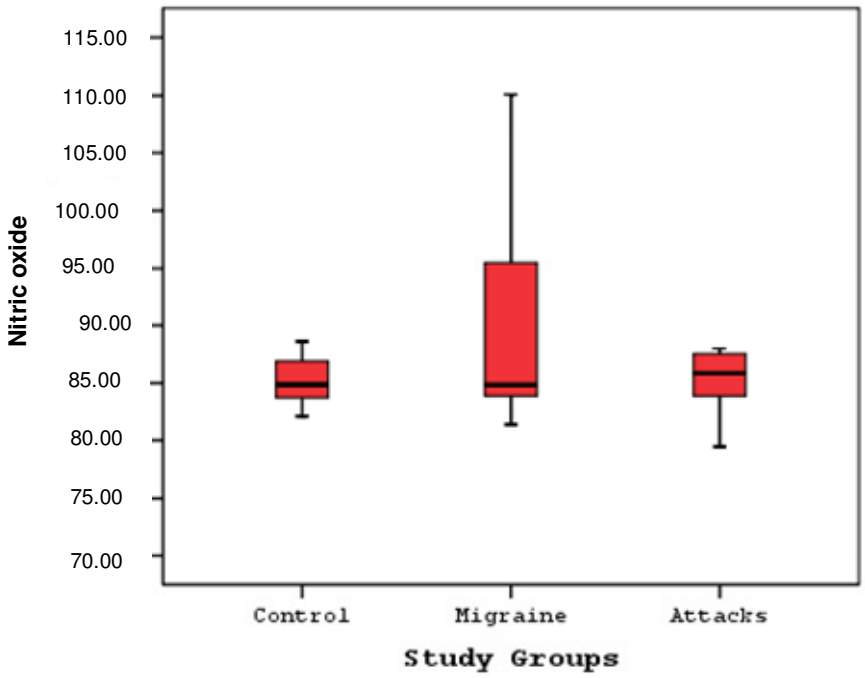

Figure 1. The distribution of values of plasma nitric oxide in the study groups. Group 1: healthy control (n = 24), Group 2: migraine patients in initial period (n = 26), Group 3: attack period (n = 24).

DISCUSSION

Migraine is a chronic disease with frequent attacks, high levels of pain and disability during attacks which causes reduced quality of life between attacks (Yilmaz et al., 2007). It is a widespread disorder, affecting about 10 to 15% general population. However, the mechanisms underlying the disease have not been clearly understood. Vascular disturbance in intracranial arteries plays a significant role in the migraine attacks. It was suggested that hemodynamic changes during the migraine attacks may be related to alterations in the level of NO. NO can precipitate the attacks by causing vasodilatation in cerebral vessels. It was shown that platelet hyperaggregation has an important role in migraine pathophysiology. Aggregated platelets produce NO levels which show a

counter effect to vasoconstrictors and finally cause vasodilatation and prevention of platelet aggregation (Yilmaz et al., 2007).

In our study, we measured NO activity during headache and headache-free period in migraine patients. Since NO is rapidly oxidized by tissue oxygen to the stable end products, nitrate (NO^{3-}) and nitrite (NO^{2-}), the best index of overall NO production is the total concentration of both nitrate and nitrite. Therefore, we measured total nitrite levels as an indicator of NO production. As similar to previous studies (D'Andrea and Cananzi, 1994; Sarchielli et al., 1996; Shimomura et al., 1999; Uzar et al., 2011), we found that plasma nitrite levels were significantly higher in migraine patients during headache-free period. Shimomura et al. (1999) showed that NO level decreased in headache-free period of migraine patients. In contrast to this study, we found slightly higher nitrite levels in headache-free period of migraine patients. In the light of our findings, we concluded that NO may be produced in plasma during headache-free period and increased NO may be related to the changes in the vascular vessels and pain during headache-free period. We would like to suggest that when the NO levels exceed a critical level, the headache-free period may begin.

On the other hand, simultaneous release of NO and of superoxide anions produces peroxynitrite anion which is a strong biological oxidant known to oxidize lipids, proteins and sulfhydryl (SH) groups particularly. In the most recent study on this issue, Taffi et al. (2005) evaluated platelet peroxynitrite levels a metabolite of nitric oxide as well as a potent oxidant in migraine patients to resolve uncertainty about NO activity in headache free period. They found increased peroxynitrite levels during headache-free period which are in concordance with our results. The main reason of increased peroxynitrite concentrations is concomitant overproduction of NO and superoxide anion, and these changes may result from platelet hyperaggregation. Therefore, increased nitrosative and oxidative stress may influence platelet function and cerebral microcirculation. It was suggested that NO may cause the headache through variations of cerebral blood flow by interacting with oxygen free radicals in migraine (Ciancarelli et al., 2003). It has also been proposed that NO may be involved in the pathophysiology of naturally occurring headaches such as migraine (Thomsen et al., 1993; Lassen et al., 1995). However, as of yet, there is no evidence that NO levels in the brain are elevated during the headache phase of migraine in humans (Lassen et al., 1997).

NO is one of the indicators of oxidative stress (Yilmaz et al., 2007). NO, a labile molecule with a half life of only a few seconds is synthesized generally in the endothelium. It is rapidly oxidized by tissue oxygen to the stable end products, nitrate and nitrite. Therefore, to the best index of overall NO production in the circulation is the total concentrations of both nitrate and nitrite (Yilmaz et al., 2007; Tain el al., 2010). NO is one of the substances that takes part in the pathophysiology of migraine (Yilmaz et al., 2007). NO is involved in the regulation of the cerebral vessels tone. NO may function as a signaling molecule in controlling neuronal activity and is important in controlling sensory inputs during migraine attack and interacting with reactive oxygen substances (ROS) which may induce headache through changes of cerebral blood flow (Ciancarelli et al., 2005).

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 nitric oxide-cyclic guanosine monophosphate (NO-cGMP) pathway in smooth vascular muscles causes vascular dilation and relaxation (Olesen, 2008; Napoli et al., 2009). 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 (Napoli et al., 2009).

It has been found that the pathways consisting of NO synthesis are activated in the experimental headache models such as nitroglycerin-induced headache (Sarchielli et al., 2000). The increase of NO may be caused by interactions with free ROS. The primary product of the interaction between NO and superoxide anion is peroxynitrite. Peroxynitrite is an aggressive and potent cellular oxidant (Gruber et al., 2009). So, increased NO with migraine patients may be associated with oxidative stress. In addition, occurrence of peroxynitrite following NO increases the effects of endothelia function (Fo¨rstermann, 2008). Marked NO increase in migraine group in our study strengthened the correlation between oxidative stress and endothelia dysfunction in pathophysiology of migraine. The findings of increased NO in migraine may lead to new therapeutic strategies, including probable antioxidant use for management of migraine. In the future, development and enhancements of existing drugs may be accompanied by increased efforts to develop truly new migraine drugs based on knowledge of the pathophysiology (Stovner et al., 2009; Farinelli et al., 2009).

Conclusıon

Results obtained indicate that the plasma nitrite levels were not altered significantly after an attack in the patients with migraine. Nitrosative and oxidative stress are increased during headache-free period in plasma. Increased oxidative damage together with increased NO level in migraine attack may effect cerebral blood flow and cause headache. These results suggested that increased level of plasma NO during headache free period may play an important role in migraine pathogenesis, and NO may serve as useful markers to show the increased oxidative stress in migraine patients. Further clinical and

biochemical studies are needed to investigate the associations between migraine, stroke and to introduce new therapeutic modalities for migraine treatment.

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