

Oxidative and antioxidative balance in patients of migraine

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Abstract. – Background: Migraine is characterized by multiple attacks of severe headaches often unilateral. The molecular mechanisms of migraine have not yet been clearly defined. Disorders of oxidant-antioxidant balance are observed in a number of acute and chronic diseases of the central nervous system. Oxidative stress is also believed to play a role in the pathogenesis of migraine. To the best of our knowledge, this is the first study investigating oxidant and antioxidant status of patients having migraine without aura (MWOA) and comparing them with those of age and sex matched healthy controls (CG).

Methods: We evaluated the Total Antioxidant Status (TAS) and Total Oxidant Status (TOS) of the plasma and Oxidative Stress Index (OSI) using a recently measurement method developed by Erel.

Results: Seventy five patients (55 Female, 20 Male) having MWOA who are free of attacks and 65 healthy volunteers (41 Female, 24 Male) (CG) were enrolled in this research. Mean age of the patients with MWOA and the control group were calculated (30.94 ± 10.37 vs 31.0 ± 9.46 years respectively; $p > 0.05$). Serum TAS levels of patients with MWOA were significantly lower than those of healthy controls (0.72 ± 0.008 vs. 0.80 ± 0.179 $\mu\text{mol Trolox equivalent/L}$; $p < 0.001$). Conversely, serum TOS values were significantly higher in patients with MWOA than in CG (15.39 ± 0.770 vs. 13.01 ± 0.471 $\mu\text{mol H}_2\text{O}_2$ equivalent/L; $P < 0.001$). The mean values of OSI were greater in patients than in controls (1.75 ± 0.59 vs. 1.56 ± 0.57 , $p < 0.023$). Total SH levels were significantly higher in the control group (MWOA: 0.24 ± 0.005 ; Controls: 0.28 ± 0.005 , $p = 0.001$). Furthermore, there was a negative correlation between the levels of Total SH and the duration of the headaches ($r: -0.426$, $p < 0.001$). Likewise; there was a positive correlation between OSI and the frequency of the headaches ($r: 0.123$, $p < 0.002$).

Conclusions: In this study, we demonstrated that the levels of total antioxidants were decreased and the levels of total oxidants and the oxidative stress index were increased in patients

with MWOA. These findings may be an evidence of exposure to potent oxidative stress in MWOA patients. Further investigations are required to clarify the role of oxidative stress in the etiopathogenesis of MWOA.

Key Words:

Migraine, headache, Oxidative stress, Antioxidants, Oxidative stress index

Introduction

Migraine is a common, disabling, primarily neurovascular disorder characterized by severe episodic headaches with systemic or neurological symptoms. The molecular mechanisms of migraine have not yet been clearly defined; but several hypotheses have been put forward. Inherited factors such as disturbances in the magnesium metabolism, calcium channelopathies, and abnormalities of mitochondria all increase the neuronal excitability leading to an impairment in the oxidative metabolism which can explain the threshold character of migraine attacks¹⁻³.

Reactive oxygen species (ROS) such as superoxide radical anions, hydroxyl radicals and hydrogen peroxides are produced during metabolic and physiological processes and harmful oxidative reactions may occur in the organism. Under certain conditions, increases in oxidants and decreases in antioxidants are inevitable, and the oxidant/antioxidant balance shifts towards oxidation. Consequently, oxidative stress is implicated in over 100 disorders^{4,5}. Disorders of oxidant-antioxidant balance underlie a number of acute and chronic diseases of the central nervous system including epilepsy and migraine⁶. The hypothesis of oxidative stress in migraine is supported by the findings in various studies⁷⁻¹⁰. Oxygen free

radicals may play a role in migraine by regulating cerebral blood flow and energy metabolism and may constitute a trigger threshold for migraine attacks¹¹.

In this study, we investigated the oxidant and antioxidant status of patients having migraine without aura (MWOA) and compared them with those of age and sex-matched healthy controls. We evaluated the Total Antioxidant Status (TAS) and Total Oxidant Status (TOS) of plasma and Oxidative Stress Index (OSI) using a recently measurement method developed by Erel¹². We hypothesized that increased oxidative stress and decreased total antioxidant response may play a role in the pathophysiology of migraine.

Materials and Methods

Subjects

This study was conducted in the Neurology Outpatient Clinic of Kafkas University, Kars, in Northeastern Turkey. The study included 75 patients with MWOA during their headache-free period. A detailed history was obtained and a thorough physical and neurological examination was performed using a predesigned structure. The diagnosis of migraine was based on the criteria established by the International Headache Society 2004¹³. The duration, frequency, localization and the severity of the headaches and the duration of migraine were evaluated. For headache severity, we used Migraine Disability Assessment (MIDAS) and VAS scales¹⁴. Pregnancy, lactation, clinically unstable medical illness, or use of medications within 3 weeks prior to the initiation of the study, were criteria for exclusion.

The control group (CG) consisted of 65 healthy subjects (including relatives of the patients, most of them were spouses of the patients and healthy volunteers from the laboratory). A thorough headache history was obtained in all the control subjects demonstrating that none of them was suffering from a headache disorder. There was no history of a medical illness in the subjects included in the study, nor had they taken any analgesics, ergot alkaloids or drugs for at least 3 weeks prior to entering in the study. None of the patients were receiving prophylactic treatment for migraine. Routine haematological and biochemical parameters were determined in both the patients and the controls. Both groups con-

sisted of non-smokers. The patients characteristics are given in Table I.

We determined the oxidative and anti-oxidative status of serum samples from migraine patients and controls. We performed routine haematological and biochemical analyses in all patients to exclude any possible systemic disorders that may affect oxidative parameters. Total cholesterol (TC) levels were determined by using commercially available assay kits (Olympus®, Hamburg, Germany) with an auto analyser (AU600®, Olympus®, Hamburg, Germany).

Approval of the Ethics Committees of Kafkas University School of Medicine and informed consents of the patients and healthy individuals were obtained.

Blood Samples

Blood samples were obtained following an overnight fast. Samples were withdrawn from a cubital vein into blood tubes and immediately stored on ice at +4°C. Serum was separated from the cells by centrifugation at 3000 rpm for 10 min. Serum samples were stored at -80°C until the time of analysis.

Measurement of Total Antioxidant Status (TAS)

TAS levels of serum was determined using commercial rel assay diagnostic kits (Gaziantep, Turkey) with an auto analyzer (Aeroset®, Abbott®, IL, USA) developed by Erel¹². In the novel method, total antioxidant response of plasma against especially potent free radical reactions, which strongly lead to oxidative damage of biomolecules such as lipids, proteins and DNA, was measured. In the assay, ferrous ion

Table I. Demographic and clinic features of patients with migraine and control group.

	MWOA	CG	p
Age (years)	30.94 ± 10.37	31.0 ± 9.46	> 0.5
Sex (F/M)	55/20	41/24	
Mean BMI	27.23	26.85	> 0.5
Migraine duration (years)	5.7 ± 5.4,		
Headache frequency (per month)	7.3 ± 5.2		
Headache duration (hour)	31.41 ± 19.56		
Mean of VAS	8.61 ± 1.0		
Mean of MIDAS	12.56 ± 6.7		

solution present in Reagent 1, is mixed with hydrogen peroxide found in Reagent 2. The sequentially produced radicals, such as brown-colored dianisidine radical cations, produced by the hydroxyl radicals, are also potent radicals. In this assay, the anti-oxidative effect of the sample against the potent free radicals' reactions initiated by the produced hydroxyl radicals was measured. The assay results were expressed as μmol Trolox equivalent/L; the precision of this assay is excellent. Accurate measurements of TAS can be obtained in as little as 10 min, making this assay eminently suitable for the clinical biochemistry laboratory.

Measurement of Total Oxidant Status (TOS)

TOS levels of serum was determined using commercial rel assay diagnostic kits (Gaziantep, Turkey) with an autoanalyzer (Aeroset®, Abbott®, IL, USA) developed by Erel¹⁵. Oxidants present in the sample oxidize the ferrous iono-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion produces a coloured complex with xylenol orange in an acidic medium. The colour intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 equiv/L).

Oxidative Stress Index

The percent ratio of the TOS to the TAS gave the oxidative stress index (OSI), an indicator of the degree of oxidative stress¹⁶⁻¹⁸. To perform the calculation, the result unit of TOS, mmol Trolox equivalent/L, was converted to μmol equivalent/L and the OSI value was calculated by the formula: OSI (arbitrary unit) = $\frac{1}{4}$ TOS (mmol H_2O_2 equiv/L)/TAS (mmol Trolox Equiv/L).

Measurement of Total Thiol (SH) Levels

The thiol level was estimated in tissue/plasma by the method of Hu et al¹⁹. A 0.2-ml sample of 10% plasma/tissue homogenate was mixed with water to make the volume up to 0.5 ml. Two millilitres of 0.3 M disodium hydrogen phosphate was added to each sample and 0.25 ml of DTNB reagent was added just before measuring the absorbance at 412 nm. Thiol groups were calculated using an absorptive of 13.600 mmol/l.

Statistical Analysis

Results were expressed as means $\pm$ standard deviation. Differences were considered significant at a probability level of $p < 0.05$. Student's *t*-test and correlation analyses were performed using SPSS for Windows, release 11.5 computer program.

Results

The demographic and clinical data of the subjects are summarized in Table II. There were no significant differences in sex, age and body mass index between patients with MWoA and controls.

Serum TAS level in patients with MWoA were significantly lower than those of healthy controls (0.72 ± 0.008 vs. 0.80 ± 0.179 μmol Trolox equivalent/L; $p < 0.001$). Additionally, serum TOS values were significantly higher in patients with MWoA than in CG (15.39 ± 0.770 vs. 13.01 ± 0.471 μmol H_2O_2 equivalent/L; $p < 0.001$). The mean values of OSI were greater in patients than in controls (1.75 ± 0.59 vs. 1.56 ± 0.57 , $p < 0.023$) (Figure 1). Total SH levels were significantly higher in CG (MWoA: 0.24 ± 0.005 ; Controls: 0.28 ± 0.005 mmol/L; $p < 0.001$).

There was a negative correlation between Total SH levels and the duration of headaches ($r: -0.426$, $p < 0.001$) (Figure 2). Likewise; there was a positive correlation between OSI and headache frequency ($r: 0.123$, $p < 0.02$) (Figure 3). But there was no correlation between TOS and headache severity or frequency.

Discussion

The pathophysiology of migraine and other headaches is still unknown; and research is mostly conducted on neurotransmitters, biochemical

Table II. Antioxidant and oxidant levels of patient with MWA and control group. Data are means $\pm$ SD.

Parameters	MWoA	CG	<i>p</i>
Serum TAS (μmol Trolox equivalent/L)	0.72 ± 0.08	0.80 ± 0.17	0.001
Serum TOS (μmol H_2O_2 equiv/L)	15.39 ± 0.77	13.01 ± 0.41	0.001
OSI (arbitrary unit)	1.75 ± 0.59	1.56 ± 0.57	0.023
Total SH (mmol/l)	0.23 ± 0.29	0.25 ± 0.28	0.001

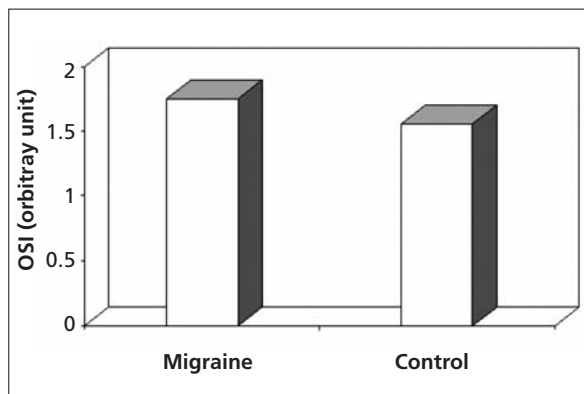


Figure 1. Comparison of OSI levels in migraine patients and healthy controls.

and vascular mechanisms. The neuro-vascular theory of migraine seizure pathogenesis is the most widely accepted one. Stimulation of the trigeminal nerve occurs via neuronal and chemical pathways, through serotonin, histamine and prostaglandins. Migraine inducing factors can act directly on these chemical mediators or via the nervous system mediators. One of the hypotheses of the origin of headache in migraine is that of

neurogenic inflammation of dura mater presented by Moskowitz et al.²⁰. According to that model, central stimulation in the trigeminal nerve endings causes an antidromic release of substance P, calcitonin gene-related peptide (CGRP) and neurokinin A, which increase the permeability of vascular walls, dilates them with a likely involvement of nitric oxide (NO) and enhances the action of blood-derived factors, such as histamine and serotonin. This leads to inflammatory reactions and blood vessel oedema, i.e. aseptic inflammation of arteries^{6,21,22}.

Oxidative stress is a term used to describe situations during which the organism's production of oxidants exceeds its capacity to neutralize them. The result can be damage to cell membranes, lipids, nucleic acids, proteins, and constituents of the extracellular matrix such as proteoglycans and collagens²³. It has been suggested that oxidative stress caused by free radicals may play a role in migraine pathogenesis^{10,24}. The enhanced ROS attack might be explained by the existence of cytokines and increased neutrophils activation in the blood of patients with migraine. Various studies demonstrated the inappropriate release of ROS such as nitric oxide (NO) and superoxide

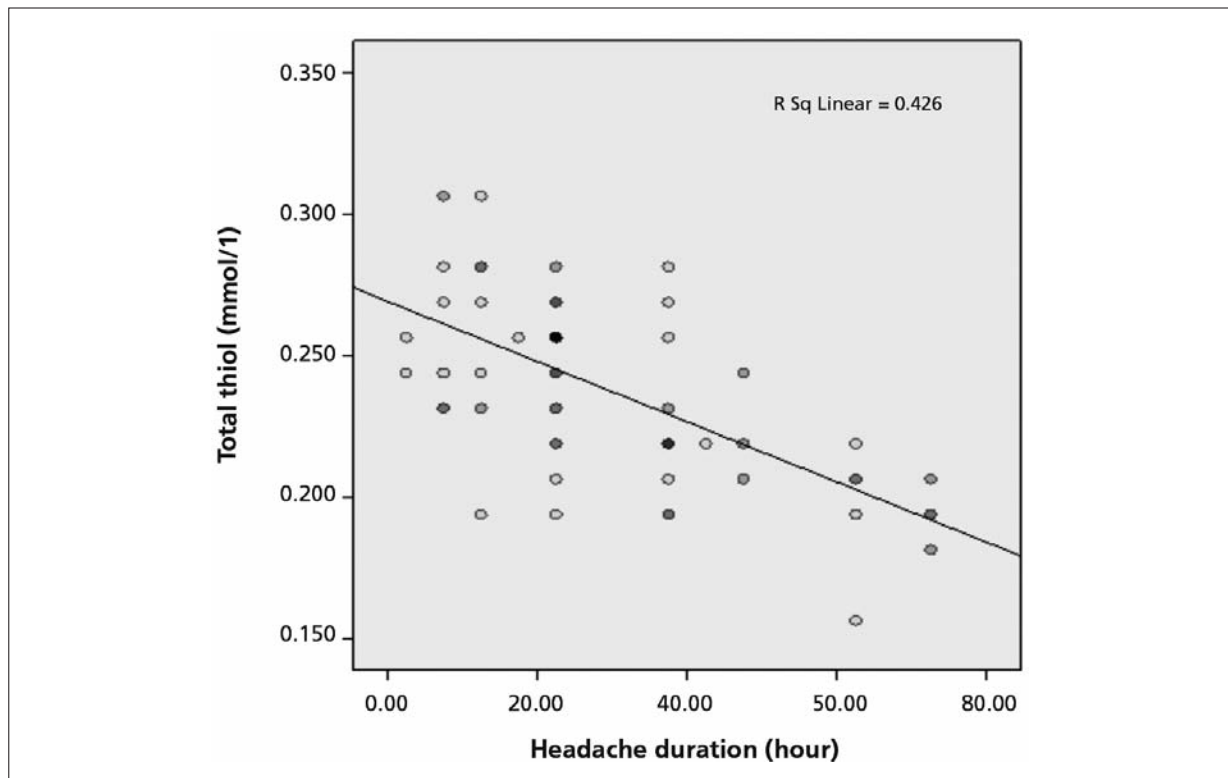


Figure 2. Correlation between SH levels and headache duration ($r: -0.426, p < 0.001$).

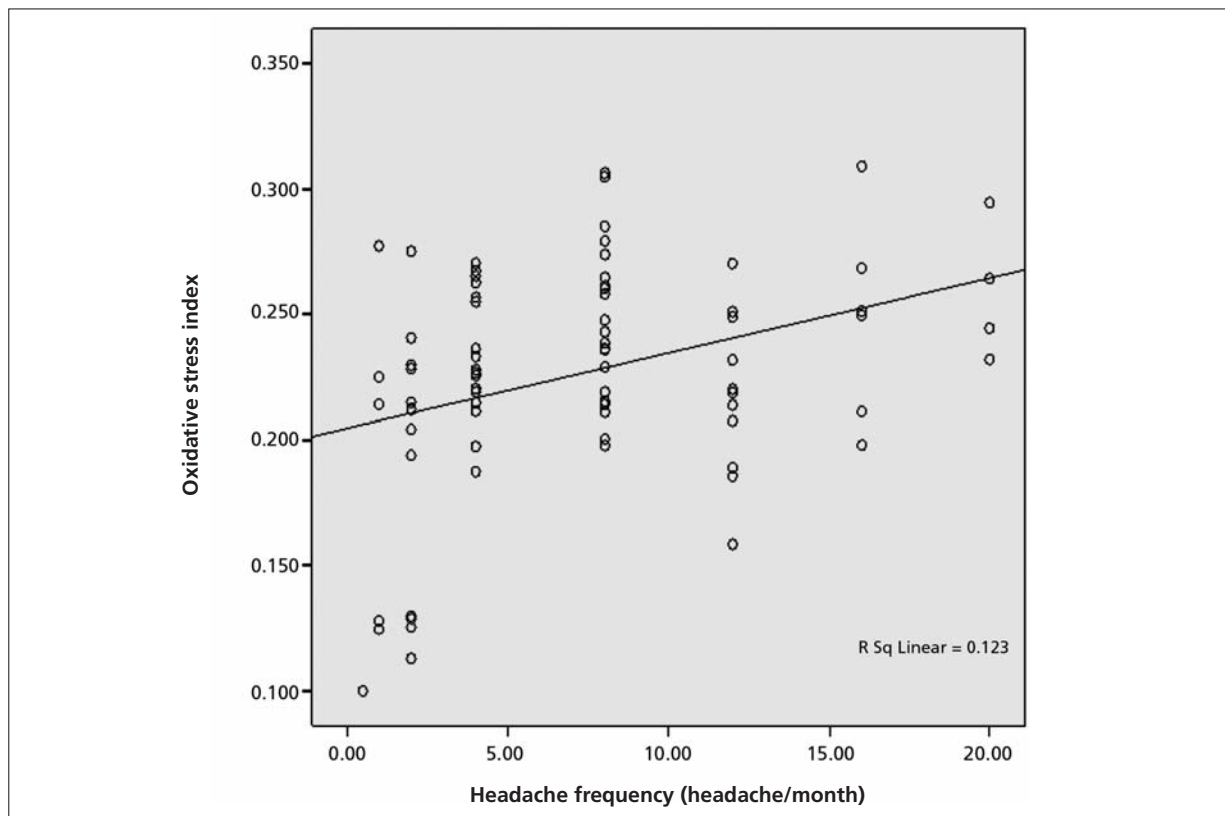


Figure 3. Correlation between OSI and headache frequency $r: 0.123, p < 0.02$.

anion from activated polymorphonuclear leucocytes, both in the bloodstream of patients with migraine^{6,8,25,26}.

Oxidative stress can be defined either as an increase in the level of oxidants and/or a decrease in the antioxidant capacity. Although determination of either oxidant or antioxidant components alone may give information about oxidative stress; determination of oxidants along with antioxidants is more useful in this context. Thus, oxidants and antioxidant capacities should be measured simultaneously to assess oxidative stress more exactly. Negative effects of oxidative stress are being tolerated by the TAS. However, sometimes the TAS cannot cope with the excessive increase of oxidants, and then the TAS will decrease and OSI will increase²⁷.

Serum thiols (SH) are physiological free radical scavengers and serve antioxidant functions by several mechanisms. Total SH is good reflection of excess free radical generation both in physiological and pathological conditions in humans²⁸. In the present study, we found OSI to be signifi-

cantly increased, and total-SH and TAS to be decreased in patients with migraine. We also detected significant negative correlations between total-SH levels and the duration of headaches in the patient group. There was a significant positive correlation between OSI and headache frequency. These findings suggest that oxidative stress may not only play a role in migraine pathogenesis but also is a triggering factor for attack severity and duration.

To the best of our knowledge, this is the first study on the balance of oxidant and antioxidant levels in patients with migraine. We found that TOS and the serum OSI values were higher and TAS levels were lower in patients with MWoA than in healthy controls. There were correlations between headache duration and oxidative/antioxidative parameters. Therefore, the decreased levels of antioxidants may be an evidence for MWoA patients exposed to potent oxidative stress. Further investigations are required to clarify the role of oxidative stress in the pathogenesis of MWoA. The possible findings of these

investigations might shed light on the development of novel therapeutic strategies for MWOA. Supplementation of regular treatment regimes with powered antioxidants may be considered in these patients.

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