

Impaired oxidative-antioxidative balance during migraine attack

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ABSTRACT

Background: Recent evidence has suggested that oxidative stress may play a role in the pathophysiology of migraine. In this study, we aimed to evaluate the oxidative-antioxidative status in sera of migraine patients from an Iranian population during migraine attacks. **Methods:** This study recruited 46 migraine patients with or without aura and 45 sex- and age-matched healthy controls. The levels of protein carbonyl (PC), malondialdehyde (MDA) and total oxidants status (TOS) were measured as the indicators of oxidative stress. The levels of total thiols (T-SH), reduced glutathione (GSH) and total antioxidant capacity (TAC) were determined as markers of antioxidant status. Oxidative stress index (OSI) was calculated by dividing TOS to TAC. **Results:** The serum levels of MDA (4.10 ± 0.70 vs. 3.20 ± 0.62 , $P = 0.003$), TOS (18.46 ± 4.06 vs. 16.21 ± 3.67 , $P = 0.007$) and OSI (1.54 ± 0.60 vs. 1.22 ± 0.46 , $P = 0.006$) were significantly higher in migraine group compared to controls, however, no statistically significant differences of PC levels were found between migraine patients and controls (0.615 ± 0.161 vs. 0.517 ± 0.126 , $P = 0.1$). In contrast, the levels of T-SH (273 ± 51.71 vs. 310.88 ± 53.32 , $P = 0.001$), GSH (28.08 ± 6.20 vs. 32.13 ± 5.8 , $P = 0.002$) and TAC (1.27 ± 0.226 vs. 1.41 ± 0.26 , $P = 0.01$) were significantly lower in migraine patients compared to healthy controls. **Conclusion:** Our study showed higher levels of oxidative stress and lower levels of antioxidant status in migraine group compared to controls, which indicates the possible role of oxidative stress in triggering migraine attacks.

Key words: Migraine, Oxidative stress, Total antioxidant capacity, Total oxidants status

INTRODUCTION

Migraine is a prevalent neurological disorder characterized by severe episodic headaches with systemic or neurological symptoms^{1,2}. There are two major types of migraines: migraines with aura and migraines without aura. Aura is experienced by about a third of migraine patients and consists of a series of transient neurological symptoms that occur in the visual system or other senses before the onset of migraine headache². Although several genetic and environmental factors have been shown to be associated with the presence or development of migraine³, molecular mechanisms of migraine attacks have not been clearly identified. One of the major factors lowering the threshold of the first headache symptom is the impairment of mitochondrial oxidative metabolism⁴. In this regard, it has been proposed that nitric oxide and oxidative stress may play a central role in migraine headache by deregulating brain blood flow⁵. In fact, oxidative stress originates from the imbalance between the production of reactive oxygen species (ROS) and antioxidants⁶. ROS are reactive oxidant molecules containing oxygen, which are formed at relatively low levels in all cells during normal metabolism and their physiological levels are

necessary to maintain normal cell function⁷. The concentration of all oxidants in biological samples is referred to as total oxidant status (TOS)⁸.

The additional amounts of ROS are removed by free radical-scavenging enzymes such as superoxide dismutase, catalase, and glutathione peroxidase⁷. Also, there are non-enzymatic antioxidants such as ascorbic acid, vitamin E, uric acid, bilirubin, reduced glutathione (GSH), and various thiol groups on protein molecules that can act to neutralize ROS⁹. The reduced sulfhydryl groups of proteins and non-protein compounds are collectively known as total thiols¹⁰ and all antioxidants present in the serum are referred to as total antioxidant capacity (TAC)¹¹. When the levels of ROS increase beyond the antioxidant capacity of the cell, a condition known as oxidative stress occurs. In such situation, free radicals may damage cellular proteins, lipids, and nucleic acids, which can potentially trigger the development of various human diseases including migraine^{12,13}. Oxidative damage to proteins can generate protein carbonyls (PC)¹⁴ and decrease free sulfhydryl groups of proteins and other thiol-containing compounds (or total thiols). The oxidation of lipids produces malondialdehyde (MDA), which is measured as one of the indicators of oxidative stress¹⁵. In some diseases, the main patho-

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physiologic basis may come from the activation of oxidative stress and inactivation of antioxidant processes^{16,17}. Studies have shown that high levels of oxidative stress are associated with an increase in migraine risk. Given the fact that both genetics and environmental factors can affect migraine and oxidative stress¹⁸, this study was designed to examine the association of non-enzymatic oxidative stress markers with migraine headache in an Iranian population sample.

METHODS

Patients

This case-control study was approved by the Ethics Committee of Zahedan University of Medical Sciences and recruited 46 migraine patients with or without aura during attacks presenting at public or private clinics in Zahedan, Iran in 2014. Migraine was diagnosed based on the International Headache Society (IHS) criteria, 2nd edition¹. This study excluded patients with the presence of other types of headaches, history of diabetes mellitus, tuberculosis, malaria, history of any types of malignancies, depression, underlying active systemic or inflammatory disorders, or history of using any vitamins or antioxidant supplements. Meanwhile, 45 sex- and age-matched healthy individuals without any history of migraine from medical students and university personnel were selected as the controls. An informed written consent form was sent to all participants. Following overnight fasting, blood samples were taken from cases and controls. Coagulation serum samples were obtained by centrifugation, which were then aliquoted for each test and kept at -70 °C until analysis. Each analysis was conducted in duplicates under similar conditions on the same day.

Biochemical assays

Chemical materials

Potassium persulfate, xylenol orange, sulfuric acid, sodium chloride, hydrogen peroxide, glycerol, sulfosalicylic acid (SSA), sodium phosphate, orthophosphoric acid, ferric chloride and TBA (2-thiobarbituric acid: 4, 6- dihydroxy-2-mercaptopyrimidine) were purchased from Merck. ABTS [2, 2-azinobis (3-ethylbenzothiazoline-6-sulfonate)], Trolox, Ortho-dianisidine, Malondialdehyde standard (1,1,3,3-tetramethoxypropane), DTNB (5,5'-dithiobis-2-nitrobenzoic acid) and reduced glutathione (GSH) were obtained from Sigma.

Total antioxidant capacity (TAC) assay

The TAC of serum was measured based on the discoloration of 2, 2-azinobis 3-ethylbenzothiazoline-6-sulfonate radical cation (ABTS^{•+}) by antioxidants present in the sample¹⁹. ABTS^{•+} was produced by incubation of 7 mM ABTS aqueous solution with 2.5 mM potassium persulfate and allowing the mixture to remain in the dark at room temperature for 12-16 hours before use. To measure sample TAC and ABTS^{•+}, stock solution was diluted in deionized water until its absorbance at 734 nm reached 0.70. One mL of diluted ABTS^{•+} was added to 10 μ L of serum, then the absorbance was read ten minutes after the initial mixing. The TAC was calculated using Trolox as a standard antioxidant and the result was expressed as μ M Trolox.

Total oxidant status (TOS) assay

The TOS levels of the serum were determined using Ereli's method that was based on the oxidation of ferrous ion-O-dianisidine complex to ferric ions by oxidants present in the sample²⁰. The ferric ions in acidic medium formed a colored complex with xylenol orange. Briefly, a solution of 900 μ L of H₂SO₄ 25 mM containing 150 μ M of xylenol orange, 140 mM NaCl and 1.35 M glycerol was mixed with 100 μ L of serum or standard TOS. Then, 45 μ L of solution containing 5 mM ferrous ion and 10 mM O-dianisidine in H₂SO₄ 25 mM was added to the mixture. After 5 minutes, color intensity was measured spectrophotometrically at 560 nm. TOS were calculated from a standard curve using hydrogen peroxide as a standard TOS and the results were expressed in μ M H₂O₂ Equivalent/L.

Oxidative stress index

Oxidative stress index (OSI) was calculated using the following equation: $OSI = [TOS (\mu\text{mol H}_2\text{O}_2 \text{ Equivalent/L})/TAC (\mu\text{mol Trolox Equivalent/L})] \times 100$.

Total thiol assay

The levels of total thiols were measured spectrophotometrically according to Hu's method with modifications performed in our laboratory as described²¹. The levels of thiols in each sample was calculated by using reduced glutathione as a standard thiol and the result was expressed in μ M.

Glutathione assay

At first, the serum was deproteinized with an equal volume of 5% sulfosalicylic acid (SSA) solution, centrifuged at 38000 rpm for 10 minutes at 4 °C to remove precipitated proteins before being assayed for

glutathione. Serum GSH was measured according to the modified method of Moron *et al.*²². Briefly, 200 μ L of serum supernatant was mixed with 2 ml of 200 mM phosphate buffer (pH 8) containing 0.6 mM 5,5'-dithiobis (2-nitrobenzoic acid). Blank samples contained 2.5% SSA, instead of the supernatant. After 5 minutes incubation at room temperature, the absorbance was read at 405 nm against the blank sample. Reduced glutathione was dissolved in 2.5% SSA and was used as a standard. Serum GSH concentration was calculated using a standard curve and the result was expressed as μ M.

Protein carbonyl assay

Before PC determination, serum protein was diluted with phosphate buffer saline (PBS) to 4 mg/mL. The levels of PC were measured by enzyme-linked immunosorbent assays (ELISA) using the commercially available kit (Cell Biolab, Inc, Company) as described by Buss *et al.* The results were presented as absorbance units in the ELISA reader.

MDA assay

MDA levels were measured according to the method of Uchiyama and Mihara²³. Briefly, 3 mL of 1% orthophosphoric acid and 1 mL of 0.6% w/v aqueous solution of thiobarbituric acid were added to 0.5 ml of serum. The mixture was heated for 45 minutes in a boiling water bath. After cooling, the mixture was centrifuged at 3000 \times g for 10 minutes and the absorbance of the upper solution was determined at 535 nm against a blank sample containing 3 mL of phosphoric acid, 1 ml thiobarbituric acid and 0.5 mL PBS. The concentration of MDA was calculated using 1,1,3,3-tetramethoxypropane standard curve and was expressed in μ M.

Statistics analysis

The results were presented as mean \pm standard deviation (SD) for quantitative variables. For the statistical analysis, the statistical software SPSS version 21.0 (SPSS Inc., Chicago, IL) was used. Numerical variables of groups were compared by the Student's unpaired t-test. Mann-Whitney U test was also used to compare the numerical variables of subgroups. Categorical variables were compared using the chi-squared test. P values of less than 0.05 were considered statistically significant.

RESULTS

Demographic and clinical properties in migraine patients and healthy controls are presented in **Table 1**.

There was no significant difference in the mean ages of migraine and control groups (27.22 ± 7.40 vs. 26.65 ± 8.20 , $P = 0.77$). Regarding to sex distribution, 67.4% of the migraine group and 66.6% of the healthy group were female and no difference in sex distribution was observed between subjects suffering from migraine and healthy individuals ($P = 0.57$).

The levels of biochemical parameters in migraine patients and healthy controls are shown in **Table 2**. The mean serum levels of MDA (4.10 ± 0.70 vs. 3.20 ± 0.62 , $P = 0.003$), TOS (18.46 ± 4.06 vs. 16.21 ± 3.67 , $P = 0.007$) and OSI (1.54 ± 0.60 vs. 1.22 ± 0.46 , $P = 0.006$) were significantly higher in migraine group than those in the healthy group. PC concentration was slightly higher in the migraine group compared to healthy controls (0.615 ± 0.161 vs. 0.517 ± 0.126), but the difference was not statistically significant ($P=0.1$). The levels of T-SH (273 ± 51.71 vs. 310.88 ± 53.32 , $P = 0.001$), GSH (28.08 ± 6.20 vs. 32.13 ± 5.8 , $P = 0.002$) and TAC (1.27 ± 0.226 vs. 1.41 ± 0.26 , $P = 0.01$) were significantly lower in patients with the migraine compared to healthy control individuals.

The results of oxidative-antioxidative balance between migraine patients with and without aura are shown in **Table 3**. There was no statistically significant difference in serum levels of MDA (4.00 ± 0.65 vs. 4.2 ± 0.78 , $P = 0.056$), TOS (18.28 ± 3.55 vs. 18.56 ± 4.36 , $P = 0.7$) and OSI (1.46 ± 0.5 vs. 1.58 ± 0.64 , $P = 0.56$) between migraine patients with and without aura. We also found no significant difference in the levels of T-SH (280.18 ± 53.07 vs. 269.56 ± 51.50 , $P = 0.55$), GSH (29.56 ± 5.57 vs. 27.30 ± 6.46 , $P = 0.23$), TAC (1.33 ± 0.255 vs. 1.24 ± 0.27 , $P = 0.3$) and PC concentration (0.574 ± 0.16 vs. 0.663 ± 0.16 , $P = 0.3$) between migraine patients with and without aura.

DISCUSSION

In the current study, it was hypothesized that oxidant increment and/or antioxidant depletion might be two major fundamentals of the pathophysiology of migraine headache. Accordingly, we determined PC, MDA, TOS and OSI as the indicators for the activation of oxidative stress and TAC, T-SH and GSH as the markers of antioxidant status. To the best of our knowledge, this is the first to study the association between oxidative stress and migraine attack in an Iranian population sample. Overall, our results revealed that migraine attacks are associated with an increase in oxidant levels and a depletion of antioxidant defenses, which suggests a shift in oxidative-antioxidative balance towards oxidative status. The results confirmed our hypothesis and are consistent with other studies. Several studies have shown the

Table 1: Demographic and clinical properties in patients with migraine and healthy controls (Mean ± SD)

Parameter	Healthy control (N = 45)	Migraine patients (N = 46)	P value
Sex (Female/Male)	30/15	30/16	0.88
Age (year)	26.65 ± 8.20	27.22 ± 7.40	0.77
Duration of migraine (years)	-	4.56 ± 2.62	-
Monthly frequency of headache	-	5.13 ± 2.25	-
Headache duration (hours)	-	29.4 ± 15.5	-

Table 2: The levels of biochemical parameters in migraine patients and healthy controls (Mean ± SD)

Parameter	Healthy control (N = 45)	Migraine patients (N = 46)	P value
Total Antioxidant capacity (mM)	1.41 ± 0.26	1.27 ± 0.226	0.01
Total oxidant status (μM)	16.21 ± 3.67	18.46 ± 4.06	0.007
Oxidative stress index (OSI)	1.22 ± 0.46	1.54 ± 0.60	0.006
Total thiol (μM)	310.88 ± 53.32	273 ± 51.71	0.001
Reduced glutathione (μM)	32.13 ± 5.8	28.08 ± 6.20	0.002
Protein carbonyl (OD)	0.517 ± 0.126	0.615 ± 0.161	0.1
MDA (μM)	3.20 ± 0.62	4.10 ± 0.70	0.003

Table 3: The levels of biochemical parameters in migraine patients with or without aura (Mean ± SD)

Parameter	Migraine with aura (N = 15)	Migraine without aura (N = 31)	P value
Sex (Female/Male)	12/4	18/12	0.35
Age (year)	28.06 ± 7.36	26.33 ± 7.60	0.52
Total Antioxidant capacity (mM)	1.33 ± 0.255	1.24 ± 0.27	0.3
Total oxidant status (μM)	18.28 ± 3.55	18.56 ± 4.36	0.7
Oxidative stress index (OSI)	1.46 ± 0.5	1.58 ± 0.64	0.56
Total thiol (μM)	280.18 ± 53.07	269.56 ± 51.50	0.55
Reduced glutathione (μM)	29.56 ± 5.57	27.30 ± 6.46	0.23
Protein carbonyl (OD)	0.574 ± 0.16	0.663 ± 0.16	0.3
MDA (μM)	4.00 ± 0.65	4.2 ± 0.78	0.56

elevated levels of MDA, a product of oxidative damage of lipids, in the blood of migraine patients when compared to controls²⁴⁻²⁶. In a study by Alp *et al.*²⁷, the levels of total antioxidants were significantly lower in migraine patients while TOS and OSI were higher when compared to healthy individuals. Gupta *et al.* showed an increase in plasma levels of MDA and total antioxidant capacity in migraine patients²⁸. Our TAC results are consistent with those of Alp *et al.*, but did not confirm the results of Gupta *et al.* Moreover, the disturbance of antioxidant enzyme activities in serum and erythrocytes of migraine patients have been reported in several studies^{15,25}.

Thiols are physiological free radical scavengers that represents approximately 53% of total antioxidants present in serum¹⁹. In the present study, low levels of GSH and T-SH in migraine patients indicate the occurrence of oxidative stress during migraine attack. Alp *et al.* also found lower levels of total thiols in migraine patients and detected a negative correlation between the duration of pain and thiol levels²⁷. Similar to the study conducted by Bernecker *et al.*, we found higher levels of PC in migraine patients compared to controls, however, the difference between the two groups was not statistically significant²⁹. Carbonyl groups of proteins can be induced by almost all types of ROS, and high levels of these proteins are reflective of high levels of oxidative stress¹⁴.

Findings of the current and previous studies suggest that oxidative stress are likely to occur during migraine attacks. Also, there is evidence that oxidative stress and nitrosative stress (another form of oxidative-antioxidative imbalance) are involved in the pathophysiology of migraine^{26,30}. Environmental factors that increase the probability of migraine attacks (so-called migraine triggers) such as alcohol, air pollutants and cigarette smoke commonly have a capacity to induce oxidative stress¹⁸. Moreover, studies have shown that Flunarizine, vitamin C, vitamin E, and coenzyme Q₁₀, which are used in migraine prevention, decrease oxidative stress via their ROS scavenger properties^{24,31,32}. These studies and our study show that irrespectively of environmental and genetic factors, migraine attack is associated with the occurrence of oxidative stress, however, it is not known how oxidative stress triggers a migraine attack. It is suggested that several oxidative stress-related mechanisms contribute to migraine pathogenesis. So far, two major theories of vascular and neurovascular origins have been suggested in migraine pathophysiology. Based on the vascular theory that was first presented by Graham and Wolff, the dilation of cerebral vessels could be a cause of migraine headache³³. One

major mechanism of vasodilation is the activation of ATP-sensitive and calcium-activated potassium channels in cerebral vascular muscle³⁴. There is evidence that ROS, such as hydrogen peroxide and peroxy-nitrite (a product of the reaction of nitric oxide with superoxide), reversibly causes cerebral vasodilation by activating ATP-sensitive potassium channels most probably through an oxidant mechanism; whereas superoxide dilates cerebral vessels by opening calcium-activated potassium channels^{35,36}. In another study, it was shown that the effect of ROS on ATP-sensitive potassium channels of the cells in the cerebral arterioles is blocked by antioxidants³⁷.

The neurovascular theory has integrated both vascular and neurological events occurring in migraine. Base on this theory, migraine headache is associated with the inflammation of the meninges, particularly the dura³⁸. During migraine attack, the trigeminal sensory nerves innervating dural blood vessels become extra sensitive and release vasoactive modulatory neuropeptides such as the neurokinins, substance P and calcitonin gene-related peptides (CGRP), which may play a role in meningeal inflammation and migraine pain³⁸⁻⁴⁰. This theory also implicates the phenomenon of cortical spreading depression as an underlying mechanism of migraine aura⁴⁰. There is evidence that increased oxidative stress contributes to various pathological states including trigeminovascular inflammation, which occurs during migraine attacks^{41,42}. It has been shown that ROS, including H₂O₂, can activate sensory neurons directly and/or indirectly by releasing endogenous CGRP as a mediator of migraine headache⁴³.

CONCLUSIONS

This study showed that oxidative-antioxidative balance shifts towards oxidative status during migraine attack. An increase in oxidant levels, either with or without the depletion of antioxidant activity, may contribute to migraine pathogenesis by affecting cerebral blood vessel diameter and by activating trigeminal sensory nerves innervating dural blood vessels.

ABBREVIATIONS

PC: Protein carbonyl
MDA: Malondialdehyde
TOS: Total oxidants status
T-SH: Total thiols
GSH: Glutathione
TAC: Aantioxidant capacity
OSI: Oxidative stress index
ROS: Reactive oxygen species
SSA: Sulfosalicylic acid

ELISA: Enzyme-linked immunosorbent assay

ABTS: 2, 2-azinobis 3-ethylbenzothiazoline-6-sulfonate

CGRP: Calcitonin gene-related peptide

COMPETING INTERESTS

Authors have no conflict of interest.

AUTHORS' CONTRIBUTIONS

Alireza Khosravi: Design of the study, acquisition of data, and writing of the article.

Abdoreza Ghoreishi: Collection of data and revision of the article.

Alireza Nakhaee: Analysis and interpretation of data.

Masoud Sadeghi: Revision of the article and final approval of the version to be published.

Zahra Arefpoor: Revision of the article.

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