

OXIDATIVE STRESS STATUS IN SERUM OF MIGRAINEURS IN ERBIL CITY-IRAQ



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ABSTRACT

Background

Migraine is one of the neurological disorders whose etiology remains elusive. Increased oxidative stress may be associated with migraine. Oxidative stress may play an important role in patients with migraine, and it can reflect the ability of tissues to free radical damage.

Objectives

To determine the oxidative stress markers levels in migraine patients' serum. This study also included the use of oxidative stress indicators to diagnose migraines.

Materials and Methods

A total of 180 subjects (100 migraines and 80 healthy controls) were included in the study; nitric oxide (NO) metabolites, malondialdehyde (MDA), and superoxide dismutase (SOD) were measured in serum.

Results

In migraineurs, the levels of SOD, NO, and MDA were significantly higher when compared to healthy controls. There was a significant positive correlation between NO with MDA and SOD. Roc curve showed that NO was a useful marker for the diagnosis of migraine. Binary logistic regression documented that NO was negatively affect the life of migraineurs.

Conclusion

Therefore, oxidative stress has a role in the pathophysiology of migraine, and NO may serve as a useful diagnostic marker in migraine patients.

Keywords: *Malondialdehyde; migraine; nitric oxide; oxidative stress; and superoxide dismutase*

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INTRODUCTION

Migraine is one of the most abundant neurological disorders characterized by headache attacks with many factors that are believed to be genetic or environmental; many aura signs appear in approximately one-third of migraineurs⁽¹⁾. Migraine has multiple effects on people under 50 years old that get worse with daily activity, and over 11% of adults have suffered from migraine and their life impacts around the world research findings^(2, 3). Migraine pathophysiology is not fully understood, but it is believed to be related to many reasons, such as the improper working of calcium ion channels, not functioning mitochondria, imperfect magnesium metabolism that raises the excitement of neurons, and delays removal of free radicals^(4, 5). Migraines could be associated with mitochondrial defects, which tend to increase the production of oxidants^(6, 7) and deregulate antioxidant enzymes⁽⁸⁾.

Blood circulation alteration of the cerebrum is a reason for high oxidant formation, causing lipid peroxidation outcomes to be collected in blood. All these changes impact increasing sensitivity to oxidative stress in migraineurs and changing its effects from moderate to severe^(9, 10). The interaction of free radicals with tissue lipids is called lipid peroxidation, a process that gives rise to several degradation metabolites; one of them is MDA, the only measurable aldehyde, so it is widely used as a biomarker of oxidative stress.

The detection of impacts of oxidative stress in migraine can be revealed by measurement of oxidative stress markers together with antioxidants⁽¹¹⁾. SODs are one of the most valuable antioxidants metalloenzymes that free up cells from oxidative stress resulting from reactive oxygen species (ROSs) formed during the process of vasospasm and vasoconstriction. Dysfunction of SOD in cerebral arteries will increase superoxide levels and decrease endothelial-dependent vasodilator reaction to acetylcholine^(12, 13).

Oxidative stress can also be studied by measuring the level of NO. NO and ROS have an impact on migraine attack threshold activation by controlling cerebral blood flow and energy metabolism; thus, they may contribute to the development of migraine⁽¹⁴⁻¹⁶⁾. The goals of the current study were to determine the impact of oxidative stress indicators on migraine sufferers through the measurement of SOD, NO, and MDA. The utilization of oxidative stress markers in the diagnosis of migraines was also under the scope of this study.

MATERIALS AND METHODS

Patients and study design

One hundred-eighty cases were enrolled in this case-control study; the cases were separated into two different groups: One hundred patients in the migraine group [77 females (77%), 23 males (23%)] and a control group which consisted of eighty healthy volunteers [63 females (79%), 17 males (21%)], we taken patients from the Rizgari Hospital's neurology department From December 2021 to April 2022.

Inclusion and Exclusion Criteria

All migraine patients identified by a neurologist using the International Headache Society Criteria⁽¹⁷⁾ were included. Patients having a history of severe hypertension, seizures, or cancer were excluded, as were those without any data or who refused to participate in the study.

Blood sample collection

From each patient, five mL of venous blood was taken and then placed in gel tubes for serum. Following centrifugation at 3000 rpm for 15 minutes to separate the serum, an aliquot was placed in a 1.5 ml Eppendorf tube to be kept at -20 °C until further analysis.

Biochemical analysis

Measurement of serum MDA was done by spectrometric calculations⁽¹⁸⁾. MDA is formed as an outcome of lipid peroxidation. First, it reacts with thiobarbituric acid (TBA) to form the MDA-TBA compound then it can be measured. In the test tube, we added 150 µL of serum with one ml of 17.5% trichloroacetic acid (TCA), then we added one ml of 0.66% TBA on; it after mixing and vortex for 1-2 min. The samples were put into a water bath for boiling at 95 °C for 45 min. After that, one ml of 70% TCA was added after enough cooling of samples at room temperature and then centrifuged for 15min at 3000 rpm after mixing. The pink-coloured supernatant was then read at 532nm by the spectrometer. Then the absorbance is converted to concentration.

Serum NO determination was done using Griess Reaction, first reported by Griess⁽¹⁹⁾. According to the procedure, salt of transient diazonium in an acidic medium will form after nitrite treatment with a diazotizing reagent, e.g., sulfanilamide. To form a stable azo compound this salt undergoes a reaction with the coupling reagent N-naphthyl-ethylenediamine

(NNED); intense purple colour was formed, and its absorbance was measured at 540nm, which directly corresponds to sample nitrate concentration⁽²⁰⁾.

Determination of serum SOD was performed through the concept given by the scientists^(21, 22). In which quercetin undergoes oxidation by O₂. Standard solutions were prepared by adding 0.8 mM TMEDA and 0.08 mM EDTA together in the clean tube in buffer solution (pH = 9) in 16 mM potassium sulfate, then 100 µl of serum with 20 µl of quercetin were added together then following 20 min the optical density measured by spectrophotometer at 406 nm.

Statistical analysis

All data are parametric since they passed normality tests (Kolmogorov-Smirnov et al. tests). The t-student tests for differences between groups and the Pearson test for correlations were used. The receiver operating characteristics (ROC) curve was used to know the usefulness of oxidative stress as a diagnostic marker. Binary logistic regression was used to know to predict the impact of oxidative stress markers on the life of migraineurs. The statistical packages used in this study were SPSS 27, MedCalc 20, and GraphPad Prism 9. A P value < 0.05 was regarded as statistically significant.

RESULTS

As shown in (Table 1, Figure 1), There was not a significant change in the age of migraine patients (34.67 ± 1.212) when compared with the control group (34.99 ± 1.285) ($P= 0.857$). A significant alteration ($P=0.027$) was observed in SOD levels in migraine patients (9.649 ± 0.938) when compared with the control group (7.420 ± 0.167) (Figure 2a).

In addition, NO levels increased significantly in migraine patients (66.76 ± 1.799) when compared with the control group (42.68 ± 3.664) ($P<0.0001$) (Figure 2b). Statistical analysis revealed that in migraine patients (18.42 ± 0.684), MDA levels changed significantly in comparison with the control group (16.74 ± 0.302) ($P=0.03$) (Figure 2c).

Next, we evaluate models to predict the quality-of-life change in migraine patients by applying binary logistic regression, including SOD, NO, and MDA. We documented that SOD ($P=.585$, OR= 0.977) and MDA ($P=0.642$, OR= 0.976) were not significantly linked, but NO levels ($P=0.01$, OR= 0.965) were significantly

associated with the quality of life in migraine patients (Table 2).

Additionally, we did a correlation between age, SOD, NO, and MDA. A significant positive correlation was found between SOD and NO ($P=0.002$, $r=0.309$), MDA and NO ($P=0.01$, $R=.256$). There was no correlation between other parameters, as found in Table 3.

In the study, to assess the diagnostic usefulness of SOD, NO, and MDA in migraine, ROC curves were drawn for 100 patients with migraine and 100 healthy controls. The significance of the SOD concentration in terms of diagnosis of migraine has 24% sensitivity and 90% specificity. The cut-off value from which diagnosis can be assumed is ≤ 5.99 , and AUC is 0.526. The calculation of the NPV turned out to be 51.6%. The PPV is 72.7% (Table 4, Figure 3).

The ROC curve of NO for diagnosing migraine was also analyzed. Its results revealed that the AUC of NO for diagnosing migraine was 0.806 (95% CI: 0.742 to 0.860), with a cut-off value of >50.39 . It had a sensitivity of 35.71% and a specificity of 93.33%, with NPV and PPV of 57.1% and 85.40%, respectively (Table 2, Figure 3). ROC curve of MDA was also obtained; it had a sensitivity of 96.94% and a specificity of 75.56%, with NPV and PPV of 95.8% and 81.20%, respectively. It was less accurate for diagnosis (AUC =0.535, and $P=0.433$).

The SOD, NO, and MDA combination achieve much higher AUC values (0.827) than each separately concerning migraine diagnosis. The NPV and PPV of this combination were 85.3% and 76.6%, respectively. The cut-off value of this serum markers combination was >0.436 . The ROC curve of serum markers is shown in Table 4, Figure 3.

Table 1. Clinical and demographic characteristics of healthy control and Migraine patients.

Parameters	Controls (Mean ±SEM)	Migraine patients (Mean ±SEM)	(P-value)
n	80	100	
Mean age	34.99 ± 1.285	34.67 ± 1.212	0.857
Gender			
Male	17	23	
Female	63	77	
Family history		Yes (50) / No (50)	
Sleep disorder		Yes (33) / No (67)	
Nausea and vomiting		Yes (62) / No (38)	
Types of Migraine			
Migraine with Aura		25	
Migraine without Aura		34	
Classic Migraine		41	
UAs			
Yes		36	
No		64	
Duration of disease		2.78 ± 0.307	
Affecting daily life		Yes (84) / No (16) (16) (50)	
SOD	7.420 ± 0.167	9.649 ± 0.938	0.027
NO	42.68 ± 3.664 34.76 3.664	66.76 ± 1.799	<0.0001
MDA	16.74 ± 0.302	18.42 ± 0.684 ± 0.612	0.03

N: number of participants, UAs: unilateral cranial autonomic symptoms, NO: nitric oxide, MDA: malondialdehyde, SOD: superoxide dismutase. SEM: standard error of the mean.

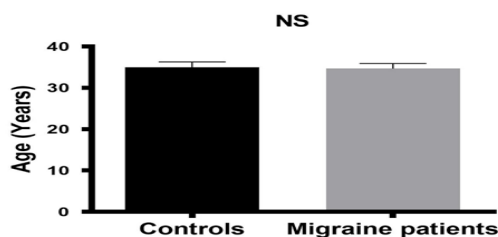


Figure. 1 Comparison of age in migraine patients and healthy controls. An independent t-test was used for the comparison. NS: non-significant.

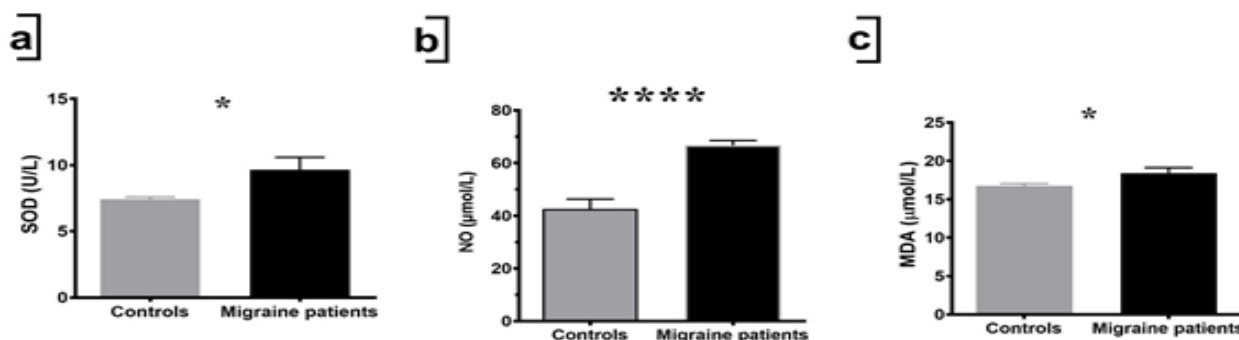


Figure. 2 Comparison of serum markers in migraine patients and healthy controls. An independent t-test was used for the comparison of (a) SOD; (b) NO; and (c) MDA. NO: nitric oxide, MDA: malondialdehyde, SOD: superoxide dismutase.

Table 2 .Binary Logistic regression of serum markers to predict the impact of migraine on quality of life.

Parameters	B	SEM	P-value	OR	95% CI for OR	
					Lower	Upper
SOD	-.023	.043	.585	.977	.898	1.062
NO	-.036	.014	.013	.965	.938	.992
MDA	-.024	.052	.642	.976	.882	1.081

B: Regression coefficient, NO: nitric oxide, MDA: malondialdehyde, SOD: superoxide dismutase. SEM: standard error of the mean. OR: odd ratio, CI: confidence interval.

Table 3 .Correlation between age and serum markers.

Parameters	Correlation	Age	SOD	NO	MDA
Age	Pearson Correlation	1	.006	.010	.010
	P-value		.951	.925	.924
SOD	Pearson Correlation	.006	1	.309**	.018
	P-value	.951		.002	.858
NO	Pearson Correlation	.010	.309**	1	.256*
	P-value	.925	.002		.012
MDA	Pearson Correlation	.010	.018	.256*	1
	P-value	.924	.858	.012	

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

NO: nitric oxide, MDA: malondialdehyde, SOD: superoxide dismutase.

Table 4. ROC curve analysis of SOD, NO, MDA, and their combinations to the diagnosis of migraine.

Variables	AUC	95% CI	P-Value	Cut-off	Sensitivity	95% CI	Specificity	95% CI	PPV	95% CI	NPV	95% CI
SOD	0.526	0.452 to 0.598	0.5424	≤5.99	24.00	16.0 - 33.6	90	81.9 - 95.3	72.7	56.7 - 84.4	51.6	48.3 - 54.8
NO	0.806	0.742 to 0.860	<0.0001	>50.39	96.94	91.3 - 99.4	75.56	65.4 - 84.0	81.2	75.0 - 86.1	95.8	88.1 - 98.6
MDA	0.535	0.461 to 0.607	0.4330	≤12.86	35.71	26.3 - 46.0	93.33	86.1 - 97.5	85.4	72.0 - 93.0	57.1	53.2 - 61.0
Combination	0.827	0.765 to 0.879	<0.0001	>0.436	88.54	80.4 - 94.1	71.11	60.6 - 80.2	76.6	70.1 - 82.0	85.3	76.7 - 91.2

AUC: area under the curve, NO: nitric oxide, MDA: malondialdehyde, SOD: superoxide dismutase. PPV: positive predictive value, NPV: negative predictive value, CI: confidence interval.

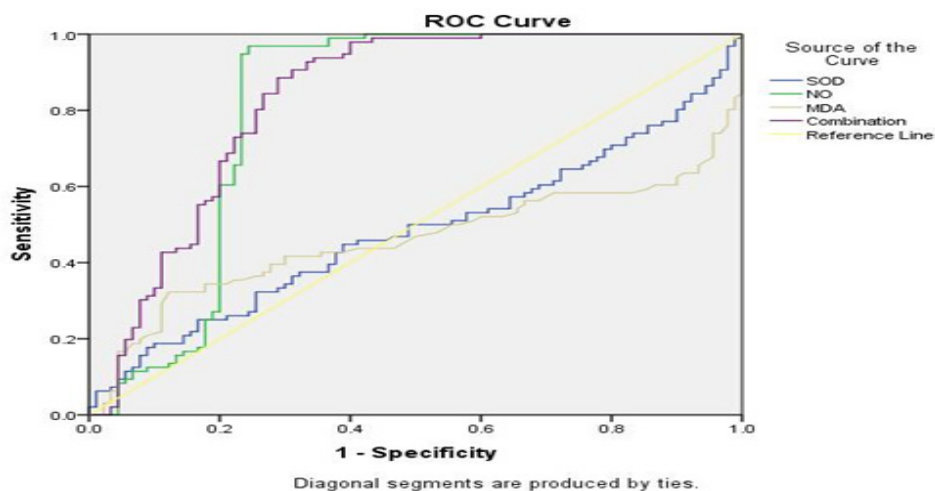


Figure. 3 ROC curve analysis of SOD, NO, MDA, and their combinations to the diagnosis of migraine. NO: nitric oxide, MDA: malondialdehyde, SOD: superoxide dismutase, ROC: receiver operating characteristics.

DISCUSSION

Migraine is a disabling neurovascular disorder whose frequency in the general population is approximately 10-15%, with a clear predominance of females⁽²³⁾. World prevalence data published in the 2015 Global Burden of Disease study show that migraine is two to three times more prevalent in women than in men and that the age prevalence peaks in both sexes between 30 and 39 years of age. Our results regarding these demographic variables were similar to those described in other investigations; female patients were the majority compared to men, and the average age coincided with the ages mentioned in different published works.

It is believed that the higher prevalence of migraine in women over men during the reproductive years would be a consequence of differential effects of male and female sex hormones since they can act as important modulators of headache, as shown by the effects of Specific hormonal events in women with migraine. This is evidenced if we consider that menstruation is known as a risk factor for migraine, being even more exacerbated during the two premenstrual days as well as in the first three days of bleeding⁽²⁴⁾.

The oxidant-antioxidant balance is an important mechanism for homeostasis in an organism. ROSs, such as superoxide radical anions, hydroxyl radicals, and hydrogen peroxide, are formed as a result of various metabolic and physiological processes, and deleterious oxidative reactions can occur. Oxidative stress is postulated to occur when an imbalance between oxidants and antioxidants is shifting towards oxidants. Oxidative stress can be defined as an increase in the level of oxidants and a decrease in antioxidant capacity. The imbalance of oxidative stress/ antioxidant status is believed to be one of the risk factors for developing migraine⁽¹⁰⁾.

In this study, the level of MDA and NO were measured as markers of oxidative stress and free radical formation. SOD was also measured as an indicator of antioxidant enzymes. The interaction of free radicals with tissue lipids is called lipid peroxidation, a process that gives rise to several degradation metabolites, one of which is MDA⁽²⁵⁾.

The level of NO was higher in migraine patients than in control. Our result concerning NO was similar to Yilmaz, Sürer⁽²⁶⁾, who documented that NO was higher in migraineurs than migraine-free subjects. The half-

life of NO was very short, so the NO measurement was difficult; it converts to nitrite (NO₂) and nitrate (NO₃); the total measurement of NO₂ and NO₃ was a mirror image of NO in this study. The contribution of NO to the pathophysiology of migraine remains elusive. Platelet aggregation and vasoconstriction are one of the risk factors in migraine, the body as negative feedback tries to increase NO as a vasodilator and decrease platelet aggregation; therefore, hemodynamic changes by platelet aggregation led to elevate NO that causes migraine attack by increasing vasodilation of cerebral arteries.

On the other hand, when the levels of free radicals, such as superoxide anion, can react with NO to produce potent free radicals, peroxynitrite (ONOO⁻). Taffi and Vignini⁽²⁷⁾ uncovered that the ONOO level was lower in migraineurs during the headache-free period than during migraine attacks. In this study, binary logistic regression revealed that NO levels associated negatively affect the life of migraineurs. The level of NO can be used as a marker for migraine diagnosis, as shown by the ROC curve in this study.

Multiple processes would be associated with oxidative stress conditions generated by hyperfibrinogenemia; the enzyme endothelial nitric oxide synthase (eNOS) can generate O₂⁻ instead of NO or O₂⁻ and NO simultaneously⁽²⁸⁾. However, NO does not reach its biological targets by reacting with O₂⁻ whose concentrations are elevated in oxidative stress. The groups of migraineurs with hyperfibrinogenemia also presented increased concentrations of L-citrulline, a co-product of the NO produced in an "equimolar" form concerning NO, showing an inverse behaviour concerning the dosed values of NO under the same experimental situation. This increase suggests that the synthesis of NO enters a pathophysiological pathway increasing oxidative stress since NO can be transformed into a significantly cytotoxic entity and to compensate for this aggression, L-citrulline increases its plasma levels, trying to supply the low availability of NO that generates functional alterations at the level of the vascular wall⁽²⁹⁾.

NO synthesis would also be mediated by the enzyme neuronal nitric oxide synthase (nNOS) and inducible nitric oxide synthase (iNOS). NO, released by satellite cells, stimulates the synthesis and release of CGRP in trigeminal neurons. In this way, activating the trigeminal ganglion in migraineurs depolarizes sensory axons, allowing vasodilation and increased permeability of

meningeal vessels with the consequent development of neurogenic inflammation in the migraineurs⁽³⁰⁾.

Superoxide dismutase (SOD) would modify its activity to control the highest levels of ROS, so the determination of the activity of this enzyme could be one of the most relevant biomarkers of the oxidative state present in patients with migraine. Its measurement would be a predictive tool to determine the degree of oxidative stress underlying this disease⁽³¹⁾.

Surprisingly, the level of SOD was elevated in Migraineurs; this finding was incompatible with Neri, Frustaci⁽³²⁾, who showed that SOD level was lower in migraine patients to compensate for this oxidative imbalance with an increase in proinflammatory and prooxidant indicators, SOD levels were increased in the migraine groups. Serum SOD values obtained in our study were higher in groups with migraine than in healthy patients. Another explanation for the elevation of SOD is to reverse the persistent oxidative stress triggered by hyperfibrinogenemia by reinforcing the body's endogenous antioxidant defense⁽³³⁾. In addition, said the increase in SOD activity would be a characteristic adaptive response of biological systems, tending to compensate for oxidative stress; the evidence for this is the presence of a positive correlation between SOD and NO levels in this study.

Conclusion, based on these findings, we can conclude that patients with migraine have a higher state of oxidative stress than healthy subjects. The NO level can be used as a marker for migraine diagnosis, as shown by the ROC curve in this study. Binary logistic regression revealed that NO levels associated negatively affect the life of migraineurs.

Conflict of interests

The authors have not revealed any conflicting interests.

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None

Ethical approval

This research was approved by the Salahaddin University-Erbil (SUE) Ethics Committee (approval number: R33-025; 94 approved on June 5, 2021).

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