Plasma Native Thiol and Lipid Hydroperoxide Levels in the Patients with A Non-Aura Migraine Attack Admitted to the Emergency Department: A Case-Control Study

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──────────────── **Abstract** ────────────────

Aim: We emphasize the importance of serum native thiol (SH), lipid peroxidation (LOOH), and ceruloplasmin (CP) levels in the management of patients with a migraine attack and clarify the possible relationships among these parameters in the emergency department (ED)

Methods: The study was designed as a case-control study which is a type of observational study by including the patients who applied to the University of Health Sciences Turkey, University Diyarbakır Gazi Yasargil Training and Research Hospital between December 2018 and July 2019. A total of 88 participants (study group, n=46 and healthy control group, n=42) consecutively admitted to the ED. with similar demographic characteristics were included in the study. SH, LOOH, and CP levels in both groups were recorded and studied.

Results: The serum SH level was significantly lower in the patient than in the control group (p=0.002). However, the LOOH and CP levels were significantly higher in the patient group than in the control group (p<0.001). A significant correlation was observed between LOOH and CP (r=0.332, p=0.002). The receiver operating characteristic analysis indicated that a serum LOOH level of 11.9 µmol/L predicted an acute migraine attack with 89% sensitivity and 71% specificity.

Conclusions: Our results suggest that the oxidative status is activated in patients with a non-aura migraine attack, and this may lead to other oxidative and inflammatory processes, with an increase in the serum LOOH level and a decrease in the native thiol level.

Keywords: Non-aura migraine, native thiol, lipid peroxidation, emergency department

Introduction

Migraine is a neurovascular disease characterized by brain and brain vascular inflammation with episodic attacks. Migraines with and without aura have been described by the International Society of Headaches. There are generally two types of migraine, migraine with aura accompanied by transient focal neurological symptoms that often start with pain, sometimes before headache, and migraine without aura, which is associated with certain symptoms (ICHD) (1). Although many theories on the pathogenesis of migraine have been proposed, it is not yet fully understood. Activation of neuropeptides and mechanisms associated with inflammation are believed to be responsible for the pathogenesis of migraine (2-4).

Thiols are compounds that scavenge free oxygen radicals. Thiols are important antioxidant agents in humans, and thiols containing the sulfur analog in alcohol are in a free or oxidized form in the plasma. Thiol groups are oxidized by disulfide bonds and reduced by reactive oxygen species (5).

Oxidative stress is responsible for migraine, and the thiol-disulfide balance is likely to deteriorate during a migraine attack. In fact, there are a limited number of studies on migraine patients with a thiol-disulfide balance (2,5). It is clear that these studies are inadequate to explain the clinical and physiopathological status of migraine.

Hydroperoxides (LOOH), which are more stable than free radicals, in serum, may be markers of oxidative stress in tissue and their levels increase in the presence of stress (5). We hypothesized that acute migraine attacks may be associated with enhanced oxidative status and that determination of the plasma levels of LOOH and native thiol (NT), which are antioxidants, may be beneficial in the management of patients with migraine in the emergency department (ED). In this study, we emphasize the importance of serum NT and LOOH levels in the management of patients with a migraine attack and clarify the possible relationships among these parameters. We also investigated the relationships of these parameters with the migraine disability assessment score (MIDAS) and determined Whether NT and LOOH were independent risk factors in patients with migraine in the ED.

Methods

Study Design

The local ethics committee approved the study and all participants gave informed written consent. The study was designed as a case-control study which is a type of observational study by including the patients who applied to the University of Health Sciences Turkey, University Diyarbakır Gazi Yasargil Training and Research Hospital between December 2018 and July 2019 (date: 2018, number: 169). All procedures were carried out in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration. A total of 46 patients with non-aura migraine consecutively admitted to the ED (Patients group: 8 males and 38 females) and 42 healthy controls (12 males and 30 females) with similar demographic characteristics were included in this study.

Inclusion criteria: 1. Admitted to the ED with headache and diagnosed with migraine; 2≥18 years of age; 3. any other known chronic or acute disease; and 4. nonsmoker and no substance dependence.

Exclusion criteria: Patients who were diagnosed with any secondary cause of headache; known febrile illness or other cardiovascular disorder; history of alcohol consumption or alcohol intake; active somatic disease in the past month; active somatic or psychiatric disease; drug use for any reason within 7 days; taking drugs such as alcohol or ecstasy, or taking drugs with suicidal intent; likely to become pregnant or suspected pregnancy; chronic disease; exposed to trauma in the last week; and 12. <18 years of age (n=13).

Clinical Evaluation

The patients were initially treated in the ED. Vital signs such as blood pressure, pulse, and body temperature were evaluated. After a migraine episode was detected, 5 cc of blood were obtained and treatment with non-steroidal anti-inflammatory drugs and/or selective 5HT1B/1D agonists (Triptan) was started. The patients were taken to quiet, low-light observation rooms with appropriate conditions. All of the patients were discharged from the ED after their symptoms were resolved. The age, sex, migraine type, duration of migraine (years), monthly frequency of migraine attacks, duration of migraine attacks (hours), and headache severity [visual analog scale (VAS)] of the patients were recorded. It was not taken into consideration whether the patient received treatment for a migraine attack. Headache severity was evaluated using a VAS (score 1-10). The diagnosis of migraine was made according to the latest diagnostic criteria classification of ICHD (1). Diagnostic criteria of the ICHD for migraine indicate that a patient should have at least five migraine attacks (untreated or unsuccessfully treated) lasting 4-72 hours with at least two of the following characteristics: 1) unilateral placement, 2) a vibrating quality, 3) moderate or severe pain intensity, and 4) aggravation that causes avoidance of daily physical activity. After arriving at the ED, the patients were evaluated by the same healthcare professional who used MIDAS to evaluate headache-related disability. The procedure involved a questionnaire to determine the effect of migraine headaches on the patient's performance routine activity. The patients were divided into three groups according to their MIDAS scores (6).

Laboratory Assessments

NT and LOOH levels in both groups were recorded as primary independent variables and demographic and clinical characteristics of the patients and other biochemical and hematological data were recorded as secondary independent variables.

A 5 mL blood sample was taken within the first hour from all patients who suffered from migraine without aura and from controls. All blood samples were placed in flat tubes and centrifuged at 3,500 rpm for 15 min to obtain the plasma or serum. All samples were stored in aliquots at -80 °C until use.

Hemogram counts were measured using an automated analyzer in K2EDTA samples (Sysmex K-1000; Block Scientific, Bellport, NY USA). Routine biochemical variables such as serum urea, creatinine, sodium, and potassium were measured using commercial kits (Abbott; Block Scientific). Serum CP was measured by the Erel (7) method, an automatic calorimetric method based on enzymatic oxidation of ferrous ion to ferric ion. Details of this method are available in Erel (7).

Ferrous ion oxidation of xylenol orange was used to assess serum LOOH levels (8). In the test, the ferric ion concentration was measured using xylenol orange. LOOH is reduced by triphenylphosphine (TPP), a specific lipid reductant. The presence or absence of TPP during pretreatment determines the difference in LOOH level. LOOH values are expressed in μmol/L (8).

NT concentrations were measured using a new and fully automated analytical method (9). In this method, the reducible disulfide bonds were reduced to form free functional thiol groups. Reductive sodium borohydride was used and extracted with formaldehyde, and the native and reduced thiol groups were determined after reacting with 5.5-dithiobis-(2-nitrobenzoic) acids. Then, the natural thiol levels were measured and calculated. The measurements were performed using a Cobasc 501 instrument (Roche Diagnostics, Mannheim, Germany).

Statistical Analysis

The demographic and clinical data of the patients were written into excel files at the time of patient admission. Serum biochemical data of the participants were also transferred to this excel file. Study calculations were performed using SPSS version 20.0 software package (Chicago, IL, USA). In the power analysis for SH, differences in the mean levels of patient and control group variables were compared. The power of the test to detect a difference between the two groups was 80% for NT. The Shapiro-Wilks test was performed to determine the normality of the distribution. Student's t-test was used for comparisons between groups of numerical variables showing a normal distribution, and an analysis of variance (ANOVA)-Tukey test for multiple comparisons within groups. Categorical variables were analyzed using the chi-squared or Fisher's Exact tests. A receiver operating characteristic (ROC) curve analysis was performed to evaluate the discriminatory ability of NT and LOOH. Binary logistic regression analyzes were used for NT and LOOH in patients with migraine attacks. P-values of <0.05 were considered indicative of significance.

Results

Of the 46 migraine patients included in the study, 8 (17.4%) were males, and 38 (82.6%) were females; 12 (28.6%) of the 42 control individuals were males, and 30 (71.4%) were females. The mean age of the migraine patients was 36.04±8.06 years, and that of the control group was 37.32±12.55 years. The sex and age distributions were similar between the groups (p=0.211, p=0.509, respectively). While the baseline clinical characteristics of the patients with acute migraine attacks are shown in Table 1, the demographic and laboratory data of the patient and control groups are in Table 2.

The serum NT level was meaningfully lower in the patient than in the control group (0.49±0.15 vs. 0.60±0.19 μmol/L, p=0.002, Table 2). However, the LOOH and CP levels were meaningfully higher in the patient group than in the control group (17.81±5.19 and 407.88±119.23 in the patient group; 10.86±3.05 and 343.24±104.83 in the control group, respectively, p<0.001, p=0.009, respectively; Table 2).

In Table 3, an ANOVA-Tukey test was performed according to the MIDAS classification to determine the

Table 1. Baseline clinical characteristics of patients with acute migraine attack

Pain localization	Number (N)	Proportion (%)
Frontal	19	(41.3%)
Occipital	7	(15.2%)
Temporal	14	(30.4%)
Verteks	6	(13.1%)
Symptomsα	Number (N)	Proportion (%)
Nausea-vomiting	39	(84.8%)
Yawning	7	(15.2%)
Irritability	15	(32.6%)
Sensitivity to light, sound or motion	25	(54.3%)
Dark circles under the eyes	4	(8.7%)
History	Mean ± SD	Minimum-maximum
Duration of disease/year	6.08 ± 2.42	1.00-12.00
Attack time/hour	19.28±10.43	6.00-48.00
Frequency of attacks/month	2.73±1.52	1.00-7.00
Pain severity (VAS)	7.65±1.40	5.00-10.00

α: Each patient had one or more symptoms, VAS: Visual analog scale, SD: Standard deviation
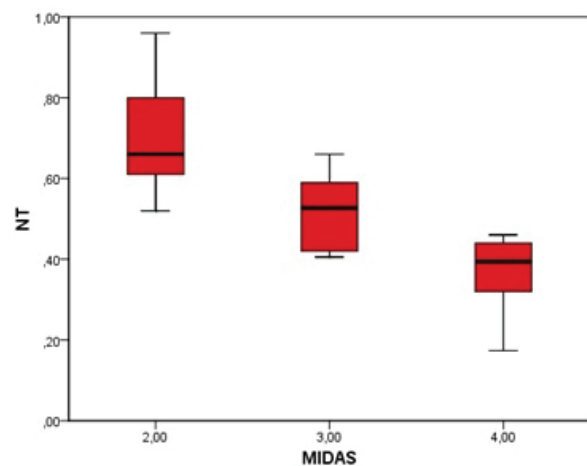

Figure 1. NT level comparison between MIDAS classification in patients with migraine attack

MIDAS: Migraine disability assessment score, NT: Native thiol

Table 2. Age, gender and baseline laboratory characteristics of patients with acute migraine attack and controls

Parameters	Migraine patients (52.2%, n=46) (n, % or mean ± SD)	Controls (47.8%, n=42) (n,% or mean ± SD)	p*
Gender			
(Male/female)	8/38	12/30	0.211
Age (years)	36.04±8.06	37.32±12.55	0.509
LOOH (µmol/l)	17.81±5.19	10.86±3.05	<0.001
NT (mmol/L)	0.49±0.15	0.60±0.19	0.002
CP (mg/dL)	407.88±119.23	343.24±104.83	0.009
LDL cholesterol (mg/dL)	109.52±34.81	114.23±31.06	0.506
Ca (mg/dL)	9.79±1.66	10.16±0.92	0.200
Glucose (mg/dL)	96.10±24.50	100.98±23.35	0.313
Urea (mg/dL)	23.64±7.50	29.79±7.85	<0.001
Creatinin (mg/dL)	0.60±0.16	0.81±0.19	<0.001
AST (U/L)	24.82±9.70	21.21±8.25	0.067
ALT (U/L)	26.89±17.52	17.52±7.34	0.063
T. Chol (mg/dL)	182.18±51.14	196.04±38.15	0.156
HDL (mg/dL)	38.28±12.61	40.40±11.99	0.426
K (mEq/L)	4.22±0.78	4.21±0.47	0.894
LDH (U/L)	286.75±101.35	257.18±94.64	0.162
Hemoglobin (g/L)	13.93±4.07	14.20±1.81	0.715
MPV (fL)	7.44±1.27	7.39±1.08	0.894
PLT (x109/L)	258.05±84.76	282.16±92.47	0.205
APTT (Sec)	34.14±4.07	31.95±7.85	0.258
PT-INR	1.31±1.28	1.10±0.21	0.295
Hs-CRP	0.542±0.210	0.580±0.170	0.453

*Student's t-test and chi-square tests. ALT: Alanine aminotransferase, APTT: Activated partial thromboplastin time, AST: Aspartate aminotransferase, Ca: Calcium, HDL: High-density lipoprotein, Hs-CRP: High-sensitivity-C-reactive protein, K: Potassium, CP: Ceruloplasmin, NT: Native thiol, LOOH: Lipid peroxidation, LDL: Low-density lipoprotein, LDH: Lactate dehydrogenase, MPV: Mean platelet volume, PLT: Platelet, PT-INR: Prothrombin time- International normalized ratio

clinical severity of patients presenting to the ED with a migraine attack. The mean NT level of the group with the most severe clinical presentation was lowest (for comparisons among three groups, p<0.001; Table 3 and Figure 1, respectively). In contrast, the mean plasma LOOH level increased in proportion to the clinical severity of migraine (p=0.002, Table 3, Figure 2).

A significant, positive correlation was observed between MIDAS score and LOOH (r=0.449, p=0.002). Conversely, the MIDAS score was significantly negatively correlated with NT level (r=-0.777, p<0.001). As seen in Figure 3, the ROC analysis indicated that a cut-off serum LOOH level of 11.9 µmol/L predicted acute a migraine attack with 89% sensitivity and 71% specificity (area under the curve=0.896; 95% confidence interval (CI): 0.832-0.960).

In binary logistic regression analyses, NT and LOOH were independent markers of a migraine attack in the ED (Table 4); the odds ratio (OR) was significantly lower for NT (OR <0.001; 95% CI, 0.00-1.04; p=0.002) and significantly higher for LOOH (OR: 1.89; 95% CI, 1.42-2.52; p<0.001)

Discussion

This is one of the few studies in which serum NT was studied in patients who presented to the ED with a migraine attack without aura. No previous study has evaluated NT and LOOH levels in patients admitted to the ED with a migraine attack without aura. Our results show that the NT level was meaningfully lower and the LOOH level was higher in patients admitted to the ED with a migraine attack without aura compared to those of healthy participants. Moreover, the NT level decreased and the LOOH level increased in proportion to the severity of the migraine attack. Also, the MIDAS score was significantly positively correlated with the LOOH level and significantly negatively correlated with that of NT. In addition, binary logistic regression analyses showed that the NT and LOOH levels were independent markers of a migraine attack in the ED.

Migraine patients are susceptible to neurovascular reactions caused by different factors. The Impaired balance between inhibition and stimulation of the nervous system leads to some clinical variations, including headache

Table 3. Serum NT, CP and LOOH levels comparison between MIDAS classification in patients with migraine attack

MIDAS	N	Mean ± SD	Minimum-maximum	ANOVA	Tukey HSD
Clas				Px	Pα
NT II	8	0.70±0.14	0.52-0.96	<0.001	II & III → <0.001
III	20	0.51±0.08	0.41-0.66	-	II & IV → <0.001
IV	18	0.36±0.09	0.17-0.46	-	III & IV → <0.001
LOOH II	8	13.75±2.55	10.78-17.30	0.002	II & III → 0.112
III	20	17.58±3.37	10.63-24.10	-	II & IV → 0.005
IV	18	20.04±5.91	12.10-32.52	-	III & IV → 0.218
CP II	8	456.09±110.25	297.86-647.50	0.426	II & III → 0.436
III	20	393.92±119.33	244.20-641.84	-	II & IV → 0.541
IV	18	401.95±123.89	268.26-678.03	-	III & IV → 0.977

Pα : Tukey test for Multiple Comparisons (the mean difference is significant at the 0.05 level), Px: One-Way ANOVA test. MIDAS: Migraine disability assessment score, LB: Lower bound, UB: Upper bound, CP: Ceruloplasmin, NT: Native thiol, LOOH: Lipid peroxidation
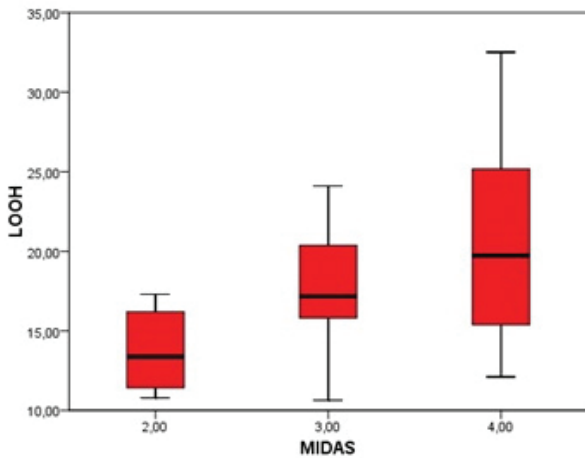

Figure 2. LOOH level comparison between MIDAS classification in patients with migraine attack
LOOH: Lipid peroxidation, MIDAS: Migraine disability assessment score

Figure 3. Receiver operating characteristic analysis for NT
NT: Native thiol, LOOH: Lipid peroxidation, LOOH: Lipid peroxidation ROC: Receiver operating characteristic

(10,11). Clinical studies have indicated that oxidative stress increases and antioxidant defense decreases in patients with migraine (12,13). The role of the acute inflammatory response caused by oxidative stress has been reported in some clinical studies in patients with a headache due to a migraine (13,14). LOOH is a primary product of lipid peroxidation, and it may be useful in predicting oxidative stress in the blood (15). Few studies have shown levels of lipid peroxidation in migraine patients or the levels of these antioxidant enzymes (12,16).

Lipid peroxidation levels can be assessed based on serum total oxidative stress measures using a recently developed method (16,17). Native thiol may be useful in determining antioxidant activity. Gumusyayla reported that NT levels were higher in migraine patients than in healthy controls (5). In the same study, NT level was reported to be similar in migraine patients with and without aura. In
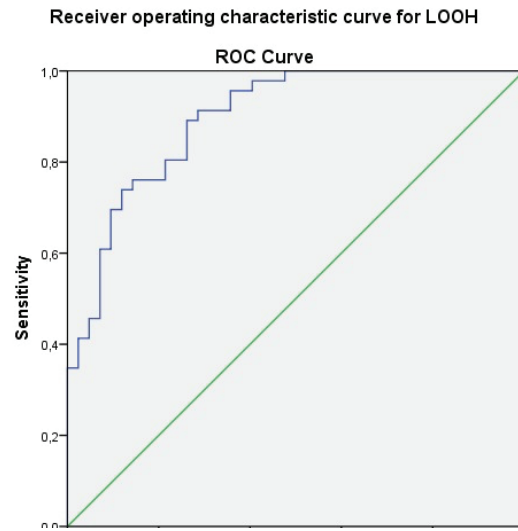
Table 4. Binary logistic regression analyses of study variables

Variables	OR 95%	CI	p	Sensitivity	Specificity
NT	<0.001	0.00-1.04	0.002		
LOOH	1.89	1.42-2.52	<0.001	84.3	85.7
CP	1.00	0.99-1.01	0.317		
Hs-CRP	0.48	0.18-1.29	0.149		

NT: Native thiol, LOOH: Lipid peroxidation, CP: Ceruloplasmin, Hs-CRP: High-sensitivity-C-reactive protein, OR: Odds ratio, CI: Confidence interval

another study, the relationships of changes in dynamic thiol-disulfide homeostasis with pain severity and duration of disease were investigated in children with migraine (14). In that study, a negative correlation was found between the NT level and headache severity in patients with both

migraine and tension-type headaches. NT levels have been reported to decrease as headache severity increases. In our study, serum NT levels were meaningfully lower in patients who presented to the ED with a migraine attack without aura than in the control group. We also classified the patients with migraine according to their MIDAS score; the mean NT level was significantly lower in patients with the most severe migraine attacks. Moreover, there was a negative correlation between the MIDAS score and NT level.

In a study of patients with coronary slow flow, the LOOH levels were higher than those in the normal population (18). Similarly, Yigit et al. (13) reported that the levels of oxidative parameters were high in patients presenting to the ED with migraine attacks. Similarly, levels of CP and LOOH, which are inflammatory markers closely associated with oxidative stress, were meaningfully higher in migraine patients than in controls in the present study. Furthermore, the level of LOOH, the main product of lipid peroxidation and a marker of the level of oxidative stress, increased meaningfully. In addition, the mean plasma level of LOOH increased in proportion to the severity of the migraine attack according to the MIDAS classification; patients with the highest MIDAS score had the highest level of LOOH. So, there was a significant, positive correlation between the MIDAS score and the LOOH level. However, the patients in the MIDAS-II, -III, and -IV groups had similar CP levels. Moreover, the NT and LOOH levels were independent markers of a migraine attack in the ED. The high serum LOOH levels suggest that oxidative stress plays a role in migraine. The oxidant state is dominant in cases of an acute attack. The increased serum levels of LOOH during migraine attack suggest that an acute inflammation event is triggered in the dominant state of oxidation. However, whether oxidative stress is a cause of migraine or the result of metabolic processes associated with this condition is unclear.

Study Limitations

The limitations of our study should be mentioned. The migraine diagnosis was based on subjective criteria. This was a single-center study with a relatively small number of participants. We used a pain severity scale for migraine patients, and we could not evaluate the effects of previously used drugs that may have affected the levels of SH, LOOH, or CP. This study was based on a single blood sample to determine NT, LOOH, and CP levels in each patient. A comparison could not be made regarding the pain location and symptoms of the patients, because the primary parameters in our study are based on the analysis of laboratory data. The fact that such a comparison was not made in the study is an important limiting factor. In our study, we can say that dehydration

may affect oxidative stress. In this respect, perhaps this is an important limitation of our study.

Conclusion

The levels of the antioxidant NT were meaningfully lower in patients with migraine without aura than in controls. Moreover, as the MIDAS score increased, the level of NT decreased and the severity of disability increased. In contrast, the LOOH level, which reflects the oxidative state, was meaningfully higher in migraine patients than in the controls. Moreover, we observed a positive correlation between MIDAS score and LOOH level. In addition, NT and LOOH levels were independent risk factors for migraine attack in the ED. Our results suggest that the oxidative status is activated in patients with a migraine, and this may lead to other oxidative and inflammatory processes, with an increase in the serum LOOH level and a decrease in the NT level.

Authorship Contributions

Concept: M.T.G., Design: O.S., Data Collection or Processing: C.G., E.D., Analysis or Interpretation: O.S., M.T.G., Literature Search: H.E., Writing: O.S., M.T.G.

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