

Copeptin and Global Oxidative Imbalance in Interictal Migraine: An Exploratory Matched Case–Control Study

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Abstract

Background and Purpose:

Migraine is associated with oxidative imbalance and stress-related neuroendocrine mechanisms. This study evaluated serum copeptin and global oxidative imbalance in patients with interictal migraine and matched healthy controls.

Methods:

This exploratory matched case–control study included 43 patients with interictal migraine and 43 age- and sex-matched healthy controls. Serum copeptin was measured by enzyme-linked immunosorbent assay, total antioxidant status (TAS) and total oxidant status (TOS) were assessed colorimetrically, and oxidative stress index (OSI) was calculated as the principal marker of global oxidative imbalance. Clinical assessments included affective symptoms, migraine-related disability, and pain severity. Correlation, receiver operating characteristic (ROC) curve, and multivariable logistic regression analyses were performed.

Results:

Patients with migraine had lower TAS and higher TOS, OSI, and copeptin levels than controls (all $p < 0.001$). After adjustment for depressive and anxiety symptoms and correction for multiple testing, copeptin was not significantly associated with clinical severity measures. OSI showed good discriminatory performance for migraine status (area under the curve (AUC) = 0.856, 95% confidence interval = 0.780–0.931), whereas copeptin showed moderate performance (AUC = 0.730, 95% confidence interval = 0.623–0.838). In exploratory multivariable models, both OSI and copeptin remained associated with migraine status after adjustment for demographic and affective variables.

Conclusions:

Interictal migraine was associated with global oxidative imbalance and elevated serum copeptin levels. OSI showed stronger association with migraine status, whereas copeptin may provide complementary information on stress-related neuroendocrine activity. These hypothesis-generating findings require validation in larger prospective cohorts.

Introduction

Migraine is a highly prevalent and disabling neurovascular disorder characterized by recurrent headache attacks accompanied by sensory, autonomic, and affective symptoms [1, 2]. Its pathophysiology is multifactorial and involves interactions among trigeminovascular activation, neuroinflammation, cortical hyperexcitability, endothelial dysfunction, and central sensitization [3].

Oxidative stress may contribute to migraine through reactive oxygen species generation, mitochondrial dysfunction, neurovascular inflammation, and cortical spreading depression [4]. Total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) provide peripheral measures of systemic redox balance. However, findings in migraine remain inconsistent, and the biological significance of these alterations during the interictal period remains unclear [5–7].

Neuroendocrine stress responses may also contribute to migraine biology. Arginine vasopressin (AVP) regulates hypothalamic stress responses and water homeostasis. Copeptin, a stable peptide derived from the same precursor as AVP, serves as a surrogate marker of vasopressinergic activity and systemic stress [8]. Elevated copeptin levels have been reported in several acute neurological disorders and headache-related conditions, including migraine [9, 10]. However, the relationship between copeptin and systemic oxidative stress markers in migraine remains insufficiently characterized, particularly during the interictal period when acute attack-related biological fluctuations are minimized.

This exploratory study aimed to evaluate serum copeptin and oxidative stress parameters in interictal migraine and matched healthy controls. OSI was defined a priori as the principal marker of global oxidative imbalance, with copeptin assessed as a complementary neuroendocrine stress marker. Secondary objectives examined associations with clinical characteristics, affective symptoms, migraine subtype, and aura status.

Methods

Design and participants

This observational, cross-sectional case–control study recruited consecutive eligible migraine patients and age- and sex-matched healthy controls at a tertiary neurology outpatient clinic between January and May 2026. Clinical assessments, questionnaires, and blood sampling were completed at enrollment; biochemical analyses were performed in May 2026 after sample collection. The Ethics Committee of Tekirdağ Namık Kemal University Faculty of Medicine approved the study (30 December 2025; protocol no. 2025.247.12.16). Procedures followed the Declaration of Helsinki, and all participants provided written informed consent before enrollment.

Patients aged 18–65 years with neurologist-confirmed migraine according to the International Classification of Headache Disorders, 3rd edition (ICHD-3), who could complete clinical assessments were eligible [11]. Exclusion criteria were neuropsychiatric disorders, rheumatologic diseases, chronic kidney or liver disease, pulmonary disease, malignancy, chronic inflammatory diseases, active infection, neurodegenerative disorders, and obesity (body mass index [BMI] ≥ 30 kg/m²).

Interictal sampling required no migraine attack or acute migraine medication use during the preceding 72 hours, confirmed by headache diaries and structured interviews. Patients receiving prophylactic treatment within the preceding 3 months or antioxidant supplements were excluded. Premenopausal women were sampled during the early follicular phase whenever feasible. Pregnancy, lactation, and hormonal therapy were exclusion criteria.

Controls were recruited from the same institution during the same period and had no history of migraine or other primary headache disorders.

Sample size was calculated using G*Power 3.1.9.6 for the Mann–Whitney U test. With an expected OSI effect size of 0.681 [12], $\alpha = 0.05$, and 86% power, 86 participants were required (43 per group).

Clinical assessment and questionnaires

All participants underwent face-to-face clinical assessment by the investigator. Demographic and clinical data, including headache frequency, disease duration, migraine subtype, smoking status, educational level, marital status, and first-degree family history of migraine, were recorded using standardized case report forms.

Migraine subtypes were classified according to ICHD-3. Chronic migraine was defined as ≥ 15 headache days/month for > 3 months, including ≥ 8 days/month with migraine features.

Depressive and anxiety symptoms, migraine-related disability, and pain severity were assessed using validated Turkish versions of the Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI), Migraine Disability Assessment Scale (MIDAS), and Visual Analog Scale (VAS), respectively.

Biochemical analyses

After overnight fasting, morning venous blood samples were collected in serum separator tubes. Serum was separated by centrifugation at $3500 \times g$ for 10 minutes and stored at -80°C until analysis.

Serum TAS and TOS were measured colorimetrically (Elabsience; catalog nos. E-BC-K801-M and E-BC-K802-M, respectively), and copeptin by enzyme-linked immunosorbent assay (Sunred; catalog no. 201-12-5463), following the manufacturers' instructions. Intra-assay and inter-assay coefficients of variation were within manufacturer-reported acceptable ranges.

TAS was converted from mmol to μmol Trolox equivalent/L before calculating OSI as follows:

$$\text{OSI (arbitrary units)} = [\text{TOS } (\mu\text{mol H}_2\text{O}_2 \text{ equivalent/L}) / \text{TAS } (\mu\text{mol Trolox equivalent/L})] \times 100.$$

Statistical analysis

Analyses were performed using IBM SPSS Statistics 25.0 (IBM Corp., Armonk, NY, USA). Normality was assessed using Kolmogorov–Smirnov and Shapiro–Wilk tests. Continuous variables were summarized as mean $\pm$ standard deviation or median (Q1–Q3), and categorical variables as n (%).

Nonnormally distributed continuous variables were compared using Mann–Whitney U or Kruskal–Wallis tests for two or ≥ 3 independent groups, respectively. Effect sizes were expressed as r . Categorical variables were analyzed using Pearson's chi-square (with Yates correction) or Fisher's exact test (with Monte Carlo estimation). Bonferroni correction was applied to pairwise comparisons.

Spearman correlations and partial correlations adjusted for BDI and BAI were calculated. Benjamini–Hochberg false discovery rate (FDR) correction was applied to multiple correlation analyses.

Receiver operating characteristic (ROC) analyses evaluated discrimination of migraine status by OSI and copeptin. The combined model used predicted probabilities from logistic regression and was evaluated in the development dataset. Areas under the curve (AUCs) with 95% confidence intervals, sensitivity, specificity, and optimal cutoffs determined using the Youden index were calculated.

Hierarchical multivariable logistic regression models were constructed to assess the associations of OSI and copeptin with migraine status. Model 1 included age, sex, smoking status, BDI, and BAI. OSI was added in Model 2, copeptin in Model 3, and both biomarkers in Model 4.

Because educational level differed between groups, a sensitivity analysis additionally adjusted Model 4 for educational level.

Multicollinearity was assessed using variance inflation factors. Model fit and performance were assessed using the Hosmer–Lemeshow test, Nagelkerke R^2 , classification accuracy, and AUC. Associations were reported as odds ratios with 95% CIs. Primary analysis variables had no missing data; no imputation was performed. Two-sided $p < 0.05$ indicated statistical significance.

Results

Of 259 potential migraine cases and 76 potential healthy controls screened, 43 participants in each group were included in the final analysis (Fig. 1).

No statistically significant between-group differences were observed in age, sex, BMI, or smoking status, whereas educational level differed ($p=0.002$). BDI and BAI scores were higher in the migraine group (both $p<0.001$; Table 1).

Table 1. Demographic and clinical characteristics of the study population

Variable	Migraine (n=43)	Control (n=43)	p	Effect size
Age, years	32 (29–33)	30 (26–34)	0.083	0.133
Female sex, n (%)	31 (72.1)	31 (72.1)	1.000	—
BMI, kg/m ²	24.1 (22.1–26.4)	23.9 (22.0–26.0)	0.589	0.058
Smoking, n (%)	21 (48.8)	13 (30.2)	0.078	—
Education level, n (%)			0.002	0.378 ^a
• Primary school	1 (2.3)	0 (0)		
• Middle/high school	12 (27.9)	1 (2.3)		
• Higher education	30 (69.8)	42 (97.7)		
BDI score	14 (8–19)	8 (5–12)	<0.001	0.386
BAI score	15 (9–24)	9 (3–14)	<0.001	0.411
VAS score	9 (8–10)	—	—	—
Headache days/month	10 (5–15)	—	—	—
Disease duration, years	12 (8–16)	—	—	—
MIDAS grade	3 (2–4)	—	—	—
Episodic migraine, n (%)	24 (55.8)	—	—	—
Chronic migraine, n (%)	19 (44.2)	—	—	—

Data are presented as median (Q1–Q3) or n (%), as appropriate. Continuous variables were compared using the Mann–Whitney U test, and categorical variables were compared using the chi-square or Fisher’s exact test, as appropriate. Effect sizes are presented as r values for continuous variables. ^a Cramér’s V is reported for education level. BMI: body mass index; MIDAS: Migraine Disability Assessment Scale; VAS: Visual Analog Scale; BDI: Beck Depression Inventory; BAI: Beck Anxiety Inventory.

Patients with migraine had lower TAS and higher TOS, OSI, and copeptin levels than controls (all $p<0.001$; Table 2; Fig. S1). OSI showed the largest effect size ($r=0.612$), followed by TAS ($r=0.489$), TOS ($r=0.447$), and copeptin ($r=0.396$).

Table 2. Oxidative stress markers and copeptin levels

Biomarker	Migraine (n=43)	Control (n=43)	p	Effect size (r)
TAS, mmol Trolox Eqv/L	0.79 (0.66–0.91)	1.05 (0.85–1.52)	<0.001*	0.489
TOS, $\mu\text{mol H}_2\text{O}_2$ Eqv/L	13.06 (10.03–22.69)	8.78 (7.71–11.81)	<0.001*	0.447
OSI	1.78 (1.12–3.15)	0.79 (0.56–1.19)	<0.001*	0.612
Copeptin, ng/mL	12.20 (5.48–15.80)	5.29 (2.91–10.36)	<0.001*	0.396

Data are presented as median (Q1–Q3). Between-group comparisons were performed using the Mann–Whitney U test. Effect sizes are reported as r values. *p<0.05. TAS: total antioxidant status; TOS: total oxidant status; OSI: oxidative stress index.

After adjustment for BDI and BAI, TAS correlated inversely with OSI (partial $r=-0.514$, $p=0.001$), whereas TOS correlated positively with OSI (partial $r=0.791$, $p<0.001$). Only these correlations remained significant after FDR correction (Table 3).

Table 3. Partial correlation analyses within the migraine group adjusted for BDI and BAI

Variables	Partial r	p	FDR-significant
TAS–TOS	0.004	0.978	No
TAS–OSI	–0.514	0.001*	Yes
TAS–MIDAS grade	–0.044	0.787	No
TAS–Copeptin	0.154	0.335	No
TAS–VAS	0.027	0.869	No
TOS–OSI	0.791	<0.001*	Yes
TOS–MIDAS grade	–0.095	0.556	No
TOS–Copeptin	–0.179	0.262	No
TOS–VAS	0.122	0.448	No
OSI–MIDAS grade	–0.145	0.364	No
OSI–Copeptin	–0.153	0.338	No
OSI–VAS	–0.030	0.853	No
MIDAS grade–Copeptin	0.064	0.692	No
MIDAS grade–VAS	0.157	0.328	No
Copeptin–VAS	–0.250	0.115	No

Partial correlations were adjusted for BDI and BAI scores. Benjamini–Hochberg false discovery rate (FDR) correction was applied for multiple testing. Correlation analyses involving MIDAS were performed only within the migraine group. *p<0.05.

Variance inflation factors were acceptable for all variables. In Model 1, only BAI was associated with migraine status. Both OSI (OR 10.96, 95% CI 2.76–43.53) and copeptin (OR 1.23, 95% CI 1.08–1.40) remained associated with migraine status in Model 4 (Table 4; Fig. S2). This model had the highest Nagelkerke R^2 (0.706) and classification accuracy (82.6%) among the evaluated models. The Hosmer–Lemeshow test did not indicate lack of fit ($p=0.978$). Additional adjustment for educational level did not change the direction or statistical significance of either biomarker association.

Table 4. Hierarchical multivariable logistic regression models for migraine status

Variable	Model 1		Model 2		Model 3		Model 4	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Sex	1.42 (0.45–4.54)	0.550	1.74 (0.42–7.22)	0.445	1.65 (0.46–5.93)	0.443	1.93 (0.38–9.71)	0.424
Smoking	2.53 (0.91–7.04)	0.075	3.37 (0.89–12.70)	0.073	2.67 (0.88–8.05)	0.082	4.22 (0.94–18.92)	0.060
Age	1.06 (0.94–1.19)	0.339	1.05 (0.92–1.20)	0.447	1.07 (0.95–1.21)	0.251	1.08 (0.93–1.26)	0.333
BDI	1.05 (0.95–1.16)	0.354	1.01 (0.90–1.15)	0.835	1.07 (0.96–1.18)	0.246	1.02 (0.89–1.17)	0.761
BAI	1.10 (1.02–1.19)	0.017*	1.10 (1.01–1.20)	0.036*	1.10 (1.01–1.19)	0.024*	1.12 (1.01–1.24)	0.032*
OSI	—	—	10.10 (2.74–37.20)	<0.001*	—	—	10.96 (2.76–43.53)	<0.001*
Copeptin	—	—	—	—	1.18 (1.06–1.30)	0.002*	1.23 (1.08–1.40)	0.002*

Model performance metrics

Model	Nagelkerke R ²	Hosmer–Lemeshow p	Accuracy (%)
Model 1	0.319	0.538	69.8
Model 2	0.612	0.961	80.2
Model 3	0.446	0.935	75.6
Model 4	0.706	0.978	82.6

Model 1 included age, sex, smoking status, BDI, and BAI. OSI was added in Model 2, copeptin was added in Model 3, and both OSI and copeptin were included in Model 4. * $p < 0.05$. OR: odds ratio; CI: confidence interval; BDI: Beck Depression Inventory; BAI: Beck Anxiety Inventory; OSI: oxidative stress index.

AUCs were 0.856 for OSI, 0.730 for copeptin, and 0.937 for the combined model, which was developed and evaluated in the same dataset (Table 5). In exploratory subgroup analyses, TAS, TOS, OSI, and copeptin differed between controls and both episodic and chronic migraine groups, whereas no significant differences were observed between the migraine subgroups after Bonferroni correction (Table S1). No significant biomarker differences were observed by aura status (Table S2). Unadjusted Spearman correlations are reported in Table S3.

Table 5. ROC curve analyses for migraine status

Marker/model	AUC	95% CI	p	Best cutoff	Sensitivity (%)	Specificity (%)
OSI	0.856	0.780–0.931	<0.001*	1.05	83.7	67.4
Copeptin	0.730	0.623–0.838	<0.001*	7.26	67.4	67.4
Combined OSI and copeptin model	0.937	0.891–0.983	<0.001*	0.40	90.7	81.4

Receiver operating characteristic (ROC) curve analyses were performed to evaluate the discriminatory performance of oxidative stress index (OSI), copeptin, and the combined OSI-copeptin model for migraine status. Optimal cutoff values were determined using the Youden index. The combined OSI-copeptin model was derived and evaluated in the same dataset; therefore, its performance should be interpreted as exploratory. * $p < 0.05$. AUC: area under the curve; CI: confidence interval.

Discussion

The present exploratory matched case–control study provides an integrated assessment of interictal serum copeptin levels and systemic oxidative stress parameters in patients with migraine. The main finding was that interictal migraine was associated with reduced antioxidant capacity, increased oxidant burden, elevated OSI, and higher copeptin levels compared with healthy controls. Among the evaluated biomarkers, OSI showed the largest effect size and remained associated with migraine status after adjustment for demographic and affective variables. Copeptin also remained associated with migraine status, although its individual discriminatory performance was moderate. These findings suggest that global oxidative imbalance and stress-related neuroendocrine activity may represent complementary biological dimensions of the interictal migraine state. However, neither marker currently supports clinical use without external validation.

Migraine is increasingly recognized as a complex neurobiological disorder involving not only trigeminovascular activation but also inflammatory, metabolic, endothelial, and stress-related pathways [2, 13]. Oxidative stress has been proposed as one of the mechanisms contributing to migraine pathophysiology through reactive oxygen species generation, mitochondrial dysfunction, neurovascular inflammation, and cortical spreading depression [6, 14]. In the present study, patients with migraine demonstrated higher TOS and OSI levels together with lower TAS levels, indicating that oxidative imbalance may persist during the interictal phase [6].

Previous studies evaluating oxidative stress markers in migraine have reported heterogeneous findings. Some studies, including recent interictal data, demonstrated reduced TAS together with increased TOS and OSI levels in patients with migraine compared with healthy controls, whereas others failed to identify significant differences in TAS, TOS, or OSI despite evidence of altered oxidative balance through other markers [4, 6, 7, 15]. Differences in study populations, migraine phases, medication exposure, biomarker methodology, and comorbid conditions may partly explain these inconsistencies. In the present study, biomarker sampling was restricted to the interictal period, and multiple potential confounders, including obesity, inflammatory disorders, recent acute medication use, and prophylactic migraine treatment, were excluded to reduce heterogeneity. Importantly, these findings should not be interpreted as establishing oxidative stress as a novel mechanism in migraine, since TAS, TOS, and OSI have been evaluated in previous migraine cohorts. Rather, the present contribution lies in the interictal assessment of global oxidative imbalance together with copeptin and affective symptom adjustment.

Among the evaluated biomarkers, OSI showed the largest effect size and the strongest between-group separation for migraine status. Moreover, OSI remained independently associated with migraine after adjustment for age, sex, smoking status, depressive symptoms, and anxiety

symptoms. This pattern supports the concept that oxidative imbalance may represent a relevant biological correlate of migraine, rather than merely reflecting coexisting depressive or anxiety symptom burden. As an index derived from the balance between oxidant burden and antioxidant defense capacity, OSI may better reflect overall redox balance in migraine. This may explain why OSI showed stronger associations with migraine status than TAS or TOS individually. Previous studies have reported elevated OSI levels or broader disturbances in oxidant-antioxidant balance in migraine cohorts, although the magnitude and consistency of these findings have varied across study populations and migraine phases [6, 7, 15, 16]. The persistence of this association after multivariable adjustment suggests that oxidative alterations are not fully explained by coexisting depressive or anxiety symptoms. Nevertheless, given the cross-sectional design and peripheral nature of the evaluated biomarkers, the observed associations should not be interpreted as evidence of causality or direct central nervous system oxidative activity.

In parallel with oxidative stress-related alterations, serum copeptin levels were elevated in patients with migraine. Copeptin, a stable surrogate marker of arginine vasopressin activity, may reflect activation of hypothalamic and stress-response pathways [8, 17]. Previous studies have reported increased copeptin levels in non-traumatic headache cohorts and migraine-related conditions, although its diagnostic value appears limited [9, 10, 18]. In pediatric migraine, copeptin has also been evaluated together with TAS, TOS, and OSI, supporting a possible link between vasopressinergic stress responses and oxidative imbalance [12]. The present findings extend this literature by showing that copeptin elevation is also detectable during the interictal period, when acute attack-related biological fluctuations are minimized. Importantly, copeptin retained its association with migraine status after adjustment for demographic and affective variables. This finding suggests that copeptin may capture a stress-related neuroendocrine component of the interictal migraine phenotype that is not fully explained by depressive or anxiety symptom burden. However, the moderate individual performance of copeptin indicates that it is unlikely to serve as a standalone clinical biomarker.

Although copeptin showed only moderate discrimination individually, the combined OSI-copeptin model yielded the highest AUC among the evaluated models. This finding is consistent with the broader view that multimarker approaches may be more informative than single biomarkers in biologically heterogeneous disorders such as migraine [19]. In this context, the combined model is consistent with partly distinct contributions of oxidative and neuroendocrine stress-related pathways. Nevertheless, the observed classification performance should be interpreted cautiously because diagnostic accuracy estimates derived from case-control biomarker studies may overestimate discrimination compared with real-world clinical settings [20]. The applicability of such biomarker combinations remains uncertain without external validation and longitudinal assessment [21]. For this reason, the combined OSI-copeptin model should be viewed as a signal for future model development rather than as a clinically applicable diagnostic tool.

Interestingly, no significant biomarker differences were identified between episodic and chronic migraine after correction for multiple testing. Similarly, no statistically significant differences in oxidative stress markers or copeptin levels were observed between patients with and without aura. Previous studies examining oxidative stress parameters across migraine subgroups have yielded inconsistent results, with some reports suggesting greater oxidative imbalance in chronic or aura-associated migraine, whereas others failed to demonstrate clear subgroup-specific differences [7, 16, 22]. Such variability may partly reflect differences in sample size, migraine phase, medication exposure, and biomarker methodology across studies. In the present cohort, the absence of significant differences between episodic and chronic migraine after correction for multiple testing suggests that the observed biomarker alterations may not be restricted to a specific migraine subtype. However, the relatively limited subgroup sample sizes, particularly in aura-related analyses, may have reduced statistical sensitivity for detecting subtle biological differences.

After adjustment for BDI and BAI and FDR correction, significant correlations were confined to TAS-OSI and TOS-OSI (Table 3). Because OSI is calculated from TAS and TOS, mathematical coupling limits the interpretation of these associations as independent biological evidence. Neither copeptin nor oxidative stress parameters were significantly associated with migraine-related disability or pain severity in unadjusted or adjusted analyses (Tables 3 and S3). Thus, the between-group biomarker differences were not accompanied by detectable associations with clinical severity within the migraine cohort. Previous studies have reported inconsistent relationships between oxidative stress parameters and migraine severity [4, 7, 23]. However, the modest sample size limits precision, and these nonsignificant findings do not exclude clinically relevant associations.

A strength of this study is the simultaneous assessment of copeptin and oxidative stress markers under interictal sampling conditions, together with adjustment for depressive and anxiety symptoms in multivariable and partial correlation analyses. The use of effect sizes, false discovery rate correction, multivariable models, and sensitivity analyses also reduced the likelihood of overinterpreting exploratory findings. The combined use of correlation, regression, and ROC-based analyses provided complementary statistical perspectives on the investigated biomarkers rather than relying solely on between-group comparisons. Nevertheless, the results should still be considered exploratory until confirmed in larger prospective and multicenter cohorts.

Several limitations should be considered when interpreting these findings. First, the sample size was modest and does not allow definitive biomarker validation. Although the sample size was adequate to detect between-group differences in the principal oxidative stress measure, the study may have been underpowered for multiple biomarker comparisons, subgroup analyses, and multivariable prediction modelling. The single-center and cross-sectional design also precludes causal inference and may limit generalizability. Biomarker measurements were obtained only during the interictal period; therefore, temporal changes across preictal, ictal, and postictal phases could not be evaluated. The inclusion of both

episodic and chronic migraine patients may have introduced clinical heterogeneity related to attack frequency, disability, central sensitization, and affective symptom burden, although exploratory subgroup analyses were performed. In addition, although the multivariable models were prespecified and supported by acceptable collinearity and goodness-of-fit diagnostics, the relatively small sample size may still have increased the risk of model overfitting, particularly for the combined biomarker model. Peripheral serum biomarkers should also be interpreted as indirect systemic correlates rather than direct measures of central nervous system processes. Finally, despite adjustment for depressive and anxiety symptoms and sensitivity analysis for educational level, residual confounding related to lifestyle, sleep, dietary, metabolic, hormonal, or other unmeasured factors cannot be fully excluded. External validation in larger prospective and multicenter cohorts is required before clinical applicability can be inferred.

In conclusion, this exploratory matched case–control study showed that interictal migraine was associated with concurrent oxidative imbalance and elevated serum copeptin levels. OSI and copeptin remained associated with migraine status after adjustment for demographic and affective variables, suggesting that global redox dysregulation and stress-related neuroendocrine activation may coexist in the interictal migraine state. However, the clinical applicability of these peripheral biomarkers remains uncertain, and validation in larger, prospective, and multicenter cohorts is required before they can be considered for clinical use.

Declarations

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Ethics Approval

This study was performed in accordance with the principles of the Declaration of Helsinki and its later amendments. The study protocol was approved by the Ethics Committee of Tekirdağ Namık Kemal University Faculty of Medicine (30 December 2025; protocol no. 2025.247.12.16).

Consent to Participate

Written informed consent was obtained from all participants before enrollment.

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Author Contribution

E.D.: Conceptualization; investigation; writing – original draft; writing – review and editing; visualization; validation; methodology; formal analysis; resources; data curation; supervision.A.A.G.: Conceptualization; methodology; supervision; writing – review and editing.Z.M.Y.K.: Investigation; resources.A.Y.: Investigation; resources.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

References

Of 259 potential migraine cases and 76 potential healthy controls screened, 43 participants in each group were included in the final analysis (Fig. 1).

No statistically significant between-group differences were observed in age, sex, BMI, or smoking status, whereas educational level differed (p=0.002). BDI and BAI scores were higher in the migraine group (both p<0.001; Table 1).

Table 1. Demographic and clinical characteristics of the study population

Variable	Migraine (n=43)	Control (n=43)	p	Effect size
Age, years	32 (29–33)	30 (26–34)	0.083	0.133
Female sex, n (%)	31 (72.1)	31 (72.1)	1.000	—
BMI, kg/m ²	24.1 (22.1–26.4)	23.9 (22.0–26.0)	0.589	0.058
Smoking, n (%)	21 (48.8)	13 (30.2)	0.078	—
Education level, n (%)			0.002	0.378 ^a
• Primary school	1 (2.3)	0 (0)		
• Middle/high school	12 (27.9)	1 (2.3)		
• Higher education	30 (69.8)	42 (97.7)		
BDI score	14 (8–19)	8 (5–12)	<0.001	0.386
BAI score	15 (9–24)	9 (3–14)	<0.001	0.411
VAS score	9 (8–10)	—	—	—
Headache days/month	10 (5–15)	—	—	—
Disease duration, years	12 (8–16)	—	—	—
MIDAS grade	3 (2–4)	—	—	—
Episodic migraine, n (%)	24 (55.8)	—	—	—
Chronic migraine, n (%)	19 (44.2)	—	—	—

Data are presented as median (Q1–Q3) or n (%), as appropriate. Continuous variables were compared using the Mann–Whitney U test, and categorical variables were compared using the chi-square or Fisher’s exact test, as appropriate. Effect sizes are presented as r values for continuous variables. ^a Cramér’s V is reported for education level. BMI: body mass index; MIDAS: Migraine Disability Assessment Scale; VAS: Visual Analog Scale; BDI: Beck Depression Inventory; BAI: Beck Anxiety Inventory.

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Table 2. Oxidative stress markers and copeptin levels

Biomarker	Migraine (n=43)	Control (n=43)	p	Effect size (r)
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TOS, $\mu\text{mol H}_2\text{O}_2$ Eqv/L	13.06 (10.03–22.69)	8.78 (7.71–11.81)	<0.001*	0.447
OSI	1.78 (1.12–3.15)	0.79 (0.56–1.19)	<0.001*	0.612
Copeptin, ng/mL	12.20 (5.48–15.80)	5.29 (2.91–10.36)	<0.001*	0.396

Data are presented as median (Q1–Q3). Between-group comparisons were performed using the Mann–Whitney U test. Effect sizes are reported as r values. * $p < 0.05$. TAS: total antioxidant status; TOS: total oxidant status; OSI: oxidative stress index.

After adjustment for BDI and BAI, TAS correlated inversely with OSI (partial $r = -0.514$, $p = 0.001$), whereas TOS correlated positively with OSI (partial $r = 0.791$, $p < 0.001$). Only these correlations remained significant after FDR correction (Table 3).

Table 3. Partial correlation analyses within the migraine group adjusted for BDI and BAI

Variables	Partial r	p	FDR-significant
TAS–TOS	0.004	0.978	No
TAS–OSI	–0.514	0.001*	Yes
TAS–MIDAS grade	–0.044	0.787	No
TAS–Copeptin	0.154	0.335	No
TAS–VAS	0.027	0.869	No
TOS–OSI	0.791	<0.001*	Yes
TOS–MIDAS grade	–0.095	0.556	No
TOS–Copeptin	–0.179	0.262	No
TOS–VAS	0.122	0.448	No
OSI–MIDAS grade	–0.145	0.364	No
OSI–Copeptin	–0.153	0.338	No
OSI–VAS	–0.030	0.853	No
MIDAS grade–Copeptin	0.064	0.692	No
MIDAS grade–VAS	0.157	0.328	No
Copeptin–VAS	–0.250	0.115	No

Partial correlations were adjusted for BDI and BAI scores. Benjamini–Hochberg false discovery rate (FDR) correction was applied for multiple testing. Correlation analyses involving MIDAS were performed only within the migraine group. *p<0.05.

Variance inflation factors were acceptable for all variables. In Model 1, only BAI was associated with migraine status. Both OSI (OR 10.96, 95% CI 2.76–43.53) and copeptin (OR 1.23, 95% CI 1.08–1.40) remained associated with migraine status in Model 4 (Table 4; Fig. S2). This model had the highest Nagelkerke R² (0.706) and classification accuracy (82.6%) among the evaluated models. The Hosmer–Lemeshow test did not indicate lack of fit (p=0.978). Additional adjustment for educational level did not change the direction or statistical significance of either biomarker association.

Table 4. Hierarchical multivariable logistic regression models for migraine status

Variable	Model 1		Model 2		Model 3		Model 4	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Sex	1.42 (0.45–4.54)	0.550	1.74 (0.42–7.22)	0.445	1.65 (0.46–5.93)	0.443	1.93 (0.38–9.71)	0.424
Smoking	2.53 (0.91–7.04)	0.075	3.37 (0.89–12.70)	0.073	2.67 (0.88–8.05)	0.082	4.22 (0.94–18.92)	0.060
Age	1.06 (0.94–1.19)	0.339	1.05 (0.92–1.20)	0.447	1.07 (0.95–1.21)	0.251	1.08 (0.93–1.26)	0.333
BDI	1.05 (0.95–1.16)	0.354	1.01 (0.90–1.15)	0.835	1.07 (0.96–1.18)	0.246	1.02 (0.89–1.17)	0.761
BAI	1.10 (1.02–1.19)	0.017*	1.10 (1.01–1.20)	0.036*	1.10 (1.01–1.19)	0.024*	1.12 (1.01–1.24)	0.032*
OSI	—	—	10.10 (2.74–37.20)	<0.001*	—	—	10.96 (2.76–43.53)	<0.001*
Copeptin	—	—	—	—	1.18 (1.06–1.30)	0.002*	1.23 (1.08–1.40)	0.002*

Model performance metrics

Model	Nagelkerke R ²	Hosmer–Lemeshow p	Accuracy (%)
Model 1	0.319	0.538	69.8
Model 2	0.612	0.961	80.2
Model 3	0.446	0.935	75.6
Model 4	0.706	0.978	82.6

Model 1 included age, sex, smoking status, BDI, and BAI. OSI was added in Model 2, copeptin was added in Model 3, and both OSI and copeptin were included in Model 4. *p<0.05. OR: odds ratio; CI: confidence interval; BDI: Beck Depression Inventory; BAI: Beck Anxiety Inventory; OSI: oxidative stress index.

AUCs were 0.856 for OSI, 0.730 for copeptin, and 0.937 for the combined model, which was developed and evaluated in the same dataset (Table 5). In exploratory subgroup analyses, TAS, TOS, OSI, and copeptin differed between controls and both episodic and chronic migraine groups, whereas no significant differences were observed between the migraine subgroups after Bonferroni correction (Table S1). No significant biomarker differences were observed by aura status (Table S2). Unadjusted Spearman correlations are reported in Table S3.

Table 5. ROC curve analyses for migraine status

Marker/model	AUC	95% CI	p	Best cutoff	Sensitivity (%)	Specificity (%)
OSI	0.856	0.780–0.931	<0.001*	1.05	83.7	67.4
Copeptin	0.730	0.623–0.838	<0.001*	7.26	67.4	67.4
Combined OSI and copeptin model	0.937	0.891–0.983	<0.001*	0.40	90.7	81.4

Receiver operating characteristic (ROC) curve analyses were performed to evaluate the discriminatory performance of oxidative stress index (OSI), copeptin, and the combined OSI-copeptin model for migraine status. Optimal cutoff values were determined using the Youden index. The combined OSI–copeptin model was derived and evaluated in the same dataset; therefore, its performance should be interpreted as exploratory. *p<0.05. AUC: area under the curve; CI: confidence interval.

Figures

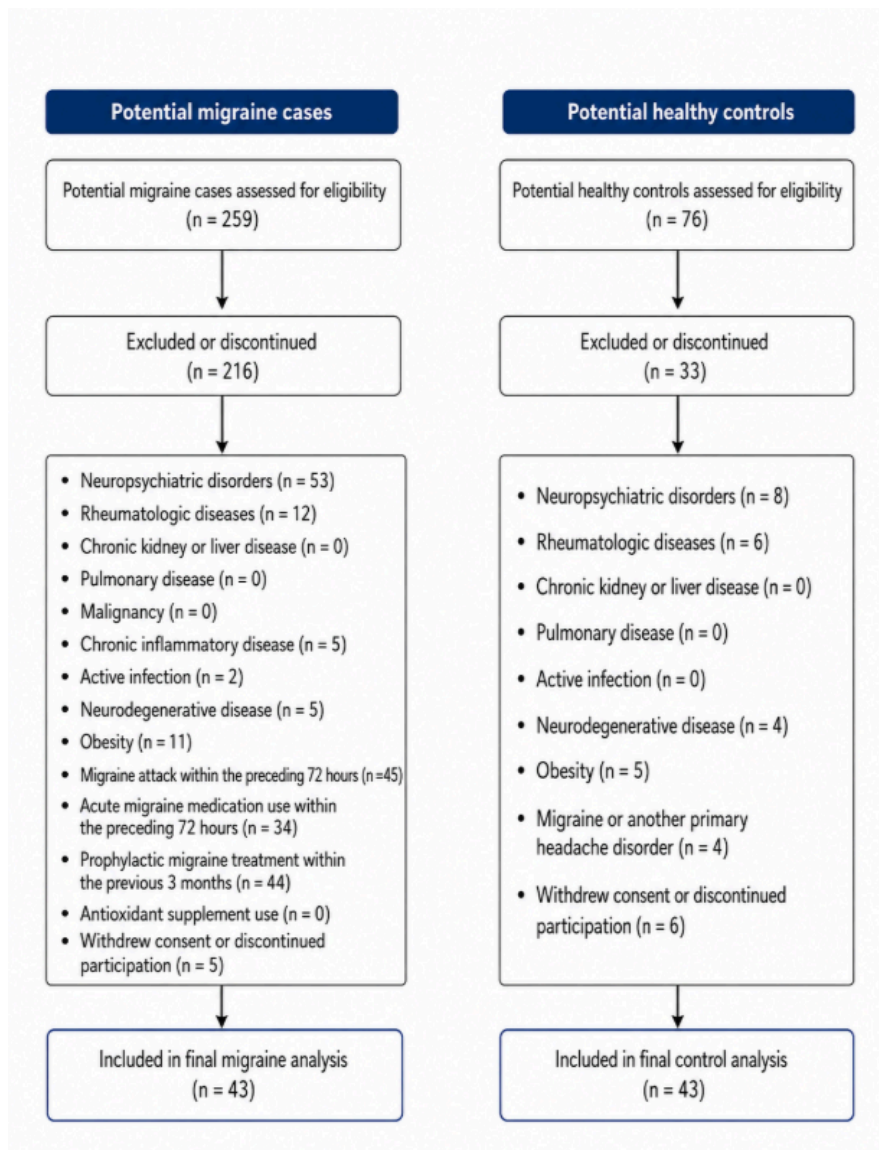


Figure 1

Flow diagram showing screening, exclusions, discontinuations, and final inclusion of migraine cases and healthy controls. The primary reason for exclusion was recorded for each participant to avoid double counting.

Supplementary Files

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