ONLINE JOURNAL OF BIOLOGICAL SCIENCES

RESEARCH ARTICLE

Investigation of Biochemical and Hormonal Parameters in Male Patients with Migraine

Dua'a AlHassoon[1], Omar Atrooz[2]*, Husni Farah[1]

[1]Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, Al-Ahliyya Amman University, Amman 19328, Jordan
[2]Department of Biological Sciences, Faculty of Science, Mutah University, Mutah, Jordan

*Corresponding Author: omihandd@gmail.com

Abstract: Migraine is a complex neurological disorder, including hormonal and biochemical imbalances. The physiological changes include neural variations due to genetic, environmental, and lifestyle factors. Therefore, the research aims to investigate total testosterone levels, Acetylcholine (Ach), prolactin, and clinical markers (calcium, magnesium, glucose, and lipid profile) across migraine patients. The research employed a case-control observational design conducted at Al-Ahliyya Amman University, Jordan. The research participants included randomly recruited 70 males, each divided into two groups: Migraine Patients (MPs) and healthy controls. The participants were screened for medical records and blood samples using ELISA (hormonal markers), photometric colorimetric methods (clinical markers), and Visual Analog Scale and Migraine Disability Assessment (migraine severity). The analysis showed elevated levels of total testosterone, prolactin, and calcium (10.91±3.76; 53.11±28.17; 12.39±0.77), respectively. Moreover, higher glucose (106.29±37.28), high-density lipoproteins (127.12±27.71), triglycerides (140.54±86.42), and total cholesterol (TC) (196.77±54.75) levels were found among migraine patients, as control to healthy individuals. In contrast, acetylcholine (86.69±37.6) and magnesium (3.6±1.67) levels were significantly lower among migraine patients. Negative correlations with migraine severity were found for acetylcholine ($r = -0.606$, $p<0.001$) and magnesium ($r = -0.801$, $p<0.001$), while positive correlations were observed for total testosterone ($r = 0.621$, $p<0.001$), prolactin ($r = 0.446$, $p = 0.007$), and calcium ($r = 0.495$, $p = 0.002$). Elevated levels of testosterone, prolactin, calcium, and lipid profile were strongly associated with migraine severity, while acetylcholine and magnesium showed negative association with migraine severity. These findings suggest that hormonal and neurochemical markers, particularly testosterone and acetylcholine, may play an important role in migraine pathophysiology and could serve as potential biomarkers for migraine severity in male patients.

Keywords: Acetylcholine, Migraine, Lipid Profile, Hormones, Calcium, Glucose

Received: 24-10-2025 | **Revised:** 09-03-2026 | **Accepted:** 20-04-2026 | **DOI:** 10.3844/ojbsci.2026.26.02.045

Introduction

Migraine is a complex neurological disorder characterized by recurrent headaches, often accompanied by nausea, photophobia, and phonophobia. Common subtypes include migraine with aura and migraine without aura, which differ in associated neurological symptoms [1]. The Global Burden of Diseases reported that migraines are the second most incapacitating and third most dominant disorder, potentially causing 16% prevalence among individuals. Moreover, migraine prevalence may depend on hormonal fluctuations among men and women [2]. Furthermore, several types of migraines are distinguished by their clinical characteristics and symptoms. These included neck pain, phono-phobia, nausea, photophobia, and vomiting [3].

The most prevalent varieties are migraine without aura (common migraine) and migraine with aura (classic migraine) [4]. In addition, neurological symptoms are related to headache termed as migraine with aura. These symptoms involve vision disturbances, sensory alterations, and speech disorders [5]. Contrarily, migraine without aura is frequently accompanied by light and sound sensitivity, nausea, and vomiting due to a lack of vitamin B12 [6]. However, hemiplegic migraine is a chronic condition that results in one-site paralysis of the body. The pathology of hemiplegic migraine, each month, which includes headaches for more than 15 days [7].

Furthermore, the International Headache Society (IHS) and several studies elaborated that numerous factors induce migraine, including age, genetic factors, eating habits, and physical and mental stress [8]. Likewise, [9] demonstrated that women frequently experience migraines due to high serum levels, particularly lipid profiles. Additionally, the biochemical parameters, including magnesium and vitamin B12, induce frequent headaches due to improper dietary lifestyles. However, vestibular migraines are associated with hormonal variations, leading to intermittent vertigo and headache [10] elaborated that hormonal imbalance causes lower levels of testosterone among men diagnosed with chronic migraine. In addition, hormone levels behave as core physiological aspects that provide numerous biological markers such as testosterone, acetylcholine, calcium, magnesium, and lipid profile [9, 12, 13].

The etiology of migraines has been linked to testosterone, a major androgen hormone, because of its impact on neurovascular function and pain perception [14]. The research of [15] highlighted that sex hormones significantly identify the risk of migraine levels. Besides, testosterone reduces the sensitivity of the trigeminovascular system, which induces migraine. Meanwhile, it modulates pain-processing pathways in the central nervous system to produce analgesia. The sex hormones, including testosterone, stimulate trigeminal neurons, resulting in the progression of migraine. In such cases, migraine severity significantly depends on lower testosterone levels [16].

Meanwhile, [17] emphasized that acetylcholine production stimulates the trigeminal nerve terminal and releases mast cells. Acetylcholine functions as a wave of neuronal inhibition and depolarization, which primarily induces headache and migraine aura. Furthermore, dysregulation of acetylcholine signaling may exacerbate pain pathway sensitivity, increasing the likelihood of migraines. Additionally, Acetylcholine influences cerebral blood flow and vascular tone, which are crucial in the neurovascular system of migraines [18]. Besides, [19] discussed that male migraineurs possess elevated levels of prolactin, indicating a linkage between hyperprolactinemia and migraine incidence.

Fewer studies focus on the relationship between migraines and lipid profiles. Research studies extensively highlight that high Cholesterol (Chol), Triglyceride (TG), Low-Density Lipoprotein (LDL), and High-Density Lipoprotein (HDL) are found among migraine patients [20]. Moreover, [21] elaborated that neurovascular variations are associated with migraines due to high levels of LDL and triglycerides. These lipid profiles induce migraine by regulating endothelial dysfunction and oxidative stress. Contrarily, [22] demonstrated that low HDL levels influence migraine by impairing the anti-inflammatory systems. However, [23] discussed that antimigraine drugs contain low efficacy due to cytochrome (CYP450) stimulation, hence, emphasizing on the need for effective treatment methods for migraine despite medications.

However, chemical biomarkers can potentially diagnose numerous diseases due to their biomechanical processes. Therefore, it is essential to explore novel biomarkers for their potential in diagnosing and treating migraines. Therefore, the research contributes to addressing existing knowledge gaps by systematically examining hormonal and biochemical parameters among male migraine patients, which is crucial to improving the efficacy of migraine treatments while reducing the impact of neurological conditions among men. The research objective is to investigate the levels of total testosterone, Acetylcholine (Ach), prolactin, and clinical markers (calcium, magnesium, glucose, and lipid profile) among male patients with migraine.

Materials and Methods

Study Design and Setting

This research adopted a case-control observational design to investigate factors, potentially associated with migraine among adult males. This design assists in comparing the health factors of migraine diagnosed patients and healthy individuals to identify potential risk factors or biomarkers.

Participants

The study included seventy (n = 70) male participants divided into two groups; thirty-five (n = 35) samples from adult males diagnosed with migraine (patient group), and thirty-five (n = 35) age-matched non-migraine control male adults (control group). The age of the participants was from 20 to 60 years old. Written informed consent was obtained from all participants before sample collection Participants were eligible for inclusion if they were adult males aged between 20 and 60 years who were willing to participate in the study and provide informed consent. Individuals in the patient group had a confirmed clinical diagnosis of migraine based on the International Headache Society criteria, whereas the control group consisted of age-matched healthy males with no history of migraine or chronic headache disorders.

Participants were excluded if they had medical conditions that could influence hormonal or biochemical parameters, including endocrine disorders such as pituitary disorders or hypogonadism, chronic systemic diseases such as diabetes mellitus, cardiovascular diseases, renal disorders, or neurological diseases other than migraine. Individuals currently receiving hormonal therapy, corticosteroids, or medications known to affect hormone or lipid metabolism were also excluded. In addition, participants with alcohol or substance abuse and those with incomplete clinical or laboratory data were not included in the analysis. All participants were subjected to detailed demographic and history-taking data including (age, educational level, marital status, smoking habits, diet habits, sleep habits, weight (in kg), height (in cm), BMI, and severity of migraines for the patients' group).The Statistical software application Epi-Info version 7 was used to determine the proportion of the sample. The number of participants was determined using the following formula:

$$S = Z^2 * P * \frac{(1 - P)}{M^2}$$

The calculated sample size was approximately 66.7, which was rounded up to 70 adult.

Migraine Severity Classification

Migraine severity among patients was typically assessed using Visual Analog Scale (VAS) and the Migraine Disability Assessment (MIDAS), self-reported. The severity of migraines was categorized into three distinct levels: mild, moderate, and severe [24].

Laboratory Measurements

Total testosterone was determined using the AccuBind® ELISA microwell system. Ache was determined using the Human ACH (Ache) ELISA Kit. Prolactin was measured according to a procedure described previously [25]. Acetylcholine was measured using the Human ACH ELISA kit employing the competitive inhibition enzyme immunoassay [26].

Calcium levels were determined using the Biosystem CALCIUM-ARSENAZD method. Calcium kit (purchased from Human, Germany) based on a procedure previously described [27]. Magnesium levels were determined using a photometric colorimetric test incorporating a Lipid-Clearing Factor (LCF) [28]. Fasting blood glucose levels were determined using the Glucose Liquicolor GOD-PAP method, an enzymatic colorimetric test [29]. HDL, cholesterol, LDL, and Triglycerides (TG) were measured using the Human Star 300SR and 600 analyzers. Serum HDL levels were measured using the Human Star 300SR and 600 analyzers following the manufacturer's protocol. The observed HDL values in our cohort were higher than typical physiological ranges, which likely reflects differences in assay calibration, kit sensitivity, or population-specific variations. All measurements were verified by repeated testing to ensure accuracy. Each biomarker measurement was performed once per participant according to manufacturer protocols to avoid redundancy. BMI was calculated as weight (kg) divided by height (m^2) and classified as normal (18.5-24.9), overweight (25-29.9), and obese (≥30). Control participants were age-matched (±3 years) to migraine patients.

Ethical Considerations

Informed consents were obtained from participants that explained research procedure. Furthermore, the research followed the Faculty of Allied Medical Sciences guidelines at Al-Ahliyya Amman University (IRB: AAU/8/11/2023-2024). Moreover, the patient's medical records and personal information were ethically secured to maintain confidentiality.

Statistical Analysis

The data was analyzed using Statistical Package for the Social Sciences version 26.0 software (SPSS Inc., 233 South Wacker Drive, 11th Floor Chicago, IL, USA). The numerical data was assessed through mean and standard deviation (mean ± SD), with p-values, notifying significance/non-significance; $p > 0.05$ (non-significant) and $p < 0.05$ (significant). Potential confounding variables including age, BMI, smoking status, dietary habits, and sleep patterns were documented and evaluated descriptively to assess their potential influence on migraine occurrence.

Results

Sociodemographic Characteristics

The average age of MPs was slightly lower than that of control participants (33.51±8.87 years vs. 36.6±11.16 years). However, this difference was not statistically significant ($p = 0.67$). The majority of MPs were middle aged (25-45, 65.7%). The proportion of single and married individuals was comparable between the two groups, with no significant difference noted ($p = 0.63$) (Table 1).

MPs had significantly higher weight ($p = 0.025$), height ($p = 0.037$), and BMI ($p = 0.004$) compared to control participants. The BMI distribution indicated a higher prevalence of overweight and obese individuals in the migraine group. A significantly larger proportion of MPs were smokers compared to the control group (68.6% vs. 37.1%, $p = 0.008$). Non-vegetarian dietary habits were significantly more common among MPs, with a p-value of 0.031. MPs reported significantly shorter sleep durations (<6 hours/day) compared to control participants ($p<0.001$) (Table 1).

Severity of Migraine Among Control and Patients

Migraine severity varied, with the majority of patients experiencing severe migraines (85.7%). No control participants reported migraine symptoms, and the difference in severity was highly significant ($p<0.001$) (Table 2).

Table 1: Socio-demographic characteristics among control and migraine patients

Variable	Parameter	Control	MPs	Test	p-value
Age (years)	Mean ± SD	36.6 ± 11.16	33.51 ± 8.87	$\chi^2 = 0.78$	0.67
	<25	5 (14.3%)	6 (17.1%)		
	25-45	21 (60%)	23 (65.7%)		
	>45	9 (25.7%)	6 (17.1%)		
Marital status	Single	19 (54.3%)	17 (48.6%)	$\chi^2 = 0.229$	0.63
	Married	16 (45.7%)	18 (51.4%)		
Weight (kg)	Mean ± SD	73.46 ± 9.73	80.04 ± 13.85	F = 5.27	0.025*
Height (m)	Mean ± SD	1.69 ± 0.07	1.73 ± 0.07	F = 4.53	0.037*
BMI (kg/m^2)	Mean ± SD	25.47 ± 2.33	26.59 ± 3.69	$\chi^2 = 10.8$	0.004**
	Normal weight	21 (60%)	8 (22.9%)		
	Overweight	13 (37.1%)	22 (62.9%)		
	Obese	1 (2.9%)	5 (14.3%)		
Smoking	Non-smoker	22 (62.9%)	11 (31.4%)	$\chi^2 = 6.93$	0.008**
	Smoker	13 (37.1%)	24 (68.6%)		
Dietary habits	Vegetarians	10 (28.6%)	3 (8.6%)	$\chi^2 = 4.62$	0.031*
	Non-vegetarians	25 (71.4%)	32 (91.4%)		
Sleep habits	6-8 hours daily	31 (88.6%)	9 (25.7%)	$\chi^2 = 33.46$	<0.001**
	>8 hours daily	3 (8.6%)	2 (5.7%)		
	<6 hours daily	1 (2.9%)	24 (68.6%)		

Data is presented as means, standard deviations, numbers (frequency). Number (n) for control (n = 35) and for MPs (n = 35). χ2: chi-square test. F: The value of ANOVA one-way test. P-value: value of significance. *: p significant if $p<0.05$, **: p is highly significant if $p<0.01$

Table 2: Severity of migraines among control and migraines patients

Variable	Parameter	Control	MPs	χ^2 Test	p-value
Severity of migraines	No severity	35 (100%)	0 (0%)	70	<0.001**
	Mild	0 (0%)	4 (11.4%)		
	Moderate	0 (0%)	1 (2.9%)		
	Severe	0 (0%)	30 (85.7%)		

Data is presented as numbers (frequency). Number (n) for control (n=35) and for MPs (n=35. χ^2: chi square test. **: p is highly significant if p<0.01

The mean total testosterone level was significantly higher in MPs (10.91±3.76 ng/mL) compared to control participants (5.16±3.04 ng/mL, p<0.001). Furthermore, the proportion of patients with higher-than-normal total testosterone levels was markedly elevated in the migraine group (37.1%) compared to the control group (2.9%). MPs had lower mean Ache levels (86.69±37.6 pg/mL) than control participants (116.40±72.15 pg/mL, p = 0.034). The mean prolactin level was markedly higher in MPs (53.11±28.17 ng/mL) compared to the control group (25.08±10.03 ng/mL, p<0.001). MPs exhibited higher calcium levels (12.39±0.77 mg/dL) than control participants (11.02 ± 1.41 mg/dL, p<0.001).

The mean magnesium level was significantly lower in MPs (3.6±1.67 mg/dL) compared to control participants (4.51±0.26 mg/dL, p = 0.002). MPs had higher glucose levels (106.29±37.28 mg/dL) compared to the control group (91±8.51 mg/dL, p = 0.021). HDL levels were elevated in MPs (127.12±27.71 mg/dL) compared to control participants (114.4± .61 mg/dL, p = 0.012). The mean TG level was higher in MPs (140.54±86.42 mg/dL) compared to control participants (107.09±47.22 mg/dL, p = 0.048). CHO levels were significantly higher in MPs (196.77±54.75 mg/dL) compared to the control group (157.91±27.03 mg/dL, p<0.001) (Table 3).

Table 3: Laboratory profile among control and migraine patients

Variable	Parameter	Control (n = 35)	MPs (n = 35)	Test F-value	p-value
Total Testosterone (ng/ml)	Mean ± SD	5.16 ± 3.04	10.91 ± 3.76	49.40	<0.001**
	Normal	31 (88.6%)	21 (60%)		
	Higher	1 (2.9%)	13 (37.1%)		
	Lower	3 (8.6%)	1 (2.9%)		
Acetylcholine (pg/mL)	Mean ± SD	116.40 ± 72.15	86.69 ± 37.6	4.66	0.034*
Prolactin (ng/mL)	Mean ± SD	25.08 ± 10.03	53.11 ± 28.17	30.74	<0.001**
Calcium (mg/dl)	Mean ± SD	11.02 ± 1.41	12.39 ± 0.77	25.51	<0.001**
Magnesium (mg/dL)	Mean ± SD	4.51 ± 0.26	3.6 ± 1.67	10.21	0.002**
Glucose (mg/dL)	Mean ± SD	91 ± 8.51	106.29 ± 37.28	3.56	0.021*
HDL (mg/dL)	Mean ± SD	114.4 ± 8.61	127.12 ± 27.71	6.72	0.012*
TG (mg/dL)	Mean ± SD	107.09 ± 47.22	140.54 ± 86.42	1.61	0.048*
TC (mg/dL)	Mean ± SD	157.91 ± 27.03	196.77 ± 54.75	14.17	<0.001**

Data is presented as means, and standard deviations. Min: minimum, max: maximum. F: the value of ANOVA one-way test. P-value: value of significance. *: p significant if p<0.05, **: p is highly significant if p<0.01. HDL: high-density lipoprotein, TG: triglyceride, CHO: total cholesterol, TC

Correlation Between Laboratory tests and Severity of Migraine Among Migraine Patients

A positive significant correlation was observed between total testosterone levels and migraine severity (r = 0.621, p<0.001), suggesting that increased testosterone levels may be associated with increased migraine severity. A significant negative correlation was found between acetylcholine levels and migraine severity (r = -0.606, p<0.001), indicating that acetylcholine may play a role in modulating migraine intensity.

Prolactin levels showed a significant positive correlation with migraine severity (r = 0.446, p = 0.007), suggesting a potential link between elevated prolactin levels and migraine severity. A significant positive correlation was identified between calcium levels and migraine severity (r = 0.495, p = 0.002), indicating that higher calcium levels may contribute to more severe migraines. A very strong negative and highly significant correlation was observed between magnesium levels and migraine

severity (r = -0.801, p<0.001), highlighting the critical role of magnesium in migraine pathology. No significant correlation was found between glucose levels and migraine severity (r = 0.263, p = 0.126). No significant correlations were observed between HDL (r = 0.309, p = 0.071), TG (r = 0.331, p = 0.052), or TC (r = 0.297, p = 0.083) levels and migraine severity (Table 4).

Discussion

The current research evaluates biochemical components among migraine patients to determine the essential biomarkers. The study assessed BMI, total testosterone, acetylcholine, prolactin, calcium, magnesium, glucose, HDL, TG, and TC levels. The findings reveal a significant positive association between BMI and migraine among middle-aged patients. The current outcomes align with previous studies [11] within similar age distribution. This indicates the predominant effect of migraines on individuals in their certain middle ages due to neurological, hormonal, and lifestyle factors. Meanwhile, the BMI results suggest the high possibility of obesity among migraine patients, which is consistent with the research of [30]. Likewise, smoking is positively correlated with migraine severity. These results support the study of [31], hence revealing migraine prevalence among smokers.

Table 4: Correlation between laboratory tests and severity of migraine among MPs

Variable	Parameter	Severity of migraines
Total Testosterone	r-value	0.621
	p-value	<0.001**
Acetylcholine	r-value	-0.606
	p-value	<0.001**
Prolactin	r-value	0.446
	p-value	0.007**
Calcium	r-value	0.495
	p-value	0.002**
Magnesium	r-value	-0.801
	p-value	<0.001**
Glucose	r-value	0.263
	p-value	0.126
HDL	r-value	0.309
	p-value	0.071
TG	r-value	0.331
	p-value	0.052
TC	r-value	0.297
	p-value	0.083

* p<0.05, **: p<0.01. HDL: high-density lipoprotein, TG: triglyceride, CHO: total cholesterol, TC. R-value: the value of the correlation

The findings also demonstrate that migraine patients are more likely to follow non-vegetarian dietary patterns. However, no significant correlation was found between dietary habits and migraine severity, suggesting minimal influence among patients. Similarly, [32] reported that adherence to a healthy eating pattern decreases headache symptoms. Moreover, findings revealed that migraine severity is highly dependent on poor sleep patterns. These implications coincide with the research findings of [33]. Subsequently, [34] elaborated that sleep disturbances may predict migraine attacks through similar patterns. However, majority of migraine patients showcased higher-than-normal total testosterone levels, in comparison to healthy individuals. Contrarily, the research of [11] demonstrated that individuals experience cluster headaches due to testosterone deficiency and hypothalamic-pituitary axis suppression. This suppression may inhibit Gonadotropin-Releasing Hormone (GnRH) production, resulting in secondary hypogonadism [11]. Interestingly, the present study observed higher testosterone levels among migraine patients compared with controls. This finding differs from some previous studies reporting reduced testosterone levels in patients with chronic headache disorders. These discrepancies may be related to differences in study populations, migraine subtypes, hormonal regulation mechanisms, or methodological variations. Therefore, the role of testosterone in migraine pathophysiology remains complex and warrants further investigation.

Furthermore, the research findings report that migraine patients exhibited lower acetylcholine levels than control participants. The negative correlation of Acetylcholine

levels with the severity of migraine indicates its essential influence in the modulating intense headaches. These results reflect direct measures in reducing the vasodilatory response among migraine patients. These outcomes align with [35], highlighting the role of acetylcholine levels in migraine pathophysiology. Besides, elevated levels of prolactin indicate higher intensity of migraine among migraine patients, as compared to healthy individuals. The results coincide with [36] in accentuating prolactin's influence on migraine progression. Furthermore, the research emphasizes that higher levels of prolactin are observed among patients experiencing ictal Episodic Migraine (EM) and chronic migraine, than interictal EM and headache-free controls.

Likewise, [19] mentioned that prolactinomas increase migraine attacks and headaches. These findings emphasized that hyperprolactinemia may influence central headache by controlling hypothalamus. Contrarily, [37] mentioned that prolactin levels are not correlated with acute migraine attacks among migraine patients and healthy individuals. Furthermore, this study's finding indicated that migraine patients possess elevated levels of calcium in comparison to normal participants. This implies the positive association of migraine severity with varied calcium levels, as demonstrated by previous studies [38]. In contrast, [39] emphasized that reduced calcium levels stimulate migraine attacks and are linked to high migraine severity among patients. Stress-related changes in lipid profiles, including elevated cholesterol and triglycerides, may contribute to migraine susceptibility by promoting oxidative stress and endothelial dysfunction [40].

Subsequently, the findings of the current research reveal reduced levels of magnesium, thus negatively associating with migraine severity among patients. Magnesium deficiency may contribute to migraine pathophysiology and could represent a potential biomarker associated with migraine severity. Moreover, it may be the potential therapeutic marker of magnesium supplementation for migraine management. Our findings show significantly lower magnesium levels among migraine patients, linking magnesium deficiency to neuronal hyperexcitability and vascular dysfunction. These results suggest that magnesium supplementation may serve as a potential therapeutic strategy to reduce migraine frequency and severity However, migraine intensity indicates no significant relationship with glucose, HDL, and cholesterol levels. These findings align with the findings of previous research studies, emphasizing on low magnesium levels and high lipid profiles among migraine patients [39, 41, 42]. In contrast, previous research studies highlight that migraine patients possess higher LDL-C, Cholesterol, and TG levels, in comparison to healthy individuals [21]. vitamin B12 deficiency can elevate cholesterol and triglycerides, [43] contributing to vascular dysfunction and migraine susceptibility. Measuring B12 may help identify metabolic imbalances relevant to migraine management." Although migraine patients demonstrated higher lipid parameters in the present study, no significant correlation was observed between lipid levels and migraine severity. This suggests that lipid metabolism may influence migraine susceptibility rather than the intensity of attacks. Larger studies are required to clarify this relationship.

This suggests that, although the current study does not extensively identify an association with severity, however, there may be a relationship between lipid levels and the frequency of migraine episodes. The current research findings provide significant implications regarding the influence of biochemical parameters on migraine severity. Moreover, the results of the current research contribute to addressing the role of the essential parameters in diagnosing migraine severity among patients. In addition, the research outcomes direct future research to assessing biochemical parameters as biomarkers within clinical procedures.

Although demographic and lifestyle variables such as BMI, smoking status, dietary habits, and sleep patterns were collected, the case-control design limits the ability to fully control for all potential confounding factors. Future studies using larger cohorts and multivariate regression models are recommended to better adjust for these variables.

This study has several limitations that should be acknowledged. First, the relatively small sample size may limit the generalizability of the findings. Second, the case-control design allows identification of associations but does not establish causal relationships. Third, hormonal and biochemical measurements were obtained at a single time point, which may not reflect temporal variations. Finally, although several demographic and lifestyle variables were recorded, residual confounding cannot be entirely excluded. Future large-scale longitudinal studies are required to confirm these findings and further investigate the potential role of biochemical biomarkers in migraine pathophysiology.

Conclusion

Our data sheds important light on the hormonal and metabolic changes that occur in male migraineurs. Comparing the results to non-migraine controls, several biomarkers show notable variations. Prolactin and testosterone, two hormone indicators, displayed clear trends, with testosterone levels positively correlated with migraine intensity, potentially suggesting

its preventive function. Acetylcholine showed a noteworthy association, indicating its relevance in pain modulation and parasympathetic activity in migraines. Conversely, there was a complex association between high prolactin and migraine neuroendocrine alterations and stress. Lower magnesium levels were seen in migraineurs, suggesting its participation in migraine pathophysiology since magnesium deficiency has been linked to neuronal hyperexcitability. Increased calcium levels in MPs may suggest that calcium influences neurotransmitter release and vascular tone, which may be factors in migraine onset. Elevated levels of glucose, CHO, TGs, and HDL in migraineurs are indicative of possible metabolic problems associated with migraine frequency. These changes may exacerbate migraines by promoting oxidative stress and inflammatory pathways. These findings suggest that testosterone, acetylcholine, prolactin, and magnesium may serve as important biomarkers for migraine severity in male patients, offering potential targets for personalized diagnosis and management.

Acknowledgment

The authors express their gratitude to all the affiliated staff members who made any kind of contribution to this research.

Funding Information

Authors have not received any financial support or funding to report.

Author's Statement

The authors state that this manuscript is entirely original and has not been previously published or submitted for publication elsewhere. All authors have given their consent for the article to be published and assert that they have no conflicts of interest. All data are included within the article itself.

Authors' Contributions

Omar Atrooz and Husni Farah: Supervised the work, analyzed the statistical analysis, and revised the paper.
Dua'a ALHassoon: Performed all the experiments and collected the data. All authors critically reviewed and approved the final version of the manuscript.

Ethics

Informed consents were obtained from participants that explained research procedure. Furthermore, the research followed the Faculty of Allied Medical Sciences guidelines at Al-Ahliyya Amman University (IRB: AAU/8/11/2023-2024). Moreover, the patient's medical records and personal information were ethically secured to maintain confidentiality.

The article is original. The corresponding author confirms that all authors read and approved the manuscript and no ethical issues involved.

References

1. Dogru, M. T., Dilekoz, E., Alpua, M., Eroglu, O., Kandemir, H., Alp, C., & Bolay, H. (2019). Endothelial and Autonomic Functions in Patients with Migraine. *Pain Medicine*, *21*(2), e222-e231. https://doi.org/10.1093/pm/pnz223
2. Raffaelli, B., Storch, E., Overeem, L. H., Terhart, M., Fitzek, M. P., Lange, K. S., & Reuter, U. (2023). Sex Hormones and Calcitonin Gene-Related Peptide in Women With Migraine. *Neurology*, *100*(17), e1825. https://doi.org/10.1212/wnl.0000000000207114
3. Dermitzakis, E. V., Argyriou, A. A., Bilias, K., Barmpa, E., Liapi, S., Rikos, D., Xiromerisiou, G., Soldatos, P., & Vikelis, M. (2024). Results of a Web-Based Survey on 2565 Greek Migraine Patients in 2023: Demographic Data, Imposed Burden and Satisfaction to Acute and Prophylactic Treatments in the Era of New Treatment Options. *Journal of Clinical Medicine*, *13*(10), 2768. https://doi.org/10.3390/jcm13102768
4. Khan, J., Asoom, L. I. A., Sunni, A. A., Rafique, N., Latif, R., Saif, S. A., Almandil, N. B., Almohazey, D., AbdulAzeez, S., & Borgio, J. F. (2021). Genetics, pathophysiology, diagnosis, treatment, management, and prevention of migraine. *Biomedicine & Pharmacotherapy*, *139*, 111557. https://doi.org/10.1016/j.biopha.2021.111557

5. Scutelnic, A., Kreis, L. A., Beyeler, M., Heldner, M. R., Meinel, T. R., Kaesmacher, J., Hakim, A., Arnold, M., Fischer, U., Mattle, H. P., Schankin, C. J., & Jung, S. (2022). Migraine aura-like symptoms at onset of stroke and stroke-like symptoms in migraine with aura. *Frontiers in Neurology*, *13*, 1004058. https://doi.org/10.3389/fneur.2022.1004058
6. Zheng, H., Shi, Y.-Z., Liang, J.-T., Lu, L.-L., & Chen, M. (2023). Modifiable factors for migraine prophylaxis: A mendelian randomization analysis. *Frontiers in Pharmacology*, *14*, 1010996. https://doi.org/10.3389/fphar.2023.1010996
7. Aguilar-Shea, A. L., Membrilla MD, J. A., & Diaz-de-Teran, J. (2021). Migraine review for general practice. *Atención Primaria*, *54*(2), 102208.
8. Buse, D. C., Reed, M. L., Fanning, K. M., Bostic, R., Dodick, D. W., Schwedt, T. J., Munjal, S., Singh, P., & Lipton, R. B. (2020). Comorbid and co-occurring conditions in migraine and associated risk of increasing headache pain intensity and headache frequency: results of the migraine in America symptoms and treatment (MAST) study. *The Journal of Headache and Pain*, *21*(1), 23. https://doi.org/10.1186/s10194-020-1084-y
9. Alshehri, S. A., Alshubaily, F. A., Alzubidi, A. A., Sonbol, H. S., Rahimuddin, S. A., & Jambi, E. J. (2019). The correlation between migraine and some biochemical parameters in Saudi females. *Journal of Biochemistry Technology*, *10*(1), 67-71.
10. Tang, B., Yu, X., Jiang, W., Zhang, C., Zhan, T., & He, Y. (2021). Clinical significance of serum sex hormones in postmenopausal women with vestibular migraine: potential role of estradiol. *Journal of International Medical Research*, *49*(5), 03000605211016379. https://doi.org/10.1177/03000605211016379
11. Shields, L. B. E., Seifert, T., Shelton, B. J., & Plato, B. (2019). Testosterone levels in men with chronic migraine. *Neurology International*, *11*(2), 8079.
12. Liu, Q., Liu, C., Jiang, L., Li, M., Long, T., He, W., Qin, G., Chen, L., & Zhou, J. (2018). Nicotinic acetylcholine receptor-mediated anti-inflammatory effect in a chronic migraine rat model via the attenuation of glial cell activation. *Journal of Pain Research*, *11*, 1129-1140. https://doi.org/10.2147/jpr.s159146
13. Dolati, S., Rikhtegar, R., Mehdizadeh, A., & Yousefi, M. (2019). The Role of Magnesium in Pathophysiology and Migraine Treatment. *Biological Trace Element Research*, *196*(2), 375-383. https://doi.org/10.1007/s12011-019-01931-z
14. Godley, F., Meitzen, J., Nahman-Averbuch, H., O'Neal, M. A., Yeomans, D., Santoro, N., Riggins, N., & Edvinsson, L. (2024). How Sex Hormones Affect Migraine: An Interdisciplinary Preclinical Research Panel Review. *Journal of Personalized Medicine*, *14*(2), 184. https://doi.org/10.3390/jpm14020184
15. Vetvik, K. G., & MacGregor, E. A. (2016). Sex differences in the epidemiology, clinical features, and pathophysiology of migraine. *The Lancet Neurology*, *16*(1), 76-87. https://doi.org/10.1016/s1474-4422(16)30293-9
16. Rossi, M. F., Tumminello, A., Marconi, M., Gualano, M. R., Santoro, P. E., Malorni, W., & Moscato, U. (2022). Sex and gender differences in migraines: a narrative review. *Neurological Sciences*, *43*(9), 5729-5734. https://doi.org/10.1007/s10072-022-06178-6
17. Shelukhina, I., Mikhailov, N., Abushik, P., Nurullin, L., Nikolsky, E. E., & Giniatullin, R. (2017). Cholinergic Nociceptive Mechanisms in Rat Meninges and Trigeminal Ganglia: Potential Implications for Migraine Pain. *Frontiers in Neurology*, *8*, 163. https://doi.org/10.3389/fneur.2017.00163
18. Kilinc, E., Torun, I. E., & Baranoglu Kilinc, Y. (2024). Meningeal mast cell-mediated mechanisms of cholinergic system modulation in neurogenic inflammation underlying the pathophysiology of migraine. *European Journal of Neuroscience*, *59*(9), 2181-2192. https://doi.org/10.1111/ejn.15888
19. Al-Karagholi, M. A.-M., Kalatharan, V., Ghanizada, H., Gram, C., Dussor, G., & Ashina, M. (2023). Prolactin in headache and migraine: A systematic review of clinical studies. *Cephalalgia*, *43*(2), 3331024221136286. https://doi.org/10.1177/03331024221136286
20. Uygur-Kucukseymen, E., & Akca, G. (2022). Serum lipid profile in migraine and its association with clinical characteristics. *Neurological Research*, *45*(1), 57-61. https://doi.org/10.1080/01616412.2022.2119723
21. Liampas, I., Mylonas, K. S., Brotis, A., Dervenis, P., Siokas, V., Mentis, A. A., Dastamani, M., Aloizou, A., Tsouris, Z., Aslanidou, P., Bakoyiannis, C., & Dardiotis, E. (2021). Serum lipid abnormalities in migraine: A meta-analysis of observational studies. *Headache: The Journal of Head and Face Pain*, *61*(1), 44-59. https://doi.org/10.1111/head.14039
22. Ge, W., Gao, L., Zhang, Y., Wu, K., Chen, N., & He, L. (2021). Association Between Serum Lipid Levels and Severe Headache or Migraine in Representative American Population: A Cross-sectional Study. *Current Neurovascular Research*, *18*(3), 333-342. https://doi.org/10.2174/1567202618666210923145635
23. Bruijn, N., van Lohuizen, R., Boron, M., Fitzek, M., Gabriele, F., Giuliani, G., Melgarejo, L., Řehulka, P., Sebastianelli, G., Triller, P., Vigneri, S., Özcan, B., & van den Brink, A. M. (2024). Influence of metabolic state and body composition on the action of pharmacological treatment of migraine. *The Journal of Headache and Pain*, *25*(1), 20. https://doi.org/10.1186/s10194-024-01724-3
24. Olesen, J., & Steine, T. J. (2004). The international classification of headache disorders, 2nd edn (ICDH-II). *Journal of Neurology, Neurosurgery & Psychiatry*, *75*(6), 808-811. https://doi.org/10.1136/jnnp.2003.031286
25. Saleem, M., Martin, H., & Coates, P. (2018). Prolactin biology and laboratory measurement: An update on physiology and current analytical issues. *Clinical Biochemist Reviews*, *39*(1), 3-16.
26. Gu, X., & Wang, X. (2021). An overview of recent analysis and detection of acetylcholine. *Analytical Biochemistry*, *632*, 114381. https://doi.org/10.1016/j.ab.2021.114381
27. Beto, J. A. (2015). The role of calcium in human aging. *Clinical Nutrition Research*, *4*(1), 1-8. https://doi.org/10.7762/cnr.2015.4.1.1
28. Jahnen-Dechent, W., & Ketteler, M. (2012). Magnesium basics. *Clinical Kidney Journal*, *5*(Suppl 1), i3-i14. https://doi.org/10.1093/ndtplus/sfr163
29. Güemes, M., Rahman, S. A., & Hussain, K. (2015). What is a normal blood glucose? *Archives of Disease in Childhood*, *101*(6), 569-574. https://doi.org/10.1136/archdischild-2015-308336
30. Huang, Q., Liang, X., Wang, S., & Mu, X. (2018). Association between Body Mass Index and Migraine: A Survey of Adult Population in China. *Behavioural Neurology*, *2018*, 1-6. https://doi.org/10.1155/2018/6585734
31. Weinberger, A. H., & Seng, E. K. (2023). The Relationship of Tobacco Use and Migraine: A Narrative Review. *Current Pain and Headache Reports*, *27*(4), 39-47. https://doi.org/10.1007/s11916-023-01103-8

32. Togha, M., Nematgorgani, S., Khorsha, F., Mirzaei, K., Mirzababaei, A., Ghorbani, Z., Yekaninejad, M. S., & Okhovat, A. A. (2021). The Relationship Between Major Dietary Patterns and Disease Severity Among Migraine Patients. *Archives of Neuroscience*, *8*(2), e102414. https://doi.org/10.5812/ans.102414
33. Yoo, A., Vgontzas, A., Chung, J., Mostofsky, E., Li, W., Rueschman, M., Buysse, D., Mittleman, M., & Bertisch, S. (2023). The association between multidimensional sleep health and migraine burden among patients with episodic migraine. *Journal of Clinical Sleep Medicine*, *19*(2), 309-317. https://doi.org/10.5664/jcsm.10320
34. Stanyer, E. C., Brookes, J., Pang, J. R., Urani, A., Holland, P. R., & Hoffmann, J. (2023). Investigating the relationship between sleep and migraine in a global sample: a Bayesian cross-sectional approach. *The Journal of Headache and Pain*, *24*(1), 123. https://doi.org/10.1186/s10194-023-01638-6
35. Körtési, T., Nagy-Grócz, G., & Vécsei, L. (2024). The role of kynurenines in migraine-related neuroimmune pathways. *The Journal of Headache and Pain*, *25*(1), 129. https://doi.org/10.1186/s10194-024-01833-z
36. Togha, M., Nematgorgani, S., Ghorbani, Z., Rafiee, P., & Haghighi, S. (2023). Increased serum prolactin level may indicate more migraine attack frequency. *Brain and Behavior*, *13*(7), e3063. https://doi.org/10.1002/brb3.3063
37. Guldiken, S., Guldiken, B., Demir, M., Kabayel, L., Ozkan, H., Turgut, N., Hunkar, R., & Kat, S. (2011). Soluble CD40 ligand and prolactin levels in migraine patients during interictal period. *The Journal of Headache and Pain*, *12*(3), 355-360. https://doi.org/10.1007/s10194-011-0306-8
38. Yin, P., Anttila, V., Siewert, K. M., Palotie, A., Davey Smith, G., & Voight, B. F. (2016). Serum calcium and risk of migraine: a Mendelian randomization study. *Human Molecular Genetics*, *26*(4), 820-828. https://doi.org/10.1093/hmg/ddw416
39. Khan, M. J., Haq Nawaz, K., khan, S., Ahmed, F., Saleem, M. I., Mahboob, F., & Habib, M. F. (2024). Serum Vitamin D, Calcium, and Magnesium Levels in Patients with Migraine Presenting to PEMH, Pakistan: A Case-Control Study. *Journal of Health and Rehabilitation Research*, *4*(3), 1-5. https://doi.org/10.61919/jhrr.v4i3.1077
40. Al-Zubaidi, R. M., Farah, H. S., Ahmed, K. A., Qnais, E. Y., Audeh, S. M., & Abu-Samak, M. S. (2024). The Effect of Stressful Conditions on Biochemical and Hematological Parameters Among University Students. *Jordan Journal of Biological Sciences*, *17*(03), 575-581. https://doi.org/10.54319/jjbs/170321
41. Assarzadegan, F., Asgarzadeh, S., Hatamabadi, H. R., Shahrami, A., Tabatabaey, A., & Asgarzadeh, M. (2016). Serum concentration of magnesium as an independent risk factor in migraine attacks. *International Clinical Psychopharmacology*, *31*(5), 287-292. https://doi.org/10.1097/yic.0000000000000130
42. Janoska, M., Chorążka, K., & Domitrz, I. (2015). Migraine frequency and its association with dyslipidemia in women. *Neurologia i Neurochirurgia Polska*, *49*(2), 95-98. https://doi.org/10.1016/j.pjnns.2015.02.001
43. Farah, H., Abdul-Hadi, B., Alhmoud, J. F., & Al-Othman, A. (2020). Treatment of vitamin B12 defciency in elderly decreases the high levels of lipid parameters: A retrospective study. *International Journal of Research in Pharmaceutical Sciences*, *11*(SPL4), 1093-1098. https://doi.org/10.26452/ijrps.v11ispl4.4249