

Correlation of circulating cell-free mitochondrial DNA levels with TFAM expression and oxidative stress markers during and after migraine attacks in women

Mohammad Mutlag Salih ¹, Shimaa Jumaa Abood ¹, Maan Hasan Salih ^{2*}, Adnan F. AL-Azaawied ²

¹Department of Biology, College of Education for Pure Sciences, University of Tikrit, Tikrit, Iraq

²Department of Biology, College of Science, University of Tikrit, Tikrit, Iraq

ARTICLE INFO

Received: 07/09/2025
Revised: 15/10/2025
Accepted: 28/10/2025
Available online: 29/07/2026
August Issue
[10.37652/juaps.2025.164886.1670](https://doi.org/10.37652/juaps.2025.164886.1670)

 CITE @ JUAPS

Corresponding author

Maan Hasan Salih
maan.hasan@tu.edu.iq

ABSTRACT

Migraine is a neurological disorder characterized by intense headaches and affects women more frequently than men. This study aimed to investigate the correlation between cell-free mitochondrial DNA (cf-mtDNA) levels, mitochondrial transcription factor A (TFAM) gene expression, and oxidative stress markers in patients with migraine. The study included a healthy control group ($n = 30$) and a patient group ($n = 27$), with blood samples collected from patients twice, during and after migraine attacks. Spectrophotometric methods were used to measure malondialdehyde (MDA), glutathione (GSH), and superoxide dismutase (SOD) levels. cf-mtDNA levels and TFAM gene expression were assessed using qRT-PCR. MDA levels were significantly higher in migraine patients than in the healthy control group ($p = 0.0108$), whereas significant decreases in GSH ($p = 0.0001$) and SOD ($p < 0.0001$) were observed in migraine patients compared with the healthy control group. In patients with migraine, TFAM gene expression decreased during headache attacks ($p = 0.04$) and after headache attacks ($p = 0.054$) compared with the control group. cf-mtDNA levels were significantly decreased in migraine patients during headache attacks ($p = 0.041$) and after headache attacks ($p = 0.002$) compared with the control group. cf-mtDNA showed a weak positive correlation with MDA ($r = 0.2715$, $p = 0.1707$) and weak negative correlations with GSH ($r = -0.2207$, $p = 0.3686$), SOD ($r = -0.3611$, $p = 0.0642$), and TFAM expression ($r = -0.3439$, $p = 0.079$). This study revealed a possible role of cf-mtDNA and TFAM gene expression in migraine, suggesting new avenues for understanding the disease.

Keywords: cf-mtDNA, Migraine, Oxidative stress, qRT-PCR, TFAM

1 INTRODUCTION

Migraine is a common genetic neurological disorder with a chronic course and unclear cause. It is characterized by episodic headaches associated with autonomic symptoms, and in some migraine patients, focal neurological deficits may occur [1]. Migraine is classified by the International Classification of Headache Disorders (ICHD) as a primary headache disorder characterized by

recurrent unilateral cephalalgia. The headache episodes have a duration of 4–72 hours, reveal a pulsating quality, and may be accompanied by symptoms such as nausea, photophobia, and/or phonophobia [2]. Approximately 14% of the global human population worldwide suffers from migraines each year, positioning this debilitating condition as the second-highest cause of the global burden of neurological disorders [3]. According to the Global

Health Estimates 2019 (GBD), migraine is considered the second common cause of disability and the first among women in the Middle Ages [4].

Migraine can seriously impact patients' professional lives [5]. Several neurobiological studies have shown that there is evidence of a correlation between migraine status and various disorders, including neurological, psychiatric, cardiovascular, gastrointestinal, metabolic, and immunological conditions [6]. Additionally, migraine has an association with familial history; patients with a family history of migraine are three times more likely to have migraine attacks [7]. Prior research, including family and twin investigations, indicates that the heritability of migraine ranges from 30% to 60% [8]. Migraine also reveals various inheritance patterns, ranging from a rare monogenic type, which accounts for approximately 0.01% [9], to more common polygenic types [10]. Previous extensive research has shown that a genome-wide association study (GWAS) of migraine has identified 123 risk loci associated with migraine disorder [11].

Women exhibit a migraine prevalence approximately three times higher than men, suggesting mitochondrial transmission, an X chromosome-linked form, or both [12]. Mitochondrial dysfunction may cause an increase in neuronal excitability, which can increase migraine susceptibility [13]. Existing research recognizes the critical role played by prooxidant and antioxidant substances as biomarkers for migraine [14]. Mitochondrial transcription factor A (TFAM) is a critical gene required for the maintenance and replication of mitochondrial DNA (mtDNA). It plays a pivotal role in cellular energy metabolism and mitochondrial homeostasis. Consequently, dysregulation of TFAM can contribute to the onset of various diseases [15, 16]. On the other hand, circulating cell-free mitochondrial DNA (cf-mtDNA) is widely recognized as a biomarker of psychobiological stress and disease, with the potential to predict the progression of various disorders [17]. Therefore, recent research focuses on mitochondrial damage to many inflammatory neurological disorders, suggesting that mtDNA release outside the cell is a common phenomenon in these cases [18].

Given the high incidence of migraine and the causes associated with the disease, this study investigates the level of cf-mtDNA and its related factors. To our knowledge, this is the first study to examine the cf-mtDNA level and its relationship with antioxidant markers and TFAM gene expression. Therefore, this study was designed to assess the effect of MDA, GSH, SOD levels,

and TFAM gene expression on cf-mtDNA in migraine patients, thereby contributing to a better understanding of the disease.

2 MATERIALS AND METHODS

2.1 Study design

A cross-sectional study was conducted to measure cf-mtDNA and antioxidant, as well as the expression of TFAM gene. This study took place from December 2024 to March 2025 at Tikrit Teaching Hospital and the Molecular Biology Laboratory, College of Science, Tikrit University, Iraq. Migraine cases were diagnosed by a neurologist according to guidelines from the criteria confirmed by the International Headache Society (IHS, 2013).

2.2 Study population

In this current study, we recruited 57 volunteers, 27 female patients previously diagnosed with migraines, and 30 healthy females. A neurologist identified migraine patients and confirmed the diagnosis using the International Classification of Diseases, 10th version G43. Blood samples were collected during and after migraine attacks. The inclusion criteria included women with premenopausal migraine, while the control group included healthy women within the same age range as the patients. Notably, the exclusion criteria included that these healthy volunteers had no family history of migraine or any neurological disorders, making them ideal for comparison with the patient group.

2.3 Serum biochemical markers

The measurement of MDA levels in serum samples from migraine patients and healthy women groups was conducted using the established Bird and Draper method [19]. The GSH level was measured in serum samples from migraine patients and healthy women groups using the Tipple and Rogers method [20]. The SOD activity was measured in serum samples from migraine patients as well as from healthy control women, using the method described by Gunduz et. al. [21].

2.4 Gene expression

Total RNA was carefully extracted from whole blood utilizing the Blood RNA Kit (Geneaid, Korea). The RNA quantity and purity were assessed using a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, USA), which provided insights into the RNA integrity. The next

step was the synthesis of complementary DNA (cDNA) using reverse transcriptases (RTs) from the Applied Biosystems cDNA Reverse Transcription Kit (USA) to convert the RNA into double-stranded cDNA. Following this, qRT-PCR was performed on an ABI Prism 7500 system (Applied Biosystems, Foster City, CA, USA), using the sensitive SYBR Green qPCR Master Mix to visualize the amplification results. In the current study, specific primers were designed for the TFAM gene: forward 5'-TGATTGAAGTTGGACGAAAGG-3' and reverse 5'-CTGGTTTCCTGTGCCTATCC-3', besides the reference gene Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) forward primer 5'-GGG ACG TGG CGT GAT-3' and reverse primer 5'-GAGTGGGTGTCGCTGTTGA-3'. The estimate of gene expression was determined using the $2^{-\Delta\Delta Ct}$ method [22]. The mean Ct value of GAPDH and TFAM was used for normalization of mRNA abundance. The x-fold change refers to the relative gene expression in population study groups.

2.5 cf-mtDNA levels

Whole Blood samples were collected in EDTA tubes to isolate integrated plasma through centrifugation for 5 minutes at 800g. Next, this plasma was transferred to other tubes and subjected to a second centrifugation protocol for 15 min. at 1000g. cf-mtDNA was extracted from 200 μ L of plasma samples isolated from migraine patients during and after headache attacks, and from healthy groups using the protocol provided by QIAamp DNA Mini Kit (Qiagen, USA). The purity and concentration of cf-mtDNA were measured using a spectrophotometer (NanoDrop 2000, Thermo Fisher, USA) at a wavelength of 260/280 nm. The qPCR reactions were carried out using SYBR Green Technology (Thermo Fisher Scientific, USA). The relative cf-mtDNA quantification was measured by calculating the ratio of mtDNA to nuclear genomic DNA, using the primers reported earlier [23]. Forward primer 5'-CACCCAAGAACAGGGTTTGT-3' and reverse primer 5'-TGGCCATGGGTATGTTGTTAA-3' were used to determine the amount of mitochondrial-NADH dehydrogenase subunit 1 (mt-ND1) in plasma samples. The mitochondrial NADH dehydrogenase subunit 1 (mt-ND1) gene was chosen as the mitochondrial marker because it is highly conserved, present in multiple copies, and provides a reliable proxy for total cf-mtDNA levels. β -actin (ACTB) was amplified using forward primer 5'-ACCCACACTGTGCCCATCTAC-3' and reverse primer 5'-TCGGTGAGGATCTTCATGAGGTA-3',

while β -actin (ACTB) served as the nuclear reference gene. The level of cf-mtDNA was computed using the $2^{-\Delta\Delta Ct}$ method.

2.6 Statistical methods

Statistical analyses were performed using GraphPad Prism version 10.4. (GraphPad Software, USA). The quantitative data are presented as the mean $\pm$ standard deviation, using descriptive statistics. One-way analysis of variance (ANOVA) was used to compare the study groups. Tukey's Honestly Significant Difference (HSD) was used to compare pairs of means. The receiver operating characteristic (ROC) curve was used to assess the ability to distinguish between healthy individuals and migraine patients. The qPCR data were computed using the $2^{-\Delta\Delta Ct}$ method. A P-value below 0.05 is considered an indicator of statistical significance.

3 RESULTS

The initial demographic and clinical characteristics are presented in Table 1. Study data regarding participants' age indicate that the mean $\pm$ standard deviation for female migraine sufferers was 37.71 ± 11.72 years, compared to 35.28 ± 9.53 years for the control group. As for the remaining demographic information of the patients, 59.26% had a family history of the disease, and 40.74% were married. Clinical data related to attack triggers were also recorded: 33.33% suffered from headaches due to light, and 66.67% from light and sound. The frequency of headache attacks varied between 26.62% who suffered from headaches twice a month, 37% who suffered from four attacks a month, and 36.38% who suffered from unlimited headaches. Regarding the direction of the headache, 70.37% reported headaches on one side, and 29.63% who suffered from headaches on both sides. The serum concentrations of MDA, GSH, and SOD were examined among the various study cohorts. As delineated in Table 3, the concentration of MDA exhibited a statistically significant elevation in the migraine patients (Mean $\pm$ SD: 5.008 ± 1.581 ; $p = 0.0108$) when compared with the healthy women (Mean $\pm$ SD: 3.836 ± 1.752). The concentration of GSH was markedly higher in the healthy group, with a Mean $\pm$ SD of 17.32 ± 5.308 and a p-value of 0.0001 in comparison to the migraine patients' group, which had a mean $\pm$ SD of 12.93 ± 3.445 . Concentrations of SOD in the migraine group showed a notable significant decrease (Mean $\pm$ SD: 2.836 ± 1.153 ; $p = < 0.0001$) compared to the healthy groups (Mean $\pm$ SD: $8.671 \pm$

3.372).

Table 1 Comparison of demographic and clinical data between female patients with migraine and healthy females

Variable	Female patients with migraine (n = 27)	Healthy females (n = 30)
Age, mean $\pm$ SD	37.71 $\pm$ 11.72	35.28 $\pm$ 9.53
Family history	Yes (59.26%) No (40.74%)	—
Marital status	Married (40.74%) Single (59.26%)	Married (56.67%) Single (43.33%)
Disease period	Under 5 years (29.63%) Above 5 years (70.37%)	—
Headache stimulation	Light (33.33%) Light and sound (66.67%)	—
Seizure frequency	Two per month (26.62%) Four per month (37%) Unlimited (36.38%)	—
Headache direction	One side (70.37%) Two sides (29.63%)	—
Drugs	Panadol (40.7%) Naproxen (37%) Zolmitriptan (22.3%)	—

Abbreviation: SD, standard deviation.

Table 2 MDA, GSH, and SOD concentrations measured in the study groups

Parameter	Migraine (n = 27) Mean $\pm$ SD	Healthy (n = 30) Mean $\pm$ SD	95% CI	DF	t-statistic	P value
MDA (nmol/mL)	5.008 $\pm$ 1.581	3.836 $\pm$ 1.752	-2.0616 to -0.2824	55	-2.640	0.0108
GSH (μ g/mL)	12.93 $\pm$ 3.445	17.32 $\pm$ 5.308	2.5250 to 7.3350	55	4.108	0.0001
SOD (μ U/mL)	2.836 $\pm$ 1.153	8.671 $\pm$ 3.372	4.4668 to 7.2032	55	8.547	< 0.0001

As shown in Figure 1A, there was a significant decrease in the expression of TFAM in female groups during headache attack (Mean $\pm$ SD: 1.249 $\pm$ 0.7093; median [IQR]: 1.167 [0.2339, 2.566]; p = 0.004) compared to the expression levels in healthy females (Mean $\pm$ SD: 2.001 $\pm$ 0.9624; median [IQR]: 1.947 [0.8269, 4.524]), while there was not a significant difference when compared between healthy groups and migraine patients after headache attract (Mean $\pm$ SD: 1.463 $\pm$ 0.8848; median [IQR]: 1.500 [0.2545, 2.870]; p = 0.054). Based on Figure 1B, the Tukey's multiple comparisons test yielded a value of 0.7523 between healthy and migraine patients during migraine attacks, and 0.5385 between healthy and migraine patients after migraine attacks. Finally, -0.2139 between migraine patients during and after migraine attacks. Figures 1C and 1D display the ROC curve for the TFAM gene expression. When comparing women patients during migraine attacks to healthy women, the AUC is 0.7395 (95%CI; 0.6112 – 0.8678) with a p -value of <0.0019. Similarly, when comparing women patients after migraine attacks to healthy women, the

AUC is 0.6432 (95%CI; 0.4951 – 0.7913) with a p -value of <0.0637.

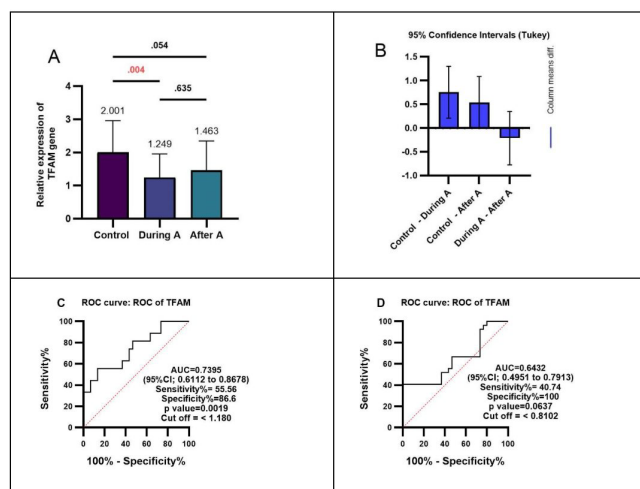


Fig. 1 A: Box plot of mean SD with P value for TFAM between migraine groups and healthy group, B: Difference between group means of TFAM gene expression between migraine group and healthy women, C: ROC analysis between migraine during attack and healthy group, D: ROC analysis between migraine after attack and healthy group.

The results of the cf-mtDNA amount were shown in Figure 2A, there was a significant increase in the amount of cf-mtDNA in female groups during headache attack (Mean $\pm$ SD: 1.563 $\pm$ 0.7244; median [IQR]: 1.475 [0.4289, 3.231]; p = 0.041) compared to its amount in healthy females (Mean $\pm$ SD: 1.081 $\pm$ 0.6164; median [IQR]: 0.9564 [0.3043, 2.986]), while there was a high significant difference when compared between healthy groups and migraine patients after headache attract (Mean $\pm$ SD: 1.788 $\pm$ 0.8645; median [IQR]: 1.744 [0.3742, 3.913]; p = 0.002). Based on Figure 2B, the Tukey's multiple comparisons test yielded a value of -0.4823 between healthy and migraine patients during migraine attacks, and -0.7071 between healthy and migraine patients after migraine attacks. Finally, -0.2248 between migraine patients during and after migraine attacks. Figures 2C and 2D display the ROC curve for the cf-mtDNA amount among study groups. When comparing women patients during migraine attacks to healthy women, the AUC is 0.71383 (95%CI; 0.6048-0.8717) with a p -value of <0.002. Similarly, when comparing women patients after migraine attacks to healthy women, the AUC is 0.7704 (95%CI; 0.6442-0.8965) with a p -value of <0.0005.

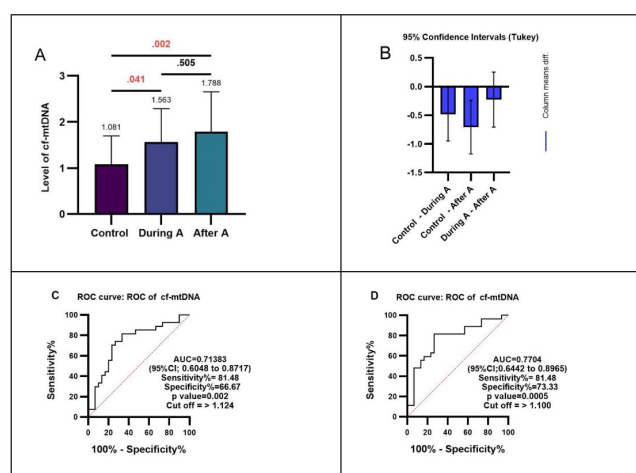


Fig. 2 A; Box plot of mean SD with P value for cf-mtDNA levels between migraine groups and healthy group, B: ROC curve of cf-mtDNA levels between migraine groups and healthy women, C; ROC analysis of cf-mtDNA levels between migraine during attack and healthy group, D: ROC analysis of cf-mtDNA levels between migraine after attack and healthy group.

The correlation between cf-mtDNA amount and antioxidant markers, as well as TFAM gene expression during migraine attacks, is shown in Figure 3. Figure 3A showed a weak positive correlation ($r = 0.2715$, $p < 0.1707$) between cf-mtDNA amount and MDA levels. From the data in Figure 3B, it was apparent that a weak negative correlation ($r = -0.2207$, $p = 0.3686$) existed between cf-mtDNA amount and GSH levels, indicating that decreased GSH levels might have been associated with increased cf-mtDNA amount. Also, there was a negative correlation ($r = -0.3611$, $p = 0.0642$) seen in Figure 3C between cf-mtDNA amount and SOD levels. Further, the results in Figure 3D indicate that a weak positive correlation ($r = -0.3439$, $p = 0.079$) existed between TFAM gene expression levels and cf-mtDNA amount.

4 DISCUSSION

To date, this may be the first study to estimate the correlation between cf-mtDNA amount and the relative TFAM gene expression in patients with migraine. Also, Oxidative stress markers are one of the important factors that correlate with mitochondrial function. Mitochondrial impairment leads to an imbalance between oxidation and antioxidants, mtDNA abnormality, mitochondrial dynamics disorder, and alters the mitophagy mechanism [24].

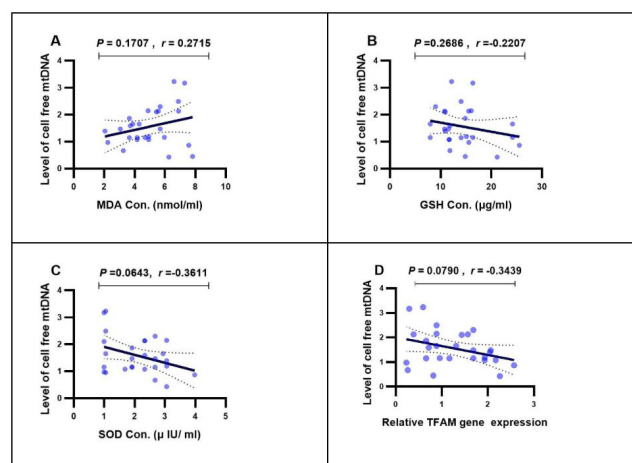


Fig. 3 The correlation between Cell-free mtDNA with A: MDA, B: GSH, C: SOD, and D: relative TFAM expression.

In the current study, serum levels of MDA, GSH, and SOD concentrations in patients and healthy women were definitively analyzed, clearly demonstrating a significant relationship between these oxidative markers and migraine progression. In accordance with these results, previous studies have demonstrated that MDA increases with migraine status [25, 26]. This study supports evidence from previous observations that GSH [27, 28] and SOD decreases during migraine [29]. During mtDNA is released from cells under stressful conditions, it is referred to as cf-mtDNA [30]. cf-mtDNA is considered a biomarker for cellular functions and is used as a biomarker for psychological stress, as well as various brain and psychiatric disorders [31].

Further analysis showed that decreased gene expression of TFAM in migraine patients. The decrease in TFAM gene expression appears to be associated with a decrease in the mitochondrial copy numbers and an increase in damaged mitochondria. Therefore, this process may lead to an increased amount of cf-mtDNA [32]. Previous studies found that the loss of TFAM in neural system cells caused the development of immature brain cells and triggered the activation of the integrated stress response in vivo [33]. Prior studies have noted that TFAM plays a crucial role in stabilizing mitochondrial DNA and preventing its escape as a form of cf-mtDNA, making it an accessible and significant therapeutic target for neurological disease [34]. A decrease in TFAM expression can lead to mitochondrial dysfunction and contribute to the development of various diseases. On the other hand, increasing TFAM expression can protect mtDNA from oxidative stress and improve mitochondrial

function [32].

The results of this study showed that the cf-mtDNA amount increased in migraine patients. Previous studies evaluating the cf-mtDNA levels as diagnostic and therapeutic biomarkers among various neurological diseases, such as schizophrenia patients [35], mild cognitive impairment, and Alzheimer' [36].

The present results were significant in at least two major respects: monitoring migraine progression and suggesting future therapeutic biomarkers.

5 CONCLUSION

This study aimed to assess cf-mtDNA levels in patients with migraine and their relationship with TFAM gene expression and oxidative stress markers. The findings showed increased cf-mtDNA levels in patients with migraine, accompanied by altered TFAM gene expression and oxidative stress marker levels. These results suggest a possible role of mitochondrial dysfunction and oxidative stress in migraine during and after headache attacks.

The main limitations of this study were the small sample size and recruitment from a single ethnic group. Despite these limitations, the study contributes to the understanding of migraine physiology during and after migraine attacks. Future studies with larger sample sizes and more diverse populations across geographic, age, sex, and ethnic groups are needed to provide more definitive evidence.

Author contributions

Sample collection and laboratory experiments were done by MMS., AF, and SJA, while MHS conducted the Statistical analysis. Each author contributed to the drafting and critical revision of the article for its intellectual content.

Funding source

No funds received.

Data availability

N/A

DECLARATIONS

Conflict of interest

The authors declare no competing interests

Consent to publish

N/A

Ethical approval

The research ethical committee at the College of Science, the University of Tikrit, Salahuddin, Iraq, approved all study procedures (No. 152 in 8/9/2024).

REFERENCES

- [1] Borończyk M, Zduńska A, Węgrzynek-Gallina J, Grodzka O, Lasek-Bal A, Domitrz I. Migraine and stroke: correlation, coexistence, dependence - a modern perspective. *The Journal of Headache and Pain*. 2025;26(1). [10.1186/s10194-025-01973-w](https://doi.org/10.1186/s10194-025-01973-w)
- [2] Arnold M. Headache classification committee of the international headache society (IHS) the international classification of headache disorders. *Cephalalgia*. 2018;38(1):1-211
- [3] Steiner TJ, Stovner LJ. Global epidemiology of migraine and its implications for public health and health policy. *Nature Reviews Neurology*. 2023;19(2):109–117. [10.1038/s41582-022-00763-1](https://doi.org/10.1038/s41582-022-00763-1)
- [4] Rubio AC, Arciniegas JA, Bolanos-Lopez JE, Gonzalez FJ, Gomez D, Mesa A, et al. Epidemiological, clinical characterization and treatment patterns of migraine patients in a Colombian cohort from 2018 to 2022. *The Journal of Headache and Pain*. 2024;25(1). [10.1186/s10194-024-01918-9](https://doi.org/10.1186/s10194-024-01918-9)
- [5] Dzoljic E, Vlajinac H, Sipetic S, Marinkovic J, Grbatinic I, Kostic V. A survey of female students with migraine: what is the influence of family history and lifestyle? *International Journal of Neuroscience*. 2013;124(2):82–87. [10.3109/00207454.2013.823961](https://doi.org/10.3109/00207454.2013.823961)
- [6] Altamura C, Corbelli I, de Tommaso M, Di Lorenzo C, Di Lorenzo G, Di Renzo A, et al. Pathophysiological Bases of Comorbidity in Migraine. *Frontiers in Human Neuroscience*. 2021;15. [10.3389/fn-hum.2021.640574](https://doi.org/10.3389/fn-hum.2021.640574)
- [7] Bicakci S, Over F, Aslan K, Bozdemir N, Saatci E, Sarica Y. Headache Characteristics in Senior Medical Students in Turkey. *The Tohoku Journal of Experimental Medicine*. 2007;213(3):277–282. [10.1620/tjem.213.277](https://doi.org/10.1620/tjem.213.277)
- [8] Polderman TJC, Benyamin B, de Leeuw CA, Sullivan PF, van Bochoven A, Visscher PM, et al. Meta-analysis of the heritability of human traits

- based on fifty years of twin studies. *Nature Genetics*. 2015;47(7):702–709. [10.1038/ng.3285](https://doi.org/10.1038/ng.3285)
- [9] van den Maagdenberg AMJM, Nyholt DR, Anttila V. Novel hypotheses emerging from GWAS in migraine? *The Journal of Headache and Pain*. 2019;20(1). [10.1186/s10194-018-0956-x](https://doi.org/10.1186/s10194-018-0956-x)
- [10] Sutherland HG, Jenkins B, Griffiths LR. Genetics of migraine: complexity, implications, and potential clinical applications. *The Lancet Neurology*. 2024;23(4):429–446. [10.1016/s1474-4422\(24\)00026-7](https://doi.org/10.1016/s1474-4422(24)00026-7)
- [11] Hautakangas H, Winsvold BS, Ruotsalainen SE, Bjornsdottir G, Harder AVE, Kogelman LJA, et al. Genome-wide analysis of 102,084 migraine cases identifies 123 risk loci and subtype-specific risk alleles. *Nature Genetics*. 2022;54(2):152–160. [10.1038/s41588-021-00990-0](https://doi.org/10.1038/s41588-021-00990-0)
- [12] Fila M, Pawłowska E, Blasiak J. Mitochondria in migraine pathophysiology – does epigenetics play a role? *Archives of Medical Science*. 2019;15(4):944–956. [10.5114/aoms.2019.86061](https://doi.org/10.5114/aoms.2019.86061)
- [13] Sparaco M, Feleppa M, Lipton R, Rapoport A, Bigal M. Mitochondrial Dysfunction and Migraine: Evidence and Hypotheses. *Cephalalgia*. 2006;26(4):361–372. [10.1111/j.1468-2982.2005.01059.x](https://doi.org/10.1111/j.1468-2982.2005.01059.x)
- [14] Jiménez-Jiménez FJ, Alonso-Navarro H, García-Martín E, Espada-Rubio S, Agúndez JAG. Oxidative Stress and Migraine. *Molecular Neurobiology*. 2024;61(10):8344–8360. [10.1007/s12035-024-04114-7](https://doi.org/10.1007/s12035-024-04114-7)
- [15] Kang D, Kim SH, Hamasaki N. Mitochondrial transcription factor A (TFAM): Roles in maintenance of mtDNA and cellular functions. *Mitochondrion*. 2007;7(1-2):39–44. [10.1016/j.mito.2006.11.017](https://doi.org/10.1016/j.mito.2006.11.017)
- [16] Yang M, Sun L, Feng X, Xu W. Mitochondrial transcription factor a as a guardian of mitochondrial integrity and emerging therapeutic target in human diseases: A review. *International Journal of Biological Macromolecules*. 2025;319:145706. [10.1016/j.ijbiomac.2025.145706](https://doi.org/10.1016/j.ijbiomac.2025.145706)
- [17] Michelson J, Rausser S, Peng A, Yu T, Sturm G, Trumpff C, et al. MitoQuicLy: A high-throughput method for quantifying cell-free DNA from human plasma, serum, and saliva. *Mitochondrion*. 2023;71:26–39. [10.1016/j.mito.2023.05.001](https://doi.org/10.1016/j.mito.2023.05.001)
- [18] Shang D, Huang M, Wang B, Yan X, Wu Z, Zhang X. mtDNA Maintenance and Alterations in the Pathogenesis of Neurodegenerative Diseases. *Current Neuropharmacology*. 2023;21(3):578–598. [10.2174/1570159x20666220810114644](https://doi.org/10.2174/1570159x20666220810114644)
- [19] Bird RP, Draper HH. In: [35] Comparative studies on different methods of malonaldehyde determination. Elsevier; 1984. p. 299–305. [10.1016/s0076-6879\(84\)05038-2](https://doi.org/10.1016/s0076-6879(84)05038-2)
- [20] Tipple TE, Rogers LK. In: Methods for the Determination of Plasma or Tissue Glutathione Levels. Humana Press; 2012. p. 315–324. [10.1007/978-1-61779-867-2_20](https://doi.org/10.1007/978-1-61779-867-2_20)
- [21] Gündüz AM, Demir H, Toprak N, Akdeniz H, Demir C, Arslan A, et al. The effect of computed tomography on oxidative stress level and some antioxidant parameters. *Acta Radiologica*. 2020;62(2):260–265. [10.1177/0284185120922135](https://doi.org/10.1177/0284185120922135)
- [22] Livak KJ, Schmittgen TD. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2CT Method. *Methods*. 2001;25(4):402–408. [10.1006/meth.2001.1262](https://doi.org/10.1006/meth.2001.1262)
- [23] Hsu CW, Yin PH, Lee HC, Chi CW, Tseng LM. Mitochondrial DNA Content as a Potential Marker to Predict Response to Anthracycline in Breast Cancer Patients: mtDNA and Drug Response in Breast Cancer. *The Breast Journal*. 2010;16(3):264–270. [10.1111/j.1524-4741.2010.00908.x](https://doi.org/10.1111/j.1524-4741.2010.00908.x)
- [24] Xu X, Pang Y, Fan X. Mitochondria in oxidative stress, inflammation and aging: from mechanisms to therapeutic advances. *Signal Transduction and Targeted Therapy*. 2025;10(1). [10.1038/s41392-025-02253-4](https://doi.org/10.1038/s41392-025-02253-4)
- [25] Mahmood LO, Abdoulrahman K. The Role of Oxidative and Nitrosative Stress in Migraine Patients. *Iraqi Journal of Science*. 2024;6781–6793. [10.24996/ijjs.2024.65.12.1](https://doi.org/10.24996/ijjs.2024.65.12.1)
- [26] Chen QW, Meng RT, Ko CY. Modulating oxidative stress and neurogenic inflammation: the role of topiramate in migraine treatment. *Frontiers in Aging Neuroscience*. 2024;16. [10.3389/fnagi.2024.1455858](https://doi.org/10.3389/fnagi.2024.1455858)
- [27] Tripathi GM, Kalita J, Misra UK. A study of oxidative stress in migraine with special reference to prophylactic therapy. *International Journal of Neuroscience*. 2017;128(4):318–324. [10.1080/00207454.2017.1374959](https://doi.org/10.1080/00207454.2017.1374959)

- [28] Zhang L, Huang J, Zhang Z, Cao Z. Altered Metabolites in the Occipital Lobe in Migraine Without Aura During the Attack and the Interictal Period. *Frontiers in Neurology*. 2021;12. [10.3389/fneur.2021.656349](https://doi.org/10.3389/fneur.2021.656349)
- [29] and Shukla DPK. Study of Oxidative Stress and Antioxidants in Chronic Migraine. *Journal of Medical Science And clinical Research*. 2018;6(5). [10.18535/jmscr/v6i5.184](https://doi.org/10.18535/jmscr/v6i5.184)
- [30] Yu M. Circulating cell-free mitochondrial DNA as a novel cancer biomarker: opportunities and challenges. *Mitochondrial DNA*. 2012;23(5):329–332. [10.3109/19401736.2012.696625](https://doi.org/10.3109/19401736.2012.696625)
- [31] Blalock ZN, Wu GWY, Lindqvist D, Trumpff C, Flory JD, Lin J, et al. Circulating cell-free mitochondrial DNA levels and glucocorticoid sensitivity in a cohort of male veterans with and without combat-related PTSD. *Translational Psychiatry*. 2024;14(1). [10.1038/s41398-023-02721-x](https://doi.org/10.1038/s41398-023-02721-x)
- [32] Zhou X, Trinh-Minh T, Tran-Manh C, Gießl A, Bergmann C, Györfi A, et al. Impaired Mitochondrial Transcription Factor A Expression Promotes Mitochondrial Damage to Drive Fibroblast Activation and Fibrosis in Systemic Sclerosis. *Arthritis & Rheumatology*. 2022;74(5):871–881. [10.1002/art.42033](https://doi.org/10.1002/art.42033)
- [33] Kuroda R, Tominaga K, Kasashima K, Kuroiwa K, Sakashita E, Hayakawa H, et al. Loss of mitochondrial transcription factor A in neural stem cells leads to immature brain development and triggers the activation of the integral stress response in vivo. *PLOS ONE*. 2021;16(7):e0255355. [10.1371/journal.pone.0255355](https://doi.org/10.1371/journal.pone.0255355)
- [34] Zhang G, Wei H, Zhao A, Yan X, Zhang X, Gan J, et al. Mitochondrial DNA leakage: underlying mechanisms and therapeutic implications in neurological disorders. *Journal of Neuroinflammation*. 2025;22(1). [10.1186/s12974-025-03363-0](https://doi.org/10.1186/s12974-025-03363-0)
- [35] Garcia-de la Cruz D, Juarez-Rojop I, Tovilla-Zarate CA, Nicolini H, Genis-Mendoza A. Circulating Cell-Free Mitochondrial DNA in Plasma of Individuals with Schizophrenia and Cognitive Deficit in Mexican Population. *Neuropsychiatric Disease and Treatment*. 2024;Volume 20:1757–1765. [10.2147/ndt.s460554](https://doi.org/10.2147/ndt.s460554)
- [36] Song BX, Beroncal E, Andreazza AC, Gallagher D, Marzolini S, Rapoport MJ, et al. Circulating cell-free mitochondrial DNA (ccf-mtDNA) and memory in mild cognitive impairment (MCI) and Alzheimer's disease (AD). *Alzheimer's & Dementia*. 2023;19:e071912

How to cite this article

Salih MM, Abood SJ, Salih MH, AL-Azaawied AF. Correlation of circulating cell-free mitochondrial DNA levels with TFAM expression and oxidative stress markers during and after migraine attacks in women . *Journal of University of Anbar for Pure Science*. 2026; 20(2):65-72. doi:[10.37652/juaps.2025.164886.1670](https://doi.org/10.37652/juaps.2025.164886.1670)