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FUNCTIONAL DISABILITY IN MIGRAINE: INFLUENCE OF BODY COMPOSITION AND DNA METHYLATION IN WOMEN WITH AND WITHOUT OVERWEIGHT

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ABSTRACT

Migraine is a disabling neurological condition whose pathophysiology involves complex inflammatory mechanisms and may be influenced by metabolic factors such as excess weight and epigenetic alterations. This case-control study aimed to investigate possible associations between body composition, DNA methylation status, and migraine severity in women with and without excess weight. A total of 22 participants were included and evaluated through clinical questionnaires (ID-Migraine and MIDAS), bioelectrical impedance analysis, and methylation assessment via PCR after bisulfite conversion. The mean age was 39.8 years, with higher average values of BMI, body fat percentage, and MIDAS scores in the overweight group. Although statistical tests did not show significant associations between weight, methylation, and functional disability (MIDAS), a trend toward a higher frequency of unmethylation among overweight individuals was observed ($p = 0.172$), as well as a slight increase in MIDAS scores with rising BMI and body fat percentage. The correlation between BMI and body fat was positive ($\rho = 0.949$; $p < 0.0001$). These findings suggest a potential modulatory role of adiposity on epigenetic mechanisms related to migraine, although limited by the small sample size. It is concluded that the interaction between obesity, methylation, and migraine warrants further investigation in future studies with greater statistical power and control for confounding variables.

KEYWORDS: Migraine; Obesity; DNA Methylation; Epigenetics; Body Composition; Inflammation.

1 INTRODUCTION

Migraine, also known as headache, is a highly prevalent neurological condition and is considered one of the leading causes of disability worldwide, particularly among women of reproductive age.[1] It is classified as a primary headache, typically characterized by unilateral pulsatile pain associated with nausea, vomiting, photophobia, and phonophobia, and may present in episodic or chronic forms.[2] Its pathophysiology involves complex mechanisms such as activation of the trigeminovascular system, release of neuropeptides like CGRP, and a neurogenic inflammatory response, supporting the notion that migraine can be understood as a systemic inflammatory disease.[3,4,5,6]

In parallel, obesity is a chronic condition with increasing global incidence, associated with low-grade inflammation, elevated adipokines, and dysregulation of metabolic pathways.[7,8] Multiple lines of evidence suggest a correlation between obesity and migraine, particularly highlighting an increased frequency and intensity of migraine attacks in individuals with excess body weight.[9,10]

Moreover, important gaps remain in literature, especially regarding the epigenetic mechanisms that may mediate this association. Recent studies suggest that changes in DNA methylation can influence the expression of inflammatory genes involved in pain pathophysiology, such as *CALCA*, *RAMP1*, *IL-6*, and *TNF-α*, thereby affecting the sensitivity of the trigeminovascular system.[11,12] From this perspective, it becomes relevant to investigate the integrated role of epigenetics and body composition, employing tools such as bioelectrical impedance analysis and molecular assays to better understand this interface.

Given this context, it is hypothesized that obesity may modulate gene expression through epigenetic mechanisms. Therefore, this study aims to evaluate migraine intensity in individuals with and without excess weight, correlating these findings with body composition parameters and DNA methylation levels. The goal is to provide support for more personalized and effective clinical approaches to managing migraine in the context of metabolic disturbances.

2 MATERIALS AND METHODS

2.1 Ethical Aspects

This project was approved by the Research Ethics Committee of the Pontifical Catholic University of Paraná under protocol number 98316718.7.0000.0020. All participants signed an informed consent form.

2.2 Study Population

Female individuals aged between 18 and 60 years, diagnosed with episodic and/or chronic migraine, with or without aura, were included. Individuals with other neurological, psychiatric, inflammatory, and/or chronic infectious diseases, as well as those presenting symptoms of acute infections at the time of data collection, were excluded.

2.3 Study Design

This was a qualitative, analytical, case-control study involving patients seen at the Academic Medical Outpatient Clinic of the Pontifical Catholic University of Paraná, Londrina campus, between 2023 and 2024. The diagnosis of migraine was established according to the International Classification of Headache Disorders.[2]

All participants underwent clinical evaluation and completed the ID-Migraine questionnaire, a self-administered tool composed of three questions. The test is considered negative for migraine when the patient answers "yes" to only one or none of the questions. When two or more answers are positive, the test demonstrates up to 92% sensitivity and 60% specificity for the diagnosis of migraine.[13]

Patient interviews were structured and based on a form electronically developed by the Headache Research Group of the Pontifical Catholic University of Paraná, Londrina campus, using the Google Forms® platform. The form included the following topics: demographic data (name, age, sex, self-declared race/ethnicity); presence of symptoms suggestive of infection at the time of assessment; diagnostic criteria for migraine with or without aura; migraine temporal profile (number of headache days per month, age at onset of migraine attacks); associated clinical features; migraine triggers; lifestyle habits (aerobic physical activity and sleep duration); comorbidities; substance use (tobacco, alcohol, illicit drugs); anthropometric data (weight, height, and waist circumference); and blood pressure.

Participants also completed a validated self-administered questionnaire: MIDAS (Migraine Disability Assessment), which evaluates migraine-related disability.[14,15]

Participants were categorized into groups with and without excess weight based on the cutoff points established by the World Health Organization (WHO), as described in the report *Physical Status: The Use and Interpretation of Anthropometry*.[16] Accordingly, individuals with a body mass index (BMI) below 25 kg/m² were classified as not overweight, while those with a BMI equal to or greater than 25 kg/m² were classified as overweight.

2.4 Assessment by Electrical Bioimpedance

Assessment of weight, body composition, and visceral adiposity was performed using bioelectrical impedance analysis. Prior to the procedure, participants were instructed to fast for at least 2 hours—including refraining from water intake—and to remove any metal objects in contact with the body.[17] Data were collected using the InBody 270 bioimpedance device.

Participants remained in an upright position for 5 minutes prior to the bioimpedance procedure, during which height was measured and hand and foot hygiene was performed to remove oil and improve contact with the 25 electrodes.[17] To ensure a more accurate assessment of body composition, bioimpedance was contraindicated for individuals with pacemakers and for women during their menstrual period.

2.5 Blood Samples

Blood collection was performed using a vacuum collection system with tubes containing the anticoagulant EDTA (ethylenediaminetetraacetic acid). Approximately 8 mL of peripheral venous blood was drawn by venipuncture in the antecubital region. Immediately after collection, the samples were gently inverted to ensure proper mixing with the anticoagulant.

The blood samples were then centrifuged at 2500 rpm for 10 minutes to separate cellular and plasma components. The leukocyte layer (buffy coat) was carefully isolated for subsequent DNA extraction.

2.6 Global DNA Methylation Analysis

For methylation analysis, the Thermo Scientific EpiJET DNA Methylation Analysis Kit (MspI/HpaII) was used, which employs the MspI and HpaII restriction enzymes to assess DNA methylation status at specific loci. Epi MspI and Epi HpaII are isoschizomers with different sensitivities to CpG methylation. When the internal CpG within the 5'-CCGG-3' tetranucleotide sequence is methylated, digestion by Epi HpaII is inhibited, whereas digestion by Epi MspI remains unaffected. The enzymatic digestion products were analyzed on 2% agarose gel stained with SYBR Safe (Invitrogen).

2.7 Statistical Analysis

Statistical analysis was conducted using Stata® software, version 16.0 (StataCorp, College Station, TX, USA). The analysis aimed to investigate associations and correlations among clinical, anthropometric, and epigenetic variables within the sample. Initially, continuous variables were described using measures of central tendency (mean) and compared between case (overweight individuals) and control (non-overweight individuals) groups.

To assess associations between categorical variables—such as weight status (overweight vs. normal weight) and methylation status (methylated vs. hypomethylated), as well as weight and MIDAS scale classification (grades 1 to 4, grouped as <3 and ≥3)—Fisher's exact test was used, given its suitability for small samples and contingency tables with asymmetric distributions.

Variables with normal distribution, such as age, body fat percentage, and MIDAS score, were analyzed using independent samples Student's t-tests, assuming equal variances, to compare methylated and non-methylated groups. For these variables, means, standard deviations, standard errors of the mean (SEM), and 95% confidence intervals were also reported.

Since the BMI variable did not follow a normal distribution ($p = 0.039$), it was evaluated using the Mann–Whitney U test, with data described by median and interquartile range. In this case, the first (Q1) and third (Q3) quartiles were used to more specifically express the dispersion according to the observed distribution.

Relationships between continuous and ordinal variables were evaluated using Spearman's rank correlation (ρ), given the nonparametric nature of the data. This approach was applied to assess correlations between BMI and MIDAS, body fat and MIDAS, and BMI and body fat.

For all statistical analyses, a significance level of 5% ($p < 0.05$) was adopted. Results below this threshold were considered statistically significant, indicating that the observed correlation or association was unlikely to have occurred by chance. Results with $p \geq 0.05$ were interpreted as not significant, indicating insufficient evidence to infer a correlation or association among the variables analyzed.

3 RESULTS

Data from a total of 22 study participants were analyzed. The mean age was 39.85 years, and the overall mean BMI was 27.74 kg/m². The overweight group exhibited higher mean values of BMI (33.8 kg/m²), MIDAS score (46.9), and body fat percentage (46.6%) compared to the control group (BMI: 21.7 kg/m²; MIDAS: 32.3; body fat: 29.1%). These findings preliminarily suggest a possible clinical pattern in which excess weight is associated with greater functional impairment due to headache. (Table 1)

Fisher's exact test was performed to investigate the association between weight status and MIDAS scale classification (grades 1 to 4, grouped as <3 and ≥3). The analysis indicated no statistically significant association between the variables ($p = 0.323$). Similarly, the analysis between weight status and methylation status (1 = hypomethylated, 2 = methylated) also showed no statistical significance ($p = 0.172$). (Table 2)

In the comparison between methylated and hypomethylated groups, MIDAS scores showed no statistically significant difference ($t = 0.29$; $p = 0.774$), indicating no association between methylation status and the severity of migraine-related functional disability. Similarly, body fat percentage (%) and BMI did not differ significantly between the groups ($t = -1.04$; $p = 0.310$ and $U = 38.0$; $p = 0.332$, respectively). (Tables 3 and 4)

The Spearman correlation between BMI and MIDAS scores yielded a coefficient of $r = 0.071$ ($p = 0.755$), indicating no significant correlation. Similarly, the correlation between body fat percentage (%) and MIDAS was also not significant ($r = -0.047$; $p = 0.834$). On the other hand, the correlation between BMI and body fat was statistically significant ($r = 0.949$; $p < 0.0001$), demonstrating a direct association between these two anthropometric variables. (Figures 1, 2 and 3)

Table 1: Descriptive Analysis of the variables age, BMI, MIDAS scores and body fat in the studied population.

Variable	Cases (Overweight)	Controls (Not overweight)	P-value
Age (years) ($\bar{x} \pm SD$)	41.08 ± 10.43	38.37 ± 15.83	0.64#
BMI (kg/m²) (Md(Q1-Q3))	32.00 (27.65 - 38.05)	22.10 (19.8 - 23.62)	< 0.001##
MIDAS ($\bar{x} \pm SD$)	46.92 ± 48.13	32.30 ± 33.12	0.42#
Body Fat (%) ($\bar{x} \pm SD$)	46.63 ± 5.37	29.10 ± 5.97	< 0.001#

 # Test T Student; ## Mann-Whitney test

Table 2: Association between the variables MIDAS, Methylation and Weight category in the study population.

Variable			P-value	
Weight Category	MIDAS < 3 (n)	MIDAS ≥ 3 (n)		MIDAS ≥ 3 (%)
Overweight	4	8	0.323	66.7%
Normal weight	1	9		90.0%

Weight Category	Hypomethylated (n)	Methylated (n)		Methylated (%)
Overweight	10	2	0.172	16.7%
Normal weight	5	5		50.0%

Fisher's Exact Test

Table 3: Descriptive Statistics and t-test between the variables MIDAS and Body Fat vs. Methylation.

Group	n	Mean	Standard Deviation	Standard Error (SEM)	95% CI Lower	95% CI Upper	Summary
MIDAS							
Hypomethylated	15	38.47	41.75	10.78	17.34	59.60	38.47 ± 41.75
Methylated	7	44.14	44.64	16.87	11.07	77.21	44.14 ± 44.64
t-test	—	0.29	—	—	—	p = 0.774	—
Body Fat (%)							
Hypomethylated	15	40.25	10.61	2.74	34.88	45.62	40.25 ± 10.61
Methylated	7	35.26	10.15	3.84	27.74	42.78	35.26 ± 10.15
t-test	—	-1.04	—	—	—	p = 0.310	—

Table 4: Mann-Whitney Test: BMI vs. Methylation (Q1–Q3).

Group	n	Median (BMI)	Q1	Q3
Hypomethylated	15	27.60	23.54	35.25
Methylated	7	23.70	22.45	26.30
Mann-Whitney U	—	—	—	U = 38.0, p = 0.33

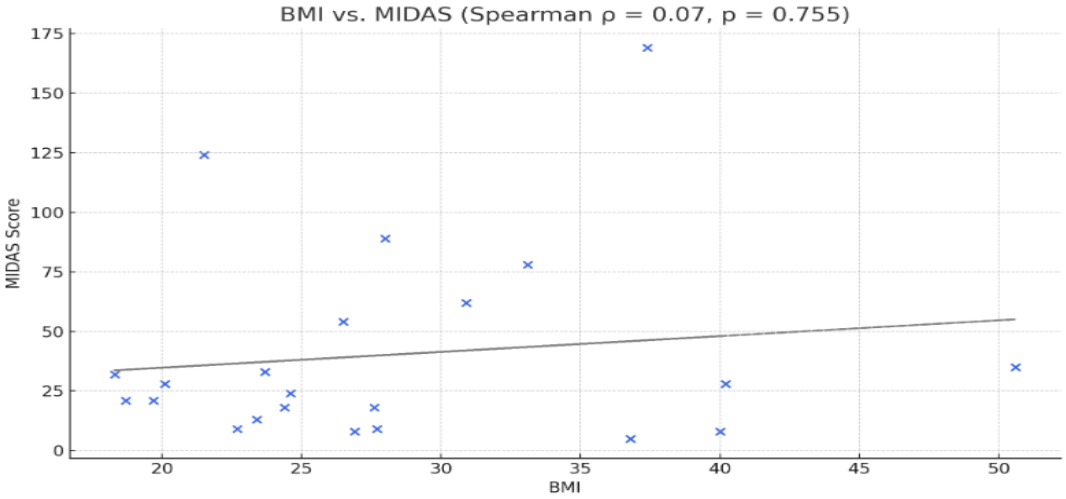

Fig. 1: Correlation between BMI and MIDAS (Spearman correlation).

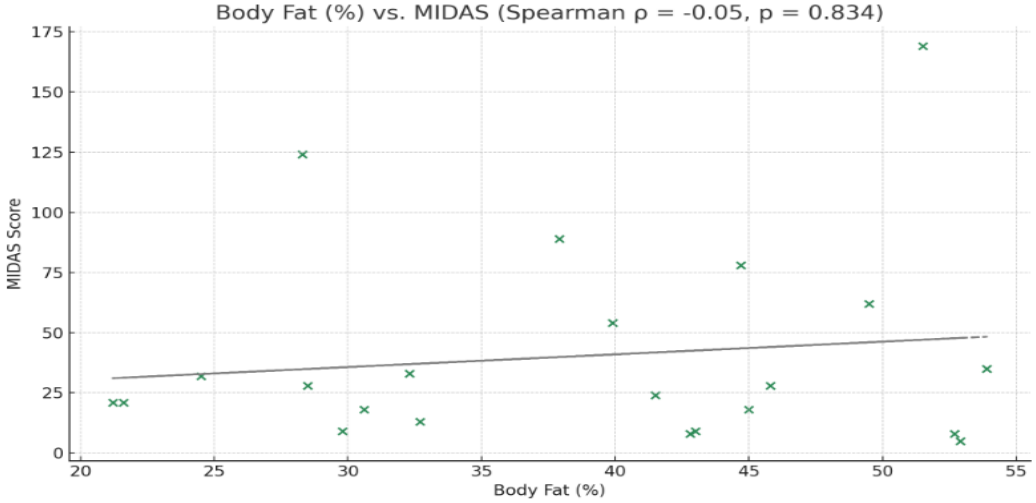

Fig. 2: Correlation between body fat and MIDAS (Spearman correlation).

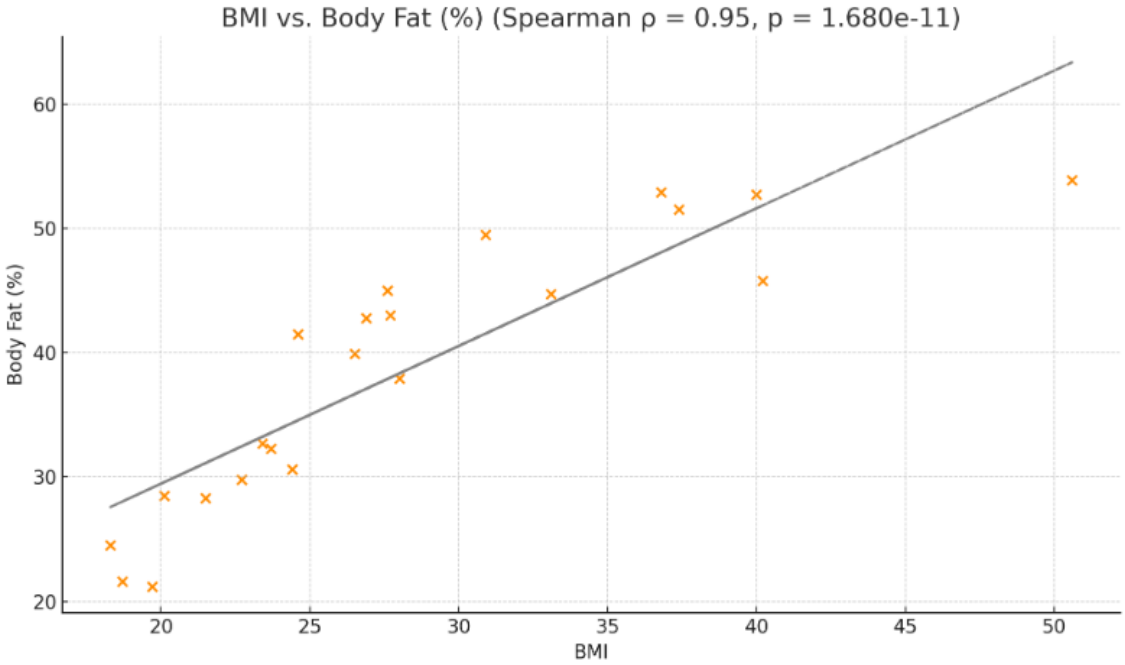

Fig. 3: Correlation between BMI and body fat (Spearman correlation).

4 DISCUSSION

This case-control study aimed to investigate possible associations between clinical and epigenetic variables, with an emphasis on methylation status, anthropometric characteristics (BMI and body fat percentage), and migraine severity, as assessed by the MIDAS scale. Although most statistical tests did not reveal statistically significant associations, relevant trends were observed that warrant discussion and further investigation in future studies with larger sample sizes.

Descriptive analysis revealed that the overweight group exhibited higher mean values of body fat percentage and MIDAS scores, as well as greater intra-group variability. In contrast, the normal weight group showed a higher relative frequency of individuals classified with greater severity on the MIDAS scale (≥3), although this difference did not reach statistical significance ($p = 0.323$), as indicated by Fisher's exact test. This finding may suggest that factors beyond excess weight—such as metabolic profiles, inflammatory responses, or methylation patterns—could influence the severity of migraine-related disability.

Regarding body weight, a trend toward a higher frequency of unmethylation was observed in the overweight group ($p = 0.172$), which may indicate a possible inverse relationship between body weight and methylation of regulatory genes involved in migraine pathophysiology. This observation aligns with hypotheses in the literature linking obesity to hypomethylation of pro-inflammatory genes.[18,19,20]

In the analysis of the effects of methylation on continuous variables, no significant differences were found between the methylated and hypomethylated groups in terms of MIDAS scores ($p = 0.774$) or body fat percentage ($p = 0.310$). Nevertheless, a trend toward a lower body fat percentage was observed among methylated individuals (mean of 35.3% vs. 40.3%), suggesting a possible role of body composition in modulating methylation, although not statistically confirmed in this sample.

BMI data did not show a significant difference between the methylation groups ($p = 0.332$). However, once again, a lower median was observed in the methylated group, reinforcing a trend toward lower adiposity associated with the presence of methylation[18,19,20], a hypothesis that warrants further exploration in larger populations using multivariate analysis adjusted for age, sex, and inflammatory profile.

A strong positive correlation was observed between BMI and body fat percentage ($\rho = 0.949$; $p < 0.0001$), confirming the internal validity of the data and the consistency between these two anthropometric variables. On the other hand, neither the correlation between BMI and MIDAS ($\rho = 0.071$; $p = 0.755$) nor between body fat and MIDAS ($\rho = -0.047$; $p = 0.834$) reached statistical significance. Nonetheless, the scatter plots with trend lines reveal a subtle upward visual trend, suggesting that as BMI and body fat percentage increase, there is a proportional rise in migraine-related disability scores (MIDAS). This supports a possible positive monotonic association, which was not statistically detected in this limited sample, but may be corroborated by findings in the scientific literature.[21,22]

Taken together, these findings suggest that, although no statistically robust associations were identified, the data point to a potential modulatory role of body composition

on methylation and inflammatory patterns, which may indirectly influence the pathophysiology of migraine.

This study presents important limitations that may have compromised the robustness of the findings, such as the small sample size, which reduces statistical power and may have obscured true associations, as well as the geographic restriction of participants, which limits the generalizability of the results. The absence of relevant clinical data, such as the duration of migraine onset and the presence of other comorbidities, also prevented adjustments that could have clarified relationships among the analyzed variables. These limitations suggest that the results should be interpreted with caution, reinforcing the need for future larger, multicenter studies with greater control of confounding variables.

Future studies with larger sample sizes, sex stratification, assessment of inflammatory biomarkers, and expanded genetic profiling are needed to better understand the interaction between adiposity, methylation, and migraine severity. Although preliminary, these findings highlight the importance of considering epigenetics and body composition in the understanding of primary headaches and suggest promising avenues for future translational research and personalized interventions in the management of migraine among populations with metabolic disorders.

5 CONCLUSION

It was observed that individuals with excess weight had higher mean MIDAS scores and body fat percentages, along with a trend toward hypomethylation, suggesting a possible role of adiposity in the epigenetic modulation of genes related to inflammation and pain.

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DECLARATION OF INTERESTS: None.

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