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Article

The Correlation Among Levels of miR-202, miR-362, IL-1β, and IL-18 in the Serum Female Patients with Migraine

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Abstract: Background and Objective: Migraine is associated with peripheral neuroinflammation, with disruptions in the epigenetic mechanisms regulating this inflammation, particularly in females. This study aimed to evaluate serum miR-202 and miR-362 levels and their relationship to IL-1β and IL-18 levels in female migraine sufferers during a migraine attack.

Materials and Methods: A case-control study was conducted with 90 participants: 50 migraine sufferers and 40 healthy individuals as an age-matched control group. Participants with active immune or inflammatory diseases or recent anti-inflammatory drug use were excluded. Serum concentrations of IL-1β and IL-18 were measured using ELISA, and relative expression of miR-202 and miR-362 was measured using qRT-PCR during an acute migraine attack.

Results: The patients showed a significant increase in IL-1β (P=0.0251) and IL-18 (P=0.0070), and a significant upregulation of miR-202 (P=0.0107) and miR-362 (P=0.0347) compared to the control group. IL-1β and IL-18 were moderately positively correlated (r=0.51, P=0.001), and miR-202 and miR-362 were significantly positively correlated (r=0.41, P=0.0427), with no significant cross-reactivity between the two groups (P>0.05).

Conclusion: The results support the existence of a dual inflammatory and epigenetic imbalance in females with migraine, and the combined profile of miR-202, miR-362, IL-1β, and IL-18 represents a promising candidate group of serological biomarkers for the molecular characterization of migraine attacks, with a correlational nature that warrants further investigation.

Keywords: Migraine; miR-202; miR-362; IL-1β; IL-18; neuroinflammation; biomarker

1. Introduction

Migraine is a chronic and complex neurological disorder that represents a significant health and economic burden globally. The latest data from the Global Burden of Disease Collaborative indicates a global prevalence of approximately 14.1%, with a clear gender disparity; the prevalence in females is 17.6% compared to 10.5% in males (Global Burden of Disease Collaborative, 2026). As a result of this disparity, migraine ranks eighth globally among causes of years lived with disability (YLDs) and second among the leading causes of disability among young adults and adults under 50 (Ashina et al., 2021).

According to the International Classification of Headache Disorders, Third Edition (ICHD-3), migraine is classified into several clinical types, most notably migraine without aura—the most common type, characterized by severe headache accompanied by nausea and photophobia—and migraine with aura, which is characterized by transient neurological and visual symptoms preceding the pain attack. Less common types include chronic migraine, hemiplegic migraine, and migraine with brainstem aura (IHS, 2018; Goadsby, 2025).

Multifactorial etiology plays a role in the pathogenesis of migraine. Genetic predisposition is estimated to contribute between 40% and 60% to increased neurological sensitivity (Sutherland et al., 2021). This genetic background interacts with environmental and physiological triggers, most notably hormonal fluctuations in women (such as estrogen), acute psychological stress, and sleep disturbances (Ramachandran, 2018; Sutherland et al., 2021). These triggers lead to activation of the trigeminal vascular system and the occurrence of sterile neuroinflammation within glial cells and synapses (Ramachandran, 2018). This inflammation is accompanied by the activation of inflammasomes which induce the maturation and release of inflammatory cytokines, mainly interleukin-1 beta (IL-1β) and interleukin-18 (IL-18). These cytokines directly contribute to central and peripheral sensitization, and to the development of the throbbing pain that is typical of a migraine attack (He et al., 2020).

In parallel to this inflammatory pathway, microRNAs (miRNAs), small non-coding RNA molecules that post-transcriptionally regulate gene expression, have emerged as important epigenetic determinants in maintaining immune and neurological homeostasis by inhibiting the translation of target genes (Andersen et al., 2016). Among these molecules, miR-202 stands out, believed to possess anti-inflammatory properties by inhibiting inflammasome activation in nerve tissue and reducing trigeminal pain sensitization (Zhang et al., 2023), and miR-362, which contributes to the regulation of the NF-κB transcription factor pathway responsible for stimulating the production of primary inflammatory cytokines in glial cells during a migraine attack (Liu et al., 2017).

Although numerous studies have identified individual abnormalities in inflammatory cytokine levels or miRNA molecules in migraine patients, the synchronic relationship and shared regulatory network that may link these epigenetic markers (miR-202, miR-362) and neuroinflammatory outputs (IL-1β, IL-18) within the same clinical setting—specifically during an acute pain attack in women—has not yet been directly investigated, representing a clear research gap that warrants further investigation. Based on the above, the current study aims to evaluate the epigenetic and inflammatory regulatory pathway of migraine in females by analyzing the correlation matrix between the relative expression levels of miR-202 and miR-362, on the one hand, and serum IL-1β and IL-18 levels, on the other, during an acute pain attack, compared to a control group of healthy females. The scientific significance of this work lies in providing a deeper understanding of the molecular mechanisms underlying neuroinflammation associated with hormonal fluctuations in women, thus paving the way for the future exploitation of this molecular network as a co-biomarker signature in accurate diagnosis, or as a targeted gene and immunotherapeutic objective.

2. Materials and Methods

Study Design

A case-control study was conducted to evaluate the relative gene expression of miR-202 and miR-362 and to measure serum concentrations of interleukin-1 beta (IL-1β) and interleukin-18

(IL-18) in female migraine patients during an acute pain attack. All cases were diagnosed by a consultant neurologist according to the International Classification of Headache Disorders, Third Edition (ICHD-3) criteria.

Study Population

The study included 90 female participants who were divided into two groups: 50 patients diagnosed with migraine, samples were collected during acute pain attack; 40 healthy, age-matched volunteers without history of migraine or any neurological or immunological disorders. The inclusion criteria were adult females who signed the informed consent to participate in the study.

Patients with auto-immune diseases, active infections, malignancies or hypertension were excluded from the study, as were patients with a history of migraine (for the control group) or patients who had used corticosteroids or anti-inflammatory medications in the two weeks prior to sample collection to minimise any potential impact on target biomarker levels. The protocol was approved by the Institutional Ethics Committee and written informed consent was taken from all participants before the start of the study.

Serum Cytokine Assay:

The Serum levels of IL-1β and IL-18 were assessed by ELISA using commercial kits (SunLong Biotech) according to the manufacturer's instructions. Optical density was measured at 450 nm and concentrations were determined from the standard curve.

RNA Extraction and Gene Expression Analysis

Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Germany). cDNA was synthesised using the High-Capacity cDNA Kit (Applied Biosystems, USA). The relative expression of miR-202 and miR-362 was determined by RT-qPCR using the primers listed in Table 1. The U6 gene was used as reference gene for normalisation. Relative gene expression was calculated using the 2^-ΔΔCt method.

Table 1: The primer sequences for selected genes in the current study.

Genes	Nucleotides sequence
miRNA 202	TTCCTATGCATATACTTCTTTG
microRNA-362	AATCCTTGGAACCTAGGTGTGAGTAA
Universal	GTGCAGGGTCCGAGGT
U6	CTCGCTTCGGCAGCACA
	AACGCTTCACGAATTTGCGT

Statistical Analysis

All statistical analyses were performed using GraphPad Prism (version 10) software. Quantitative data were expressed as mean ± standard deviation (Mean±SD). The Independent Samples t-test was applied for comparing the group of patients in a migraine attack with the control group. The level of statistical significance was set at $p < 0.05$. The Pearson Correlation Coefficient was used to analyse the correlation between the gene expression levels of miR-202 and miR-362 and the serum concentrations of IL-1β and IL-18, which were visualised by the Correlation Heatmap.

3. Results

Statistical analysis revealed a significant and concurrent increase in serum inflammatory cytokine levels in migraine patients compared to the healthy control group, as shown in Figure 1. Specifically, the concentration of interleukin-1 beta (IL-1β) was significantly higher ($P = 0.0251$) in the patients, with a mean of 188.4 ± 58.38 pg/ml, compared to 165.6 ± 35.93 pg/ml in the healthy control group. In Figure 2, interleukin-18 (IL-18) exhibited an even more significant upward trend ($P = 0.0070$), with a mean concentration of 148.5 ± 37.56 pg/ml in the patients, compared to 126.3 ± 34.61 pg/ml in the healthy control group.

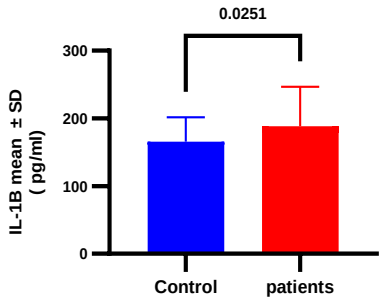


Figure (1): Comparison of serum interleukin-1 beta (IL-1β) concentration levels in the healthy control group and the migraine patient group, measured in pg/ml using ELISA. The bars represent the mean ± standard deviation (Mean ± SD). The numerical value at the top (0.0251) indicates a statistically significant difference between the two groups at a significance level of ($P < 0.05$).

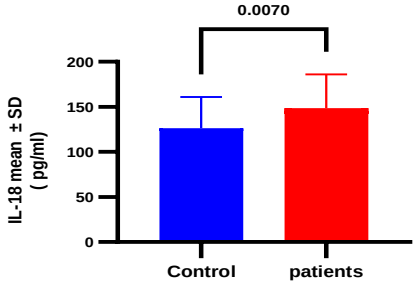


Figure (2): Comparison of interleukin-18 (IL-18) concentration levels in the serum of a healthy group (Control) and a group of migraine patients (Patients), measured in units (pg/ml) using (ELISA) technology. The graphs represent the mean ± standard deviation (Mean ± SD). The numerical value at the top (0.0070) indicates a highly statistically significant difference between the two groups at a significance level ($P < 0.01$).

The results of the (RT-qPCR) examination, as shown in Figure (3), showed a statistically significant increase in the relative gene expression of both markers in the serum of migraine patients compared to the healthy group. With regard to miR-202, a significant increase ($P = 0.0107$) was recorded in the mean and median expression in patients, reaching (2.044 ± 0.6855, and 1.997 respectively) compared to the healthy group (1.600 ± 0.4349, and 1.698), with a peak reading of (3.018) recorded in the affected patients. In Figure (4), miR-362 showed a statistically significant concurrent upward pattern ($P = 0.0347$); The mean and median concentrations of these genes were higher in the patient group (2.326 ± 0.7657 and 2.229, respectively) compared to healthy individuals (1.856 ± 0.5794 and 2.007), with a wider dynamic range (Range: 3.300) and a peak reading of 4.095 in the patients. These results confirm the high co-expression of these two genes, which is associated with the pathophysiology of migraine.

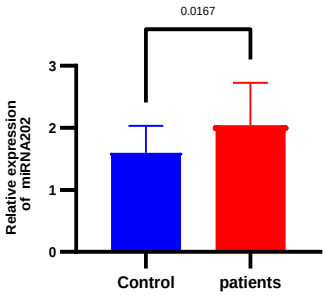


Figure (3): Comparison of relative gene expression levels of MicroRNA 202 in the serum of the healthy control group and the patient group. The bars represent the mean ± standard deviation (Mean ± SD). The numerical value at the top (0.0107) indicates a statistically significant difference between the two groups at a significance level of ($P < 0.05$).

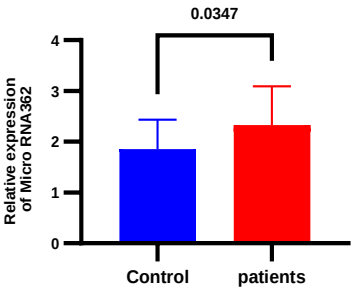


Figure (4): Comparison of relative gene expression levels of MicroRNA 362 in the serum of the healthy control group and the patient group. The bars represent the mean ± standard deviation (Mean ± SD). The numerical value at the top (0.0347) indicates a statistically significant difference between the two groups at a significance level of ($P < 0.05$).

Correlation analysis, represented in the heatmap matrix (Figure 5), revealed varying degrees of correlation between the studied variables in the serum of migraine patients. The color gradient in the figure, from purple (-1.0) to red (1.0), indicates the direction and strength of the relationship, and the values within the boxes represent the correlation coefficient (r). The analysis recorded a moderately strong and statistically significant positive correlation between the inflammatory cytokines IL-1β and IL-18 (r = 0.51, P = 0.001), coinciding with a statistically significant positive correlation between the gene expression levels of miR-202 and miR-362 (r = 0.41, P = 0.0427). In contrast, no statistically significant correlations (P > 0.05) were found between microRNA levels and cytokines. The probability values for the association of miR-202 with IL-1β and IL-18 were (P = 0.480) and (P = 0.423), and for the association of miR-362 with them were (P = 0.425) and (P = 0.962), respectively, indicating independent regulatory pathways for both cytokines and microRNAs despite their concurrent elevation in the patients' serum.

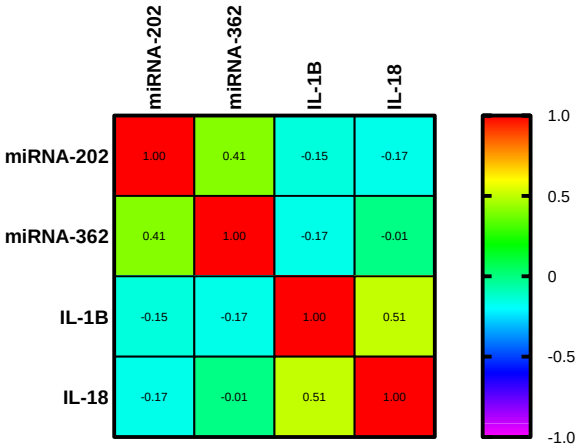


Figure 5: The figure (Correlation Heatmap) shows the correlation coefficients (r) between the gene expression levels of miR-202 and miR-362 and the levels of the inflammatory cytokines IL-1β and IL-18 in serum. The color gradient from purple (-1.0) to red (1.0) indicates the direction and strength of the correlation. The numerical values within the boxes represent the correlation coefficient (r). The values (P = 0.001) and (P = 0.0427) denote statistically significant correlations.

4. Discussion

In this study, we examined serum levels of interleukin-18 (IL-18) and interleukin-1β (IL-1β), as well as the gene expression of miR-202 and miR-362, in women with migraine during an attack, compared to healthy women. The results of this study demonstrate an association between inflammatory markers and epigenetic regulation in the pathophysiology of migraine in women.

The marked serum elevation of IL-1β and IL-18 levels in patients is closely consistent with previous literature classifying migraine as a chronic neuroinflammatory disorder (Martami et al., 2018). Stimulation of the trigeminovascular system leads to the release of these key pro-inflammatory cytokines, directly contributing to migraine pain signaling and promoting peripheral and central vascularization (Togha et al., 2022). Our current findings are fully consistent with the study by (Ying et al.,2021), which confirmed a simultaneous elevation of both IL-1β and IL-18 in the serum of female migraine sufferers. This is attributed to the close association between these two cytokines, as they are both products of the activation of the shared NLRP3 inflammasome pathway. The study by (Fidan et al.,2022) further supports these findings,

as researchers observed a significant increase in serum IL-18 levels during migraine attacks and correlated these elevated levels with pain intensity and attack duration in patients, thus supporting the role of this specific cytokine as a direct stimulator of the trigeminovascular system.
In the study (Sarchielli et al.,2020) no statistically significant difference was found in peripheral (serum) IL-1β concentration in patients versus healthy individuals during the interictal phase, although in some studies, there was significant variation. Thus, it can be assumed that the release of this cytokine is a localised, temporary event limited to the acute migraine attack (ictal phase). However, a study by (Domitrz et al.,2023) showed variation in cytokine behaviour, with a small increase in IL-1β, but no significant increase in serum IL-18 levels in random samples of migraine patients without gender differentiation. Thus, the cumulative increase of IL-18 may be a biological feature associated with female hormonal specificity and cyclical oestrogen changes rather than a general phenomenon of all migraine patients.
MicroRNAs play a pivotal role as epigenetic regulators by controlling gene expression after transcription (O'Brien et al., 2018). Among these molecules, MicroRNA-362 (miR-362) and MicroRNA-202 (miR-202) have emerged as vital components in maintaining cellular homeostasis and regulating physiological responses within the nervous system. miR-362 is primarily involved in regulating cell proliferation and differentiation pathways, and communication between immune and neuronal cells, thus influencing the regulation of neuroinflammation associated with migraine attacks (Gallelli et al., 2020; Reschke et al., 2021). On the other hand, miR-202 is closely associated with the regulation of hormonal functions and estrogen fluctuations (Domitrz et al., 2023), and with modulating the severity of inflammatory responses by directly influencing the production of neuropathic pain-inducing inflammatory cytokines in the trigeminal vascular system (Edvinsson et al., 2019; Ramachandran, 2018). Given their high stability in biological fluids, studying their serum expression levels contributes to understanding the molecular mechanisms underlying neurovascular disorders and supports their potential as promising molecular biomarkers for the diagnosis and monitoring of migraine progression (Andersen et al., 2024).
Based on the foregoing, these data provide supporting evidence for (Gallelli et al., 2020) proposal of a microRNA-cytokine axis that reflects disease severity and reinforces the potential of this combined system as a promising combination biomarker for migraine patients.
The current findings, which recorded significant elevations in serum inflammatory cytokine levels (IL-1β and IL-18) in migraine patients, are consistent with the findings of (Tan et al., 2021; Pereira et al., 2024). Their studies confirmed a chronic systemic inflammatory state reflected by increased production of pro-inflammatory cytokines resulting from the activation of innate immune pathways in these patients. The current finding of overexpression of miR-202 and miR-362 is consistent with the hypothesis of (Chen et al., 2023), who found a clear epigenetic disturbance in serum microRNAs during migraine attacks and attributed this to their role in regulating neurovascular sensitivity. In contrast, this study partially differs from that of (Domitrz et al., 2023) while their study recorded a slight increase in IL-1β, they found no significant increase in serum IL-18 levels in random samples of non-sex patients. This difference can be explained by the fact that our current study focused exclusively on female patients, supporting the hypothesis that the combined elevation of IL-18 and miR-202 is a biomarker associated with female hormonal specificity and cyclical estrogen fluctuations, rather than a general characteristic observed in males. Similarly, our results contradict those of(Li et al.,2022), who reported a decrease in serum levels of certain regulatory microRNA molecules. This discrepancy is attributed to differences in the nature and size of the samples, the geographic and ethnic diversity of the patients, and the varying time periods for sample collection between the acute and interictal phases of migraine attacks.
Based on the above, the results of this study provide strong molecular evidence of the pivotal role played by the interconnected biological network consisting of (miR-202/miR-362/IL-1β/IL-18) in shaping the pathological mechanism of migraine in females. The confirmed statistically significant serum elevation of IL-1β and IL-18 does not merely represent a biomarker of persistent neuroinflammation and activation of the trigeminal vasculature system, but rather

reflects a deeper molecular defect driven by loss of epigenetic control resulting from disruption of expression of miR-202 and miR-362, which fail to suppress the NLRP3 inflammasome pathway. Despite the notable discrepancy in the international literature regarding the levels of these cytokines between and during seizure periods, this quadruple synergy, combined with hormonal specificity for females, demonstrates the efficacy of this integrated system as a promising and highly sensitive biomarker. These conclusions open new therapeutic and diagnostic horizons based on targeting epigenetic and immune pathways simultaneously, which paves the way for the development of personalized medicine strategies to manage migraine attacks and reduce their severity in women more effectively. Without sources

The relationship between the levels of miR-202, miR-362, IL-1β, and IL-18 in the blood serum of female patients with migraine.

The identified relationships between the levels of miR-202, miR-362, IL-1β, and IL-18 in the blood serum of female patients with migraine highlight an important interconnected neuroinflammatory pathway that influences both susceptibility and severity of migraine attacks. Overall, clinical clinical studies analyzing these specific biomarkers show that proinflammatory cytokines (IL-1β and IL-18) are positively associated with each other, are directly regulated by co-expressed microRNAs (such as miR-202 and miR-362), and closely correspond to clinical parameters such as attack frequency and pain intensity.

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