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Inflammatory Cytokine Signatures Are Associated With Disease Burden and Comorbidity of Episodic Migraine and Endometriosis

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Abstract

Objectives

The aim of this study was to characterize inflammatory cytokine profiles in women diagnosed with episodic migraine, endometriosis, or both conditions and to determine how these cytokine patterns relate to symptom severity and functional impact, to identify potential biological markers distinguishing comorbid cases from single-diagnosis cases.

Methods

Female patients with only episodic migraine, only endometriosis, or both conditions were enrolled. Plasma levels of proinflammatory cytokines were measured, and correlations with clinical parameters were analyzed.

Results

Women with episodic migraine had elevated levels of IL-1β, IL-6, and TNF-α compared with healthy controls, with even higher levels in those with both migraine and endometriosis, indicating a synergistic effect on systemic inflammation. IL-1β correlated with headache frequency and disability while IL-6 and TNF-α were linked to migraine severity and pain. Women with endometriosis alone did not show similar cytokine elevations, suggesting that inflammation is particularly amplified in comorbidity. Changes in leukocyte distribution further supported a unique immune activation profile in the comorbid group.

Discussion

These findings reveal novel biological evidence of a shared inflammatory endotype in women suffering from both conditions, which may contribute to the increased burden and comorbidity, highlighting the need for integrative diagnostic and management approaches.

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Supplementary Material

Introduction

Chronic inflammation is increasingly recognized as a shared pathogenic mechanism in both migraine and endometriosis—2 prevalent, debilitating conditions with substantial impacts on quality of life. Migraine, once viewed primarily as a vascular disorder, is now understood as a neuroinflammatory disease involving activation of the trigeminovascular system and elevated levels of proinflammatory cytokines such as IL-6, TNF-α, and CGRP.[1] Similarly, endometriosis is characterized by ectopic endometrial tissue growth, driven by a proinflammatory peritoneal environment and resulting in pain and infertility.[2] Epidemiologic data suggest a strong comorbidity between

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migraine and endometriosis, with co-affected individuals experiencing more severe gynecologic disease and heightened pain.[3-5] This overlap points to shared inflammatory pathways, particularly involving IL-6 and TNF-α, and may be potentially modulated by hormonal fluctuations such as estrogen levels. Despite these insights, the inflammatory profiles specific to individuals suffering from both conditions remain poorly defined. Thus, the aim of this study was to characterize the inflammatory cytokine signatures of patients with comorbid migraine and endometriosis and to assess their correlation with clinical outcomes.

Methods

Participants

This study enrolled 36 female patients admitted to the Endometriosis Unit and the Headache Center at Tor Vergata University Hospital of Rome and diagnosed with only episodic migraine (eMG-O) (n = 11), with only endometriosis (EM-O) (n = 12), or with concomitant episodic migraine and endometriosis (eMG-EM) (n = 13). The inclusion criteria were women of premenopausal age (18–50 years), with a diagnosis of migraine made according to the criteria of the International Headache Society (IHS ICHD-3, 2018)[6] and of endometriosis according to the ESHRE guidelines,[7] as reported.[8] All participants were evaluated at the time of enrollment by a gynecologist who confirmed the diagnosis of endometriosis based on the combination of clinical symptoms and transvaginal ultrasound imaging (TVS). According to available ESHRE guidelines, these TVS direct features can be considered diagnostic and do not require a surgical confirmation.[7] The exclusion criteria were hormonal therapy in the past 6 months; a history of other neurologic or psychiatric conditions; altered consciousness, organ failure, or severe infectious diseases that could have precluded the clinical pathway; the presence of generalized medical disorders (history of renal, thyroid, or liver disease; cancer; diabetes; cardiovascular disorders); and unavailability to fill in self-administered clinical scales. Healthy control participants (n = 12) were age-matched and without any history of neurologic, psychiatric, or internal conditions or cancer. All patients underwent detailed medical history collection related to their main demographic and clinical characteristics. All patients underwent blood tests at the time of enrollment, during the proliferative phase of the menstrual cycle. Cell leukocyte counts were also reported for all participants. The Table provides demographic, clinical, and immune characteristics of the study population.

ELISA

Tumor necrosis factor-alpha (TNF-α), interleukin-1beta (IL-1β), and interleukin-6 (IL-6) levels were determined by measuring absorbance at 450 nm through specific ELISA kits (ThermoFisher Scientific), according to the manufacturer's instructions. Cytokine concentrations were calculated from the absorbance values of the samples, plotted on a standard calibration curve as percentage of controls. Detection limits of ELISA tests were found to be <0.01 pg/mL for TNF-α, <0.05 pg/mL for IL-1β, and <0.05 pg/mL for IL-6.

Statistical Analysis

All data were expressed as mean ± SEM. Differences between groups were compared using one-way ANOVA, followed by a post hoc Bonferroni test. Correlations between cytokine levels and clinical parameters were analyzed using the Spearman rank correlation coefficient. Differences were considered significant when $p < 0.05$. The value of n reported within figure legends represents the number of human participants. All statistical analyses were performed with GraphPad Prism 10.1.

Standard Protocol Approvals, Registrations, and Patient Consents

The study was performed according to the guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of the University Hospital of Rome, Tor Vergata. Written informed consent was obtained from all the participants involved in the study (protocol number 119/21).

Data Availability

Anonymized data not published within this article will be made available by request from any qualified investigator.

Results

Proinflammatory Cytokines Are Amplified in Episodic Migraine and Its Comorbidity With Endometriosis

The results revealed that the levels of all the 3 analyzed proinflammatory cytokines followed a similar trend in the different groups (Figure 1). In particular, TNF-α, IL-1β, and IL-6 were significantly increased in the episodic migraine–only (epMG-O) and episodic migraine + endometriosis (epMG-EM) groups compared with healthy controls (HCs) while no significant difference was observed in the endometriosis-only (EM-O) group. Of interest, patients with epMG-O showed higher levels of TNF-α and IL-1β levels, but not of IL-6, compared with those with EM-O, whereas patients affected by both disorders were characterized by higher amounts of IL-1β and IL-6, but not TNF-α, compared with those with epMG-O. Although there were differences in mean age between groups, cytokine levels did not correlate with age (eFigure 1).

Proinflammatory Cytokines Differentially Associate With Clinical Severity in Episodic Migraine and Migraine-Endometriosis Comorbidity

To further explore the inflammatory profile associated with episodic migraine and endometriosis, we analyzed the relationship between cytokine levels (TNF-α, IL-1β, and IL-6) and clinical parameters, including headache-related disability, disease duration, and leukocyte counts. In the epMG-O group, despite TNF-α showing no significant correlations in

Table Main Demographic and Clinical Characteristics of the Study Population

	eMG-O (n = 11)	EM-O (n = 12)	eMG + EM (n = 13)
Age (y)	42.9 ± 12.6	30.3 ± 7.1	35.2 ± 8.6
Weight (kg)	64.1 ± 4.9	58 ± 11.8	60.3 ± 10.2
Body mass index (kg/m^2)	23.2 ± 1.7	21.5 ± 2.6	22.0 ± 2.5
Menarche (y)	12.2 ± 1.0	11.4 ± 1.2	12.1 ± 1.7
Migraine disease duration (y)	13.1 ± 5.0	—	14.7 ± 14.6
Monthly migraine days	10.3 ± 4.1	—	7.0 ± 5.0
Headache intensity (VAS)	8.7 ± 0.6	—	8.7 ± 1.2
Migraine with aura, n (Y/N)	2/11	—	1/13
Pure menstrual migraine	—		—
Menstrually related migraine	3/11		4/13
Nonmenstrual migraine	8/11		9/13
Cutaneous allodynia, n (Y/N)	7/11	—	2/13
Analgesics drugs			
NSAID	8/11		13/13
Triptans	3/11		—
Pharmacologic preventive treatment	—	—	—
HIT-6 score	67.2 ± 3.9	—	61.1 ± 15.9
MIDAS score	21.1 ± 5.6	—	21.0 ± 10.7
Cycles (regular/irregular)	7/2	10/2	7/6
Menstruation (normal/abundant/scanty)	11/0/0	6/5/1	5/7/1
Prior hormonal therapy[a] (Y/N)	5/11	6/12	9/13
Dysmenorrhea (VAS)	—	5.1 ± 4.3	8.5/13
Dyspareunia (VAS)	—	3 ± 3.1	3.4/13
Dyschezia (VAS)	—	0.5 ± 1.8	1.4/13
Dysuria (VAS)	—	—	1.4/13
Intestinal disorders, n (Y/N)	—	1/12	2/13
Dysmenorrhea, n (Y/N)	—	7/12	11/13
Dyspareunia, n (Y/N)	—	6/12	6/13
Pelvic pain, n ((Y/N)	—	—	1/13
Dyschezia, n (%)	—	1/12	2/13
Dysuria, n (Y/N)	—	—	2/13
Infertility (primary/secondary), n (Y/N)	—	1/12	2/13
Endometrioma, n (Y/N)	—	4/12	3/13
Adenomyosis, n (Y/N)	—	8/12	7/13
Endometriosis, n (Y/N)	—	3/12	4/13
Endometriosis + adenomyosis, n (Y/N)	—	—	5/13
WBC (10^3/mL)	7.1 ± 1.6	6.6 ± 2.8	6.9 ± 0.8
Neutrophils (10^3/mL)	4.2 ± 1.3	3.7 ± 2.0	4.0 ± 0.3

Continued

Table Main Demographic and Clinical Characteristics of the Study Population *(continued)*

	eMG-O (n = 11)	EM-O (n = 12)	eMG + EM (n = 13)
Lymphocytes (10^3/mL)	1.9 ± 0.4	2.2 ± 0.9	2.3 ± 0.5
Monocytes (10^3/mL)	0.5 ± 0.1	0.5 ± 0.10	0.4 ± 0.09
Eosinophils (10^3/mL)	0.1 ± 1.0	0.2 ± 0.06	0.1 ± 0.10
Basophils (10^3/mL)	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.03
NLR	2.4 ± 0.60	1.7 ± 0.90	1.7 ± 0.2

Abbreviations: eMG-O = episodic migraine only; EM-O = endometriosis only; HCs = healthy controls; HIT-6 = Headache Impact Test; MG-EM = migraine and endometriosis; MIDAS = Migraine Disability Assessment Test; n = number; N = No; NLR = neutrophil-to-lymphocyte ratio; NSAID = nonsteroidal anti-inflammatory drug; WBC = white blood cell; y = years; Y = yes.
Data are reported as mean ± SD.
[a] >6 months before enrollment.

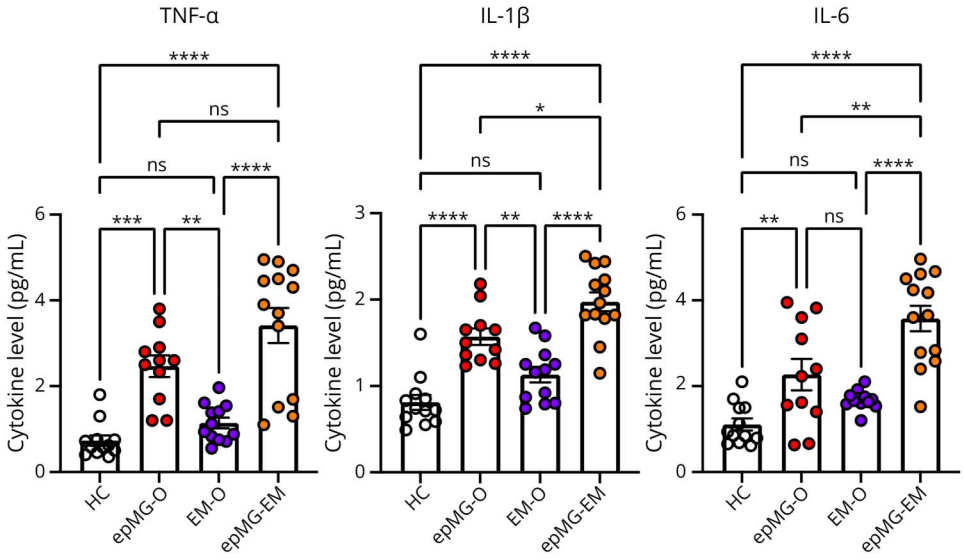
the epMG-O group (eFigure 2), the levels of IL-1β were significantly associated with migraine-related disability, including HIT-6 and MIDAS scores, as well as headache frequency and neutrophil-to-lymphocyte ratio (NLR) (Figure 2A and eFigure 3), pointing to systemic immune involvement. IL-6 was positively associated with headache frequency (Figure 2B) but not with other clinical or hematologic parameters (eFigure 4).

By contrast, among patients with epMG-EM, TNF-α levels were significantly associated with HIT-6 scores, lymphocyte count, and NLR (Figure 2C and eFigure 5), highlighting its potential role in the inflammatory profile of comorbid patients. Furthermore, even if IL-1β did not significantly associate with any clinical or immune markers in the epMG-EM group (eFigure 6), IL-6 emerged as the most clinically relevant cytokine in this group, correlating with multiple parameters including HIT-6 scores, MIDAS scores, headache frequency, and dysmenorrhea severity, as well as lymphocyte count and NLR (Figure 2D and eFigure 7).

Discussion

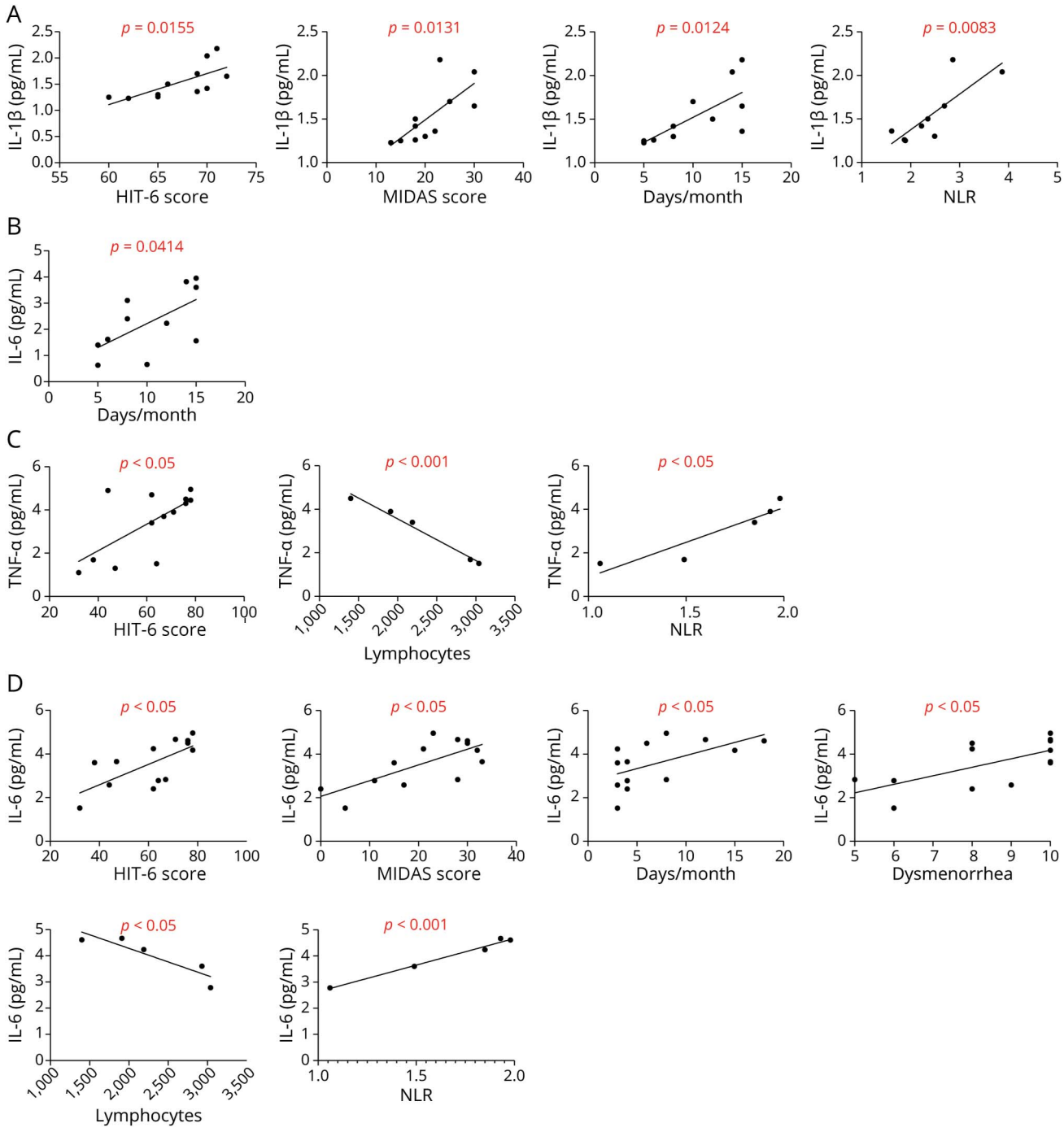
This study uncovers distinct inflammatory profiles in women with episodic migraine (epMG) and those with comorbid endometriosis (epMG-EM), focusing on the roles of TNF-α, IL-6, and IL-1β. Our findings suggest that endometriosis may act as a trigger or amplifier of migraine through systemic inflammation, highlighting overlapping inflammatory pathways in both conditions. Endometriosis is starting to increasingly be considered as a chronic inflammatory disease, with elevated cytokines such as IL-1β, IL-6, and TNF-α contributing to pain, lesion growth, and immune

Figure 1 Cytokines in Patients With eMG and EM



Histograms of TNF-α, IL-1β, and IL-6 plasma levels in healthy controls (HCs), patients with episodic migraine (epMG-O), patients with endometriosis (EM-O), and patients with episodic migraine + endometriosis (epMG-EM). Data are presented as mean pg/mL ± SEM. *$p \leq 0.5$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$ determined by one-way ANOVA, followed by the Bonferroni multiple comparison test.

Figure 2 Correlations Between Cytokine Levels and epMG-O or eMG + EM Clinical Parameters



Correlation plots of plasma TNF-α, IL-1β, and IL-6 with HIT-6 scores, MIDAS scores, days/month, and dysmenorrhea as well as the count of lymphocytes and NLR in patients with only episodic migraine (A and B) or in patients with comorbidity episodic migraine-endometriosis (C and D). Data were compared by linear regression analysis ($p < 0.05$). epMG-O = episodic migraine only; MIDAS = Migraine Disability Assessment Test; NLR = neutrophil-to-lymphocyte ratio.

dysregulation. These cytokines are also implicated in migraine, where they modulate pain signaling and neuroinflammation.[9-12] Our study supports the notion that shared inflammatory pathways may underlie the high comorbidity between migraine and endometriosis. Indeed, IL-1β was significantly associated with migraine severity (HIT-6 and MIDAS scores) and headache frequency in women with epMG only, suggesting its role in migraine burden through immune activation. This cytokine also correlated with NLR, a marker of systemic inflammation. However, this association was absent in the epMG-EM group, possibly because of dominant roles of TNF-α and IL-6 in comorbidity. In the epMG-EM group, TNF-α was significantly associated with migraine-related disability and with lymphocyte count and NLR. This

indicates its heightened role in systemic immune activation when both diseases coexist. TNF-α is known to drive lesion invasiveness and systemic inflammation in endometriosis, while also modulating nociceptive signaling in migraine.[9,13] IL-6 emerged as a key player in comorbidity. In participants with epMG-EM, IL-6 correlated with headache severity, frequency, and dysmenorrhea as well as lymphocyte count and NLR, suggesting that IL-6 might play a central role in both peripheral and central sensitization mechanisms driving migraine and endometriosis symptoms. The interplay between inflammation, estrogen, and pain sensitization is a crucial element of this comorbidity.[14,15] Fluctuations in estrogen also affect cytokine production, supporting the theory of hormonal influence on inflammation-related migraine severity. Although the migraine-only group is higher in age than the other 2 groups, clinically, these findings suggest that targeting proinflammatory cytokines such as TNF-α and IL-6 may be beneficial, particularly in the comorbid population. Biologics and anti-inflammatory agents used in other chronic inflammatory diseases may hold promise for treating migraine-endometriosis comorbidity. Hormonal treatments used in endometriosis could also modulate migraine severity indirectly via inflammation pathways. Future longitudinal studies and expanded cytokine profiling are needed to validate and build on these findings.

Author Contributions

M. Albanese: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. V. Ceci: analysis or interpretation of data. G. Carrera: analysis or interpretation of data. A. Selntigia: major role in the acquisition of data. C. Exacoustos: major role in the acquisition of data. M. Tiberi: analysis or interpretation of data. S. Saracini: analysis or interpretation of data. A. Matteocci: analysis or interpretation of data. N.B. Mercuri: drafting/revision of the manuscript for content, including medical writing for content. V. Chiurchiù: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data.

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Disclosure

The authors report no relevant disclosures. Go to Neurology.org/NN for full disclosures.

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