MCP-1 and IL-8 as Pain Biomarkers in Fibromyalgia: A Pilot Study

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Abstract

Objective. Although fibromyalgia (FM) is traditionally a non-inflammatory condition, emerging data also suggest that FM has an immunologic component. Previous studies have reported that peripheral blood concentrations of two chemokines (i.e., interleukin-8 [IL-8] and monocyte chemotactic protein-1 [MCP-1]) were elevated in FM patients compared with normal controls. We sought to determine the longitudinal relationships of changes in the levels (picogram/mL) of IL-8 and MCP-1 with changes in the severity of FM-related pain.

Design. Secondary data analysis of a cohort of 16 FM subjects who provided blood samples at two time points: week 1 and week 12.

Setting. Urban rheumatology clinic practices.

Patients. Individuals who met the American College of Rheumatology 1990 criteria for FM.

Outcome Measures. Changes from week 1 to week 12 of the following variables: Brief Pain Inventory (BPI) pain severity and plasma concentrations of IL-8 and MCP-1.

Results. Change in BPI pain severity was significantly associated with changes in IL-8 and MCP-1 plasma concentrations. Specifically, for each unit increase in the change of BPI pain severity, IL-8 increased by 2.5 pg/mL ($P = 0.03$) and MCP-1 increased by 9.4 pg/mL ($P = 0.006$). None of the covariates (i.e., body mass index, medications, severity of depression, and overall FM burden) were significantly associated with either chemokines.

Conclusion. Although preliminary, our findings raise the hypothesis that IL-8 and MCP-1 may be involved in the pathogenesis of FM. If replicated in a larger study, IL-8 and MCP-1 may assist in determining prognosis and in monitoring of treatment response.

Key Words. Chemokines; IL-8; MCP-1; Biomarkers; Chronic Pain; Fibromyalgia

Introduction

Fibromyalgia (FM) is a common disorder that is characterized by diffuse pain, generalized fatigue, cognitive complaint, and sleep disturbance. In 2005, approximately five million people in the United States have FM [1]. The diagnosis of FM is challenging because there are no laboratory tests to confirm the condition. Currently, the diagnosis of FM is based on the presence of chronic pain and other unexplained medical symptoms [2] and the presence of at least 11 out of 18 tender points (1990 American College of Rheumatology criteria) [3].

In the last 15 years, the neurobiology of FM has mostly focused on central sensitization. Central sensitization refers to enhanced responsiveness of neurons in the central nervous system that leads to pain amplification. A summary of experimental pain studies has shown that FM patients show increased sensitivity to mechanical, thermal, and electrical stimuli [4].

Although evidence for the role of central sensitization in FM is well supported in the literature, emerging data also suggest that FM is a neuroimmunoendocrine disorder. Elevated corticotrophin-releasing hormone (CRH) levels in both cerebrospinal fluid (CSF) and blood have been reported in FM patients [5,6]. Increased numbers of activated mast cells have been observed in association with...
immunoglobulin G (IgG) deposits in skin biopsies of patients with FM [7,8]. Substance P, whose CSF levels are increased in FM [9,10], has been shown to stimulate mast cells to release chemokines including monocyte chemotactic protein-1 (MCP-1) and interleukin-8 (IL-8) [11]. More importantly, elevated levels of MCP-1 and IL-8 in the peripheral blood of FM patients have now been reported separately by at least two different research laboratories [12–15].

In the current report, we sought to determine the longitudinal relationships of changes in the levels of IL-8 and MCP-1 with changes in the severity of FM-related pain. We hypothesized that increasing levels of IL-8 and MCP-1 would be correlated with worsening FM-related pain severity. We based our hypothesis on the following: 1) the putative role of IL-8 and MCP-1 in animal models of pain [16,17]; 2) the emerging literature on the immunologic basis of FM [18]; and 3) the consistent reported elevation of peripheral IL-8 and MCP-1 levels in prior cross-sectional studies in FM [12–15].

Methods

This is a secondary data analysis of a 12-week randomized clinical trial (RCT) of the effect of cognitive behavioral therapy (CBT) on nociceptive responsivity and clinical symptoms in FM patients [19]. Details of the trial design and treatment intervention have previously been published [19]. In summary, subjects who satisfied the eligibility criteria and gave written informed consent were randomized to one of two treatment arms: six weekly sessions of CBT in addition to usual care (UC) or UC alone. Subjects were evaluated at three time points: week 1 (study entry), week 6 (post-intervention), and week 12 of the study time line. Assessments included completion of web-based self-administered questionnaires including a detailed drug history. Blood sample collection, while not a requirement, was done at the same time of the day for each participant at week 1 and week 12.

Study procedures, including written informed consent, were approved by Indiana University-Purdue University Indianapolis Institutional Review Board.

Participants

All 28 subjects met the following inclusion criteria: fulfilled the American College of Rheumatology classification criteria for FM [3], had a Fibromyalgia Impact Questionnaire (FIQ)-physical impairment score ≥2, FIQ-pain score ≥4, and had been on stable doses of FM-related medications for at least 4 weeks. We enrolled only female subjects and excluded those who had diabetes, peripheral neuropathy, demyelinating disorders, and inflammatory rheumatic diseases. Subjects were allowed to take all their FM-related medications (i.e., anti-depressant, anticonvulsant and opiates) until the day of the assessment, except for non-steroidal anti-inflammatory drugs (NSAIDs), which were discontinued 48 hours before the assessment.

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Symptom-Related Questionnaires

Brief Pain Inventory (BPI)

The BPI is a pain assessment tool that has been proven reliable, valid, and responsive to change among patients with chronic non-malignant pain [20–22]. BPI measures pain severity, which is the average of four items asking about worst, least, and current pain in the past week, and current level of pain.

Patient Health Questionnaire 8-item depression scale (PHQ-8)

PHQ-8 is a brief self-administered scale, which assesses major depressive disorder core symptoms and allows a score (range: 0–24) based on the total number and severity of depressive symptoms noted over the previous 2-week period [23–27]. A PHQ-8 score ≥10 represents clinically significant depression.

FIQ

The FIQ is a 10-question survey, which assesses the impact that FM has on daily living activities as well as pain level, depression, tiredness, and anxiety. FIQ scores range from 0 to 100 with higher FIQ scores representing worse illness impact. The FIQ has been used in many studies and has consistently proven to be a reliable measure of the impact that FM-related symptoms has on daily living [28,29].

Quantitative Assay of Chemokines

Although measuring chemokines in the CSF and ascertaining mRNA cytokine production are far superior to simple plasma determination, we have chosen simple plasma determination of chemokine levels for three reasons: 1) accessibility of blood; 2) potential use in the clinical setting (e.g., quicker turnaround of test result and ease of use); and 3) lower cost.

At week 1 (study entry) and week 12, a standardized blood sample was taken, centrifuged (2,000 U/min) at 4°C and stored at −80°C. The blood samples were analyzed for plasma levels of IL-8 (picogram/mL) and MCP-1 (picogram/mL) using the BD Cytometric Bead Array (BD Pharmingen, San Diego, CA, USA) Chemokine Kit.

Statistical Analysis

In this report, the dependent variables were changes in the plasma concentrations of IL-8 and MCP-1 from week 1 to week 12. The independent variables included the following: changes in BPI pain severity, FIQ, and PHQ-8 from week 1 to week 12; body mass index (BMI) and use of medications (by class) at study entry; and treatment group assignment (CBT vs UC). Univariate general linear models were used to determine the associations of the independent variables with changes in the levels of IL-8 and MCP-1. Variables that were found to be significant at the
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Table 1  Associations of clinical variables with changes in levels of interleukin-8

<table>
<thead>
<tr>
<th>Δ Interleukin-8</th>
<th>Univariate Analysis</th>
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<th>Multivariate Analysis†</th>
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<tr>
<td></td>
<td>Parameter Estimate</td>
<td>P Value</td>
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<td></td>
<td>(Standard Error)</td>
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<td>(Standard Error)</td>
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<tr>
<td>Δ BPI pain severity</td>
<td>2.5 (1.1)</td>
<td>0.04</td>
<td>2.5 (1.0)</td>
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<tr>
<td>Δ PHQ-8 depression</td>
<td>0.2 (0.3)</td>
<td>0.4</td>
<td>-3.8 (2.3)</td>
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<tr>
<td>Δ FIQ</td>
<td>0.08 (0.1)</td>
<td>0.5</td>
<td></td>
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<tr>
<td>Body mass index at study entry</td>
<td>0.04 (0.2)</td>
<td>0.8</td>
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<td>Medications use at study entry†</td>
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<tr>
<td>Antidepressant</td>
<td>-0.7 (3.1)</td>
<td>0.8</td>
<td></td>
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<tr>
<td>Anticonvulsant</td>
<td>-3.7 (2.7)</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Opiate</td>
<td>-0.2 (2.9)</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>NSAIDs</td>
<td>-2.2 (2.8)</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Group assignment (CBT/usual care)</td>
<td>0.8 (2.9)</td>
<td>0.8</td>
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Δ represents the change in the specified variable from week 1 to week 12.
† Variables with P value ≤ 0.2 in the univariate analyses were incorporated into multivariate regression.
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<tr>
<th>Parameter Estimate (Standard Error)</th>
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0.20 level were then included in the multivariate analyses. Significance in the multivariate analyses was determined at the 0.05 level.

The primary objective of the study was to investigate the change from week 1 to week 12; therefore, the primary analyses were limited to subjects with available blood samples at week 1 and week 12. We used Wilcoxon Rank Test for continuous variables and Fisher’s Exact Test for categorical variables to compare baseline characteristics of subjects with available blood samples at both time points vs those with samples at one or no time points.

Results

The previously published RCT consisted of 28 FM subjects [19]. Sixteen (57%) of the 28 subjects had blood samples at both time points and, therefore, comprised the study population of interest. Of the 12 subjects who were excluded from the analyses, eight subjects had blood samples at week 1 only and four subjects had none at either time points. More subjects in the UC group from the parent RCT were included in the analyses by virtue of the fact that they have blood samples at two time points. In the parent RCT (N = 28), there were 13 subjects (46%) from the UC group and 15 subjects (54%) from the CBT group. In the current analyses, 10 subjects (62%) were from the UC group and six subjects (37%) were from the CBT group.

The 16 female participants had a median (25th, 75th percentile) age of 53 years (45.0, 56.5); 69% were white and 69% had at least a high school education. At study entry, the participants had median disease duration of 11.5 years (6.0, 16.5); BMI of 31.5 (27.2, 38.2) kg/m²; 50% were on NSAIDs; 44% were on anticonvulsants; 56% were on opiates; and 69% were on antidepressants. The median PHQ-8 depression score was 13.5 (8.5, 15.0) with 10 subjects (62%) having clinically significant depression. Whereas the median FIQ score was 65.9 (55.3, 74.9), the median BPI pain severity score was 6.2 (5.2, 7.7). In terms of baseline demographic and clinical characteristics (including severity of depression and levels of IL-8 and MCP-1), no significant differences (P ≥ 0.22) were noted between the study population of interest and the other 12 subjects who were excluded from the analyses.

Table 1 shows the associations between the clinical variables and the week 1 to week 12 change in IL-8. In the univariate analyses, only the week 1 to week 12 change in BPI pain severity was statistically significant (P = 0.04). PHQ-8 depression, FIQ, BMI, and medication use were not associated with the change in IL-8. On multivariate analysis, the change in BPI pain severity remained significantly (P = 0.03) associated with the change in IL-8, even after controlling for the use of anticonvulsant. Specifically, for each unit increase in the change in BPI pain severity, the change in IL-8 concentration increased by 2.5 pg/mL. The change in BPI pain severity explained 27.8% of the variation of the change in IL-8. Figure 1 shows the linear relationship between the change in BPI pain severity and the change in IL-8 plasma concentration. The correlation of these two change variables was r = 0.5 (P = 0.03). As seen in Figure 1, one subject (subject #2129) reported no change in BPI pain severity but had a large increase in the plasma concentration of IL-8. Excluding this outlier from the analysis, the change in BPI pain severity remained significantly associated with the change in IL-8 concentration (parameter estimate 2.6, P = 0.004).

Table 2 shows the associations between the clinical variables and the week 1 to week 12 change in MCP-1. In the
univariate analyses, only change in BPI pain severity reached statistical significance ($P = 0.01$). PHQ-8 depression, FIQ, BMI, and medication use were not associated with the change in MCP-1. On multivariate analysis, the change in BPI pain severity remained significantly associated with the change in MCP-1, even after controlling for the use of anticonvulsant. Specifically, for each unit increase in the change in BPI pain severity, the change in MCP-1 concentration increased by 9.4 pg/mL. The change in BPI pain severity explained 38.9% of

Table 2: Associations of clinical variables with changes in levels of MCP-1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Univariate Analysis</th>
<th>Multivariate Analysis $^\dagger$</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Parameter Estimate</td>
<td>$P$ Value</td>
</tr>
<tr>
<td></td>
<td>(Standard Error)</td>
<td></td>
</tr>
<tr>
<td>$\Delta$ BPI pain severity</td>
<td>9.3 (3.1)</td>
<td>0.01</td>
</tr>
<tr>
<td>$\Delta$ PHQ-8 depression</td>
<td>1.0 (1.0)</td>
<td>0.4</td>
</tr>
<tr>
<td>$\Delta$ FIQ</td>
<td>0.3 (0.4)</td>
<td>0.3</td>
</tr>
<tr>
<td>Body mass index at study entry</td>
<td>0.2 (0.6)</td>
<td>0.7</td>
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<tr>
<td>Medications use at study entry $^\ddagger$</td>
<td></td>
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<tr>
<td>Antidepressant</td>
<td>5.1 (9.7)</td>
<td>0.6</td>
</tr>
<tr>
<td>Anticonvulsant</td>
<td>$-$11.6 (8.6)</td>
<td>0.2</td>
</tr>
<tr>
<td>Opiate</td>
<td>8.3 (8.9)</td>
<td>0.4</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>$-$1.1 (9.1)</td>
<td>0.9</td>
</tr>
<tr>
<td>Group assignment (CBT/usual care)</td>
<td>$-$3.0 (9.3)</td>
<td>0.8</td>
</tr>
</tbody>
</table>

$\Delta$ represents the change in the specified variable from week 1 to week 12.

$^\dagger$ Variables with $P$ value $<0.2$ at the univariate analysis were incorporated into multivariate regression.

$^\ddagger$ Modeled as binary variables, where yes = 1 and no = 0.

BPI = brief pain inventory; PHQ-8 = patient health questionnaire-8; FIQ = fibromyalgia impact questionnaire; NSAIDs = non-steroidal anti-inflammatory drugs; CBT = cognitive behavioral therapy; MCP-1 = monocyte chemotactic protein-1.
the variation of the change in MCP-1. Figure 2 shows the linear relationship between the change in BPI pain severity and the change in MCP-1 plasma concentration. The correlation of these two change variables was \( r = 0.6 \) (\( P = 0.0009 \)). As seen in Figure 2, one subject (subject #2129) reported no change in BPI pain severity but had a large increase in the plasma concentration of MCP-1. Excluding this outlier from the analysis, the change in BPI pain severity remained significantly associated with the change in MCP-1 concentration (parameter estimate 9.8, \( P = 0.0006 \)).

**Discussion**

In this secondary data analysis, increasing levels of IL-8 and MCP-1 correlated with worsening severity of pain. Whereas self-report pain in FM was longitudinally associated with changes in peripheral blood chemokine concentrations; depression, BMI, and use of medications were not related to the immune markers. Our findings, albeit preliminary, support the growing literature on the immunologic aspect of FM.

Cytokines/chemokines and its relationship with pain was first observed in 1988 when Wallace et al. noted the development of FM-like pain among cancer patients after treatment with interleukin-2 therapy [30]. Since then, many studies have looked at different cytokines in FMS patients [12–15,31,32]. Although two previous studies have reported reduced or normal levels of chemokines in FM [14,33], a greater number of studies have shown elevated plasma concentrations of IL-8 and MCP-1 [12–15,34]. Based on these latter studies and the current report, it is reasonable to hypothesize that IL-8 and MCP-1 are involved in the pathogenesis of FM.

Taken together, a theoretical model of the pathogenic role of IL-8 and MCP-1 can be described as follows: 1) there are increase amount of skin mast cells in FM [7,8]; 2) substance P and CRH, both elevated in the CSF of FM patients [5,6,9,10], enters the periphery and activate skin mast cell [11,35,36]; 3) activated skin mast cells release pro-inflammatory molecules including IL-8 and MCP-1 [37–41]; 4) because of the anatomic interaction between mast cells and sensory nerves [42,43], pro-inflammatory molecules activate the sensory nerves (c-fibers); and 5) persistent low-grade inflammation in the mast cell-sensory nerve local milieu may contribute to increased tonic nociceptive input into the spinal cord that results in augmented pain processing and central sensitization [44]. In support of our theory, it is important to note that mast cells production of cytokines has also been described in complex regional pain syndrome (CRPS). Specifically, increased blister fluid level of mast cell derived tryptase [45] and elevated blood concentration of IL-8 level [46] have both been reported in CRPS.

This is the first study in FM that showed longitudinal relationships between the severity of clinical pain and the levels of chemokines. Except for one study by Wang et al.
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[34], previous FM studies that looked into cytokines/chemokines were cross-sectional in nature with measurements done only at a single time point. Although Wang et al.’s study had a longitudinal study design the authors did not assess the association between changes in chemokine concentrations with changes in clinical pain severity from time 1 to time 2 [34].

Our study has several limitations. First, the small sample size and the absence of pain-free controls are two major limitations of our study. As such, the study results should be viewed as preliminary and warrants confirmation in a larger cohort of FM patients along with a control group. Second, 43% (N = 12) of the original 28 subjects were excluded from our primary analyses, and therefore could have biased our study results. However, the magnitude of bias was likely minimal because we noted no differences in the baseline characteristics (including depression severity) of those with blood samples at two time points (N = 16) vs those without (N = 12). Third, concomitant medications and depression status may have confounded our study findings. Residual confounding due to medications was likely minimal because medication types and doses were stable throughout the 12-week study period. On the other hand, although depression has been associated with higher concentrations of pro-inflammatory cytokines, a recent meta-analysis of cytokines in major depression has found that IL-8 levels were not significantly different among the depressed and non-depressed subjects [47]. More recently, Lehto et al. have actually documented lower (and not higher) levels of MCP-1 and IL-8 in majorly depressed group compared with the healthy controls [48]. Finally, given that FM is a heterogeneous disorder, our results may not apply to all FM patients. For example, compared with FM subjects with normal growth hormone (GH) response to exercise, only FM subjects with defective GH response have elevated pre-exercise levels of IL-8 [49].

While our study does not implicate a cause and effect relationship, the longitudinal associations of FM-pain severity with the plasma concentrations of IL-8 and MCP-1 raise the question that these two chemokines maybe involved in the pathogenesis of FM. If our findings are replicated in a larger cohort study, IL-8 and MCP-1 may facilitate the prediction of prognosis and monitoring of treatment response in the future.

Acknowledgments

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References


