

Original Article

Elevated Serum MCP-1/CCL2 Levels are Linked with Disease Severity in Patients with Fibromyalgia Syndrome

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Background: Elevated monocyte chemotactic protein-1/chemokine C-C motif ligand 2 (MCP-1/CCL-2) levels have been identified in fibromyalgia (FM) patients.

Aim: The current study was conducted to examine the potential association among serum MCP-1/CCL2 levels with disease severity of FM.

Study Design: Cross-sectional study

Methods: Seventy-nine female FM patients and 75 healthy normal controls were incorporated in our study. Serum MCP-1/CCL2 levels were explored by enzyme linked immune sorbent assays. The existence of tender points was evaluated based on the standardized manual tender point examination. The pressure pain thresholds at knees and bilateral trapezius muscles were measured by the Algometer. The visual analogue scale (VAS) and revised Fibromyalgia Impact Questionnaire (FIQ) were utilized to assess degree of pain and functional abilities.

Results: Serum MCP-1/CCL2 levels were suggestively greater in FM patients compared with healthy controls (151.6±31.9 pg/mL vs 103.3±25.2 pg/mL, $P<0.001$). Severe FM patients had significantly higher CCL2 levels in serum than mild and moderate FM patients (173.1±21.9 pg/mL vs 151.0.0±35.1 pg/mL, $P=0.01$). Moderate FM patients revealed markedly augmented levels of CCL2 in serum

compare to mild patients (151.0±35.1pg/mL vs 133.3±23.9 pg/mL, $P=0.03$). Serum CCL2 levels were positively associated with tender point scores ($r=0.455$, $P<0.001$). Additionally, serum CCL2 levels were also positively associated with the pressure pain thresholds in both knees and bilateral trapezius muscles (knees: $r=-0.349$, $P=0.002$; trapezius muscles: $r=-0.318$, $P=0.004$). Lastly, we found elevated serum CCL2 levels were also positively associated with VAS ($r=0.368$, $P=0.001$) and FIQ score ($r=0.401$, $P<0.001$).

Conclusion: Elevated serum MCP-1/CCL2 levels are linked with disease severity of FM. Therapeutic interventions inhibiting MCP-1/CCL2 in FM deserves additional studies.

Keywords: Monocyte chemotactic protein-1; chemokine C-C motif ligand 2; fibromyalgia; disease severity

Fibromyalgia (FM) is a complicated chronic disorder that influences nearly 6% of the adults and is mainly characterized by broad pain (1,2). FM also includes accompanying symptoms in patients, for example non-restorative sleep, tiredness, poor physical conditioning, diminished cognition, stiffness, depression, and balance mutilation (3), leading to the significant economic burden due to high prevalence of work loss (4).

Even though the causes of FM remain unidentified, sensitization of the central nervous system (CNS) and musculoskeletal dysregulation has been regarded as two vital factors in the advancement of FM (5), indicating that the reason of great level of pain is the magnification of the sensory inputs from CNS to musculoskeletal system.

In recent years, immunological dysfunction and inflammation have been identified to have imperative roles in FM (6). As one type of important inflammatory cytokines, chemokines are comprised of a family of small soluble molecules of around 70 amino acid residues with a molecular weight of 7–12 kDa (7). Chemokine ligands are separated into four families (C, CC, CXC, CX3C) as per cysteine residue regions, and every ligand produces specific impacts on numerous cells via the stimulation of ligand-specific receptors (8). Chemokine ligands and receptors are widely expressed throughout organ tissues and cells, under physiological as well as pathological conditions (9). They regulate chemotaxis as well as the stimulation of numerous kinds of populations of leukocytes, and hence are major controllers of leukocyte traffic (10). Under pathological conditions, chemokines play pivotal roles on broad reactions of inflammatory as well as immune responses via the chemoattraction of innate plus adaptive immune cells (11).

The monocyte chemotactic protein 1 (MCP-1), also acknowledged as CCL2 (chemokine CC motif ligand 2), is a chemotactic cytokine under the CC chemokine family (12). CCL2/MCP-1 acts as an important factor for the recruitment as well as trafficking of mononuclear and immune cells to inflammation sites (13) and has pivotal functions in the development of chronic inflammatory syndromes such as osteoarthritis, atherosclerosis, rheumatoid arthritis, and multiple sclerosis (14-16). Recent studies have indicated the potential role of MCP-1/CCL-2 in FM. High levels of MCP-1/CCL-2 have been detected in FM patients compared with controls (17). In addition, myoblasts can secrete MCP-1 whereas treatment with MCP-1 instigated secretion of IL-1 β (17). In a rodent experimental study, MCP-1/CCL2 caused long-lasting mechanical hyperalgesia as well as induced a state of chronic sensitization to other algogens (18), through functioning on its binding receptor CCR2. MCP-1/CCL2 is also involved in the initiation of persistent muscle pain after repetitive exposures to stressful stimuli (18). In addition, injection of MCP-1/CCL2 into the gastrocnemius muscle led to a dosage and time dependent reduction in mechanical nociceptive threshold (18).

All the above-mentioned studies implicated that MCP-1/CCL-2 plays an imperative function in the pathogenesis as well as advancement of FM. Nevertheless, none of the studies have investigated the relationship among serum MCP-1/CCL-2 levels and disease severity of FM. Thus, the purpose of the current study was to determine if circulating MCP-1/CCL-2 levels are correlated with severity in FM patients.

Materials and Methods

Study Patients

From Sep 2017 to Jan 2019, 79 patients diagnosed with FMS satisfying the American College of Rheumatology criteria of FMS were allowed to participate in this study (19). Patients were excluded if they have active inflammatory or autoimmune diseases; unstable medical or psychiatric illness; hemorrhaging or active bleeding; thrombosis or angina pectoris; pregnancy or lactation; heart disease; substance abuse in the last year; lumbar or cervical disk disease; and back or neck injuries with 6 months. All the criteria were pre-evaluated and confirmed by our senior doctors before enrollment. Healthy controls consisted of 75 healthy women receiving regular body examination during the same period. Gathered data also comprised height as well as weight measurements, which were utilized to calculate body mass index (BMI). The current study was approved by the local Institutional Review Board. Patients provided signed informed consents.

Definition of tender point score

The existence of tender points was evaluated based on the standardized manual tender point examination (19). The number of tender points was recorded at 18 designated places on the body, and the extent of every tender point was evaluated by the following: 0, no tenderness; 1, mild tenderness (identified response when asked); 2, moderate tenderness (spontaneous response); and 3, severe tenderness (can not bear and moving away). Hence, the probable number of tender points ranged between 0 and 18, whereas the probable total score ranged between 0 and 54. In our study, tender point scores between 0-18 were regarded as mild, whereas 19-36 defined as moderate and 37-54 regarded as severe. The tender points of all patients were assessed by two experienced independent therapists. *Kappa* value was recorded for the consistency of the tender point calculation.

Assessment of pressure pain thresholds (PPTs)

The PPTs at knees and bilateral trapezius muscles (joint and non-joint) were measured using a Wagner Force 10 FDX Algometer (Wagner Instruments, USA). The 1-cm² rubber algometer probe was located right at each selected site by the physician. Pressure was augmented at a rate of 0.50 kgf per second till the presence of pain, which was defined as the PPT. Low PPTs at knee joint were regarded as indicators of peripheral sensitization, whereas low PPTs at knee as well as trapezius muscles were regarded as markers of central sensitization (20). To acquire the mean PPT for each site, trials were repeated for three times. We averaged the PPTs acquired on both sides during all three tests.

Definition Of Clinical Severity

Clinical assessment of FM was evaluated by the VAS (21) and revised version of Fibromyalgia Impact Questionnaire (FIQ) (22). For VAS, patients were shown a 10-cm ruler that was described as ranging from no pain to the worst presumable pain on the right. Patients were requested to indicate the point alongside the ruler suggesting their extent of pain. Scores were recorded by determining the distance

between the 0 cm and the indication, with scores ranging from 0 to 10. The total score of revised FIQ ranges from 0 to 100 including three items: functional activity (0-30 points) including nine items, overall impact condition (0-20 points) with two items, and symptomatic severity (0-50 points) containing ten items (22). The higher scores of FIQ indicate worse status.

Laboratory Examination

Serum was acquired from the blood drained by antecubital vein puncture at 8:00 o'clock before breakfast. The blood extractions of all participants were done by a senior nurse. Following extraction, blood samples for serum isolation were kept for 20 minutes at room temperature. The blood was centrifuged at 1500 g for 10 min. Then, the serum samples were aliquoted and kept at -80°C before measurement. Serum MCP-1/CCL2 concentrations (R&D Systems, MN, USA) were investigated using commercially available kits according to the suppliers' instruction manuals. The detection range was 31.2 - 2,000 pg/mL. The intra-assay and inter-assay CV for MCP-1/CCL2 were 4.9% and 5.9%, respectively. Each sample examination was repeated three times and the average result was calculated.

Statistical Analysis

Data analysis was performed with Graphpad 6.0 software (USA). Values are given as means \pm standard deviations (SD) or median. The Shapiro–Wilk test was utilized for normal distribution and Levene's test was carried out for equality of variance. Group comparisons were calculated using One-way ANOVA for parametric data or Kruskal–Wallis test for nonparametric statistics. The Spearman or Pearson analysis was carried out to test correlation of CCL2 levels with other parameters. A two-tailed test was done $P < 0.05$ meant statistical significance. The statistical power ($1 - \beta$) was computed using IBM SPSS 21.0 Software. The result was calculated depending on the achieved data of various mean serum MCP-1/CCL2 levels, standard error, and enrolled numbers of patients in each group. Statistical power was considered strong when > 0.8 . The formula was depicted as below (23):

$$1 - \beta = \varphi(z - z_{1-\alpha/2}) + \varphi(-z - z_{1-\alpha/2}), z = (\mu_A - \mu_B) / \sigma \left(\frac{1}{n_A} + \frac{1}{n_B} \right)$$

Results

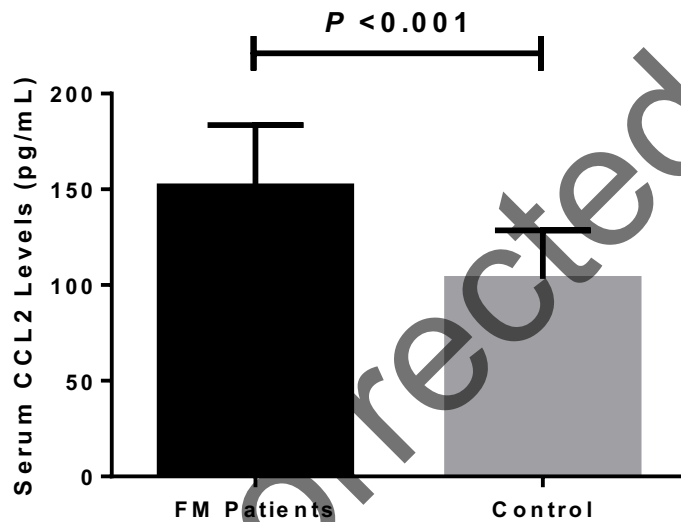
Demographic Data

The statistical power was 0.97 after quantification. Demographic data of all subjects enrolled in the current study were depicted in Table 1. The mean age of FM females was 42.4 ± 11.7 years, ranging from 22 to 65 years and average age of healthy females was 41.9 ± 11.5 years. There were no substantial dissimilarities of age between both groups ($P = 0.178$). Also, the difference of BMI between FM females and healthy controls did not reach significance (23.2 ± 2.0 kg/m² vs 22.8 ± 1.9 kg/m², $P = 0.084$). Serum MCP-1/CCL2 concentrations were suggestively greater in FM patients compared with healthy individuals (151.6 ± 31.9 pg/mL vs 103.3 ± 25.2 pg/mL, $P < 0.001$) (Table 1; Figure 1).

Table 1 Demographic Characteristics

	FM patients (n=79)	Healthy controls (n=75)	P value
Age (Y)	42.4 ±11.7	41.9 ± 11.5	0.178
BMI(kg/m ²)	23.2±2.0	22.8±1.9	0.084
VAS score	4.1±1.5	/	/
Mild/Moderate/Severe	27/28/24	/	/
FIQ function score	18.8±6.0	/	/
FIQ overall impact score	12.0±3.5	/	/
FIQ symptom score	36.9±5.9	/	/
Serum CCL2 levels (pg/mL)	151.6±31.9	103.3±25.2	<0.001

BMI: body mass index; VAS: Visual Analogue Scale; FIQ: Fibromyalgia Impact Questionnaire

**Figure 1.** Comparison of serum CCL2 levels among FM patients and control

Correlation of serum MCP-1/CCL2 levels with tender point score

The *Kappa* value was 0.84 after assessment of tender point scores. According to the classification of disease severity based on the total point scores, 27 patients were defined as mild, whereas 28 were as moderate and 24 as severe. Serum CCL2 levels were suggestively greater in moderate FM patients compare to mild FM females (151.0±35.1pg/mL vs 133.3±23.9 pg/mL, $P=0.03$). Severe FM patients had markedly elevated serum CCL2 levels than moderate FM patients (173.1±21.9 pg/mL vs 151.0±35.1 pg/mL, $P=0.01$) and mild FM patients (173.1±21.9 pg/mL vs 133.3±23.9 pg/mL, $P<0.001$) (Figure 2A). Lastly, we found that serum CCL2 levels were suggestively and positively related to total tender points ($r=0.455$, $P<0.001$) (Figure 2B).

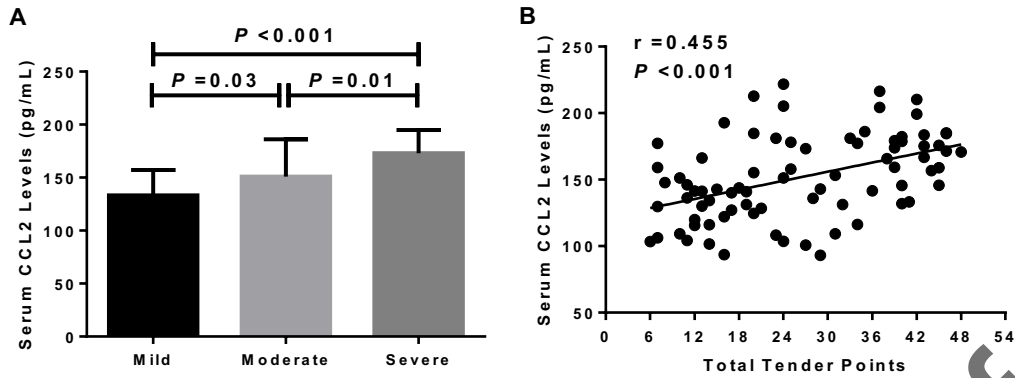


Figure 2. A. Assessment of serum CCL2 levels amongst FM patients with different disease extent B. Relationship of serum CCL2 levels with total tender points in FM patients

Correlation of serum MCP-1/CCL2 levels with PPTs

To further explore the systemic MCP-1/CCL2 levels on pain sensitization, we tested the pressure pain on knee and trapezius muscle as mentioned above. We found average PPTs of knee and trapezius muscles were suggestively lesser in FM patients compare to controls (average knee PPT: 1.34 ± 0.16 kg/cm² vs 1.53 ± 0.15 kg/cm², $P < 0.001$; average trapezius muscle PPT: 2.02 ± 0.18 kg/cm² vs 2.10 ± 0.18 kg/cm², $P = 0.004$) (Figure 3A, 3B). In addition, we found elevated serum MCP-1/CCL2 levels were related to average PPTs of knee and trapezius muscles (knee: $r = -0.349$, $P = 0.002$; trapezius muscles: $r = -0.318$, $P = 0.004$) (Figure 3C, 3D).

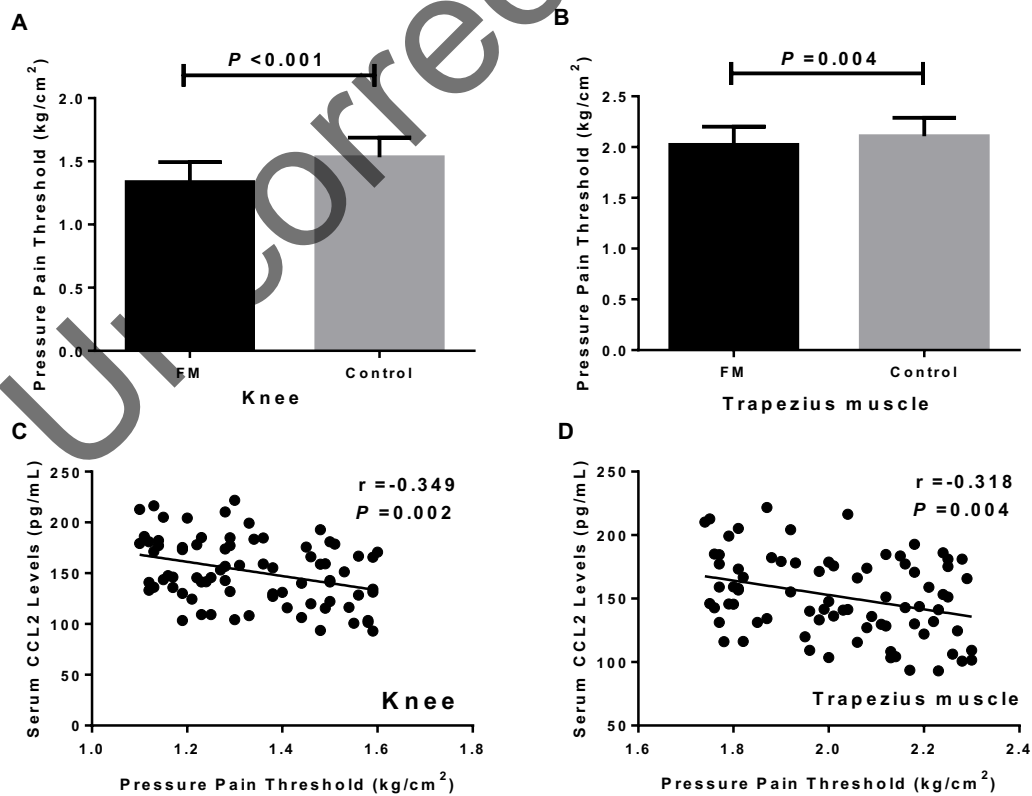


Figure 3. A. Assessment of average knee pressure pain threshold between FM patients and controls B. Comparison of average trapezius muscle pressure pain threshold between FM patients and controls C. Relationship of serum CCL2 levels with average knee pressure pain threshold in FM patients D. Relationship of serum CCL2 levels with trapezius muscle knee pressure pain threshold in FM patients

Correlation of serum MCP-1/CCL2 levels with clinical severity

We last examined the relationship between serum MCP-1/CCL2 levels with clinical severity distinguished by VAS score and FIQ scale. We found that serum MCP-1/CCL2 intensities were positively related to VAS score ($r=0.368$, $P=0.001$) (Figure 4A). In addition, serum MCP-1/CCL2 intensities were also positively related to revised FIQ function ($r=0.399$, $P<0.001$) (Figure 4B) and symptom score ($r=0.401$, $P<0.001$) (Figure 4D). Although the differences between serum CCL2 levels and FIQ overall impact score did not achieve significance, there still existed slight correlation ($r=0.217$, $P=0.054$).

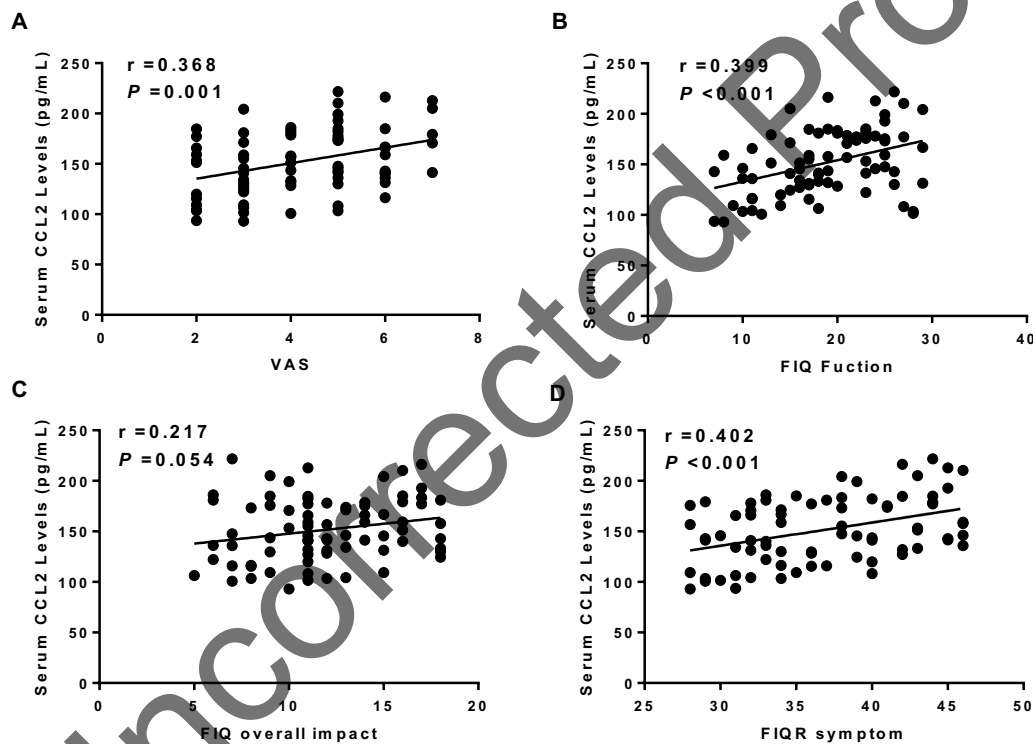


Figure 4. A. Relationship of serum CCL2 levels with VAS score in FM patients B. Relationship of serum CCL2 levels with FIQ function score in FM patients C. Relationship of serum CCL2 levels with FIQ overall impact score in FM patients D. Relationship of serum CCL2 levels with FIQ symptom score in FM patients

Discussion

The current investigation seeks to inspect the relationship of serum MCP-1/CCL2 levels with disease severity in females with fibromyalgia syndrome. We observed that serum MCP-1/CCL2 concentrations were positively associated with higher tender point score, lower PPTs and clinical severity in FM females. Our findings along with other previous studies indicated a critical involvement of MCP-1/CCL2 in FM pathophysiological process.

In recent years, examination of particular as well as detectable biomarkers that might help in accurately recognizing susceptible individuals, verifying disease diagnosis and expediting treatment, has been regarded as a novel method in FM research (24). The development of potential biomarkers has confirmed to be mainly relevant in neurological research where small molecules are vital in neurochemical metabolism and play crucial roles as neurotransmitters and signaling modulators (25). Advancements in the basic as well as clinical research over the years have validated the idea that FM is not only a central sensitivity disorder related to abnormal pain processing, but that a broader methodology is required—comprising a systemic angle—in view of a substantial influence of the inflammatory reaction (6). It is now generally anticipated that an array of inflammatory markers, connected to systems biology approaches, will transpire with well-define phenotypes, increasing the understanding of pathophysiological process of FM (26). Recently, the presence of a few inflammatory biomarkers in peripheral blood has been documented in FM, possibly revealing new visions into inflammation in the FM (27,28). And the activated pain-related nervous system that characterizes fibromyalgia is dependent on modulation partially by cytokines and chemokines (29).

In the current study, we first observed that serum MCP-1/CCL2 levels were suggestively greater in FM patients compared with controls, which is consistent with the previous study (17). In a previous study, plasma levels of MCP-1/CCL2 were also elevated in women exposed to continued psychosocial stress (30). Pain is the main indication and allodynia as well as hyperalgesia are usual indications in FM. We next found that serum MCP-1/CCL2 levels were positively related to tender point score, VAS score and FIQ score. Previous study has found that MCP-1/CCL2 as well as its respective receptor CCR2 is both upregulated in numerous subpopulations of sensory neurons (31). Stimulation of CCR2 by MCP-1/CCL2 causes membrane depolarization, activates action potentials as well as sensitizes nociceptors via transactivation of transient receptor potential channels TRPA1 and TRPV1 (31). All these studies indicate that MCP-1/CCL2 participates in pain in FM.

This study has certain limitations. First, our study was done only in included female FM patients; investigation of MCP-1/CCL2 levels in male patients would help to obtain an accurate conclusion. Second, the current study only included Chinese people. Therefore, the findings might not be directly valid to individuals of other ethnicities. Third, only serum MCP-1/CCL2 levels were examined, examination of other potential chemokines might offer vital information. Last and important, the current study was designated as a cross-sectional study. Consequently, no inferences concerning cause and effect associations can be reached. Hence, prospective longitudinal studies are needed to record disease advancement and describe the potential function of MCP-1/CCL2 in FM.

Collectively, we showed suggestively elevated systemic expression of MCP-1/CCL2 and demonstrated a significantly positive relationship with the extent of disease severity in Chinese FM females. Our findings might help to understand the function of MCP-1/CCL2 in the pathogenesis of FM.