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ORIGINAL ARTICLE

# Fecal calprotectin levels in patients with fibromyalgia: A cross-sectional study

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***ABSTRACT***

**Objectives:** This study aimed to evaluate levels of fecal calprotectin (FC), a biomarker that helps distinguish irritable bowel syndrome from inflammatory gut disorders, in patients with fibromyalgia syndrome (FMS) and compare them to healthy controls.

**Patients and methods:** The cross-sectional study was carried out on patients diagnosed with FMS according to the ACR (American College of Rheumatology) 2016 classification and healthy controls between January 2021 and February 2022. FMS patients were grouped according to the absence (Group 1) or presence (Group 2) of gastrointestinal symptoms. A third group of healthy controls without gastrointestinal complaints (Group 3) was included. Demographic data, comorbidities, medications, symptom severity scale, and widespread pain index scores were recorded. All subjects were asked to provide stool samples for the measurement of FC levels.

**Results:** A total of 100 subjects were included in the study. There were 33 patients (4 males, 29 females, mean age: 46.9±10.6; range, 22 to 69 years) in Group 1, 32 patients (2 males, 30 females, mean age: 48.5±11.0; range, 22 to 73 years) in Group 2, and 35 patients (11 males, 24 females, mean age: 39.2±13.1; range, 22 to 67 years) in Group 3. Group 2 had significantly higher levels of FC compared to Group 3 ($p<0.05$). The number of patients with positive FC values was similar between the three groups. Symptom severity scale and widespread pain index scores were significantly worse in Group 2 compared to Group 1 ($p<0.05$).

**Conclusion:** Irritable bowel syndrome usually coexists in many patients with FMS, and this may cause a misdiagnosis or delay in the diagnosis of organic gastrointestinal conditions. The value of FC in screening and diagnosis of organic disease in FMS patients needs further evaluation.

***Keywords:*** Abdominal pain, fecal calprotectin, fibromyalgia syndrome, irritable bowel syndrome.

Fibromyalgia syndrome (FMS) is a chronic widespread pain condition that is often accompanied by sleep disturbances, fatigue, cognitive symptoms, and somatic symptoms.[1] These somatic symptoms may cause a delay in diagnosis and require a detailed clinical assessment to avoid misdiagnosis. In addition, most patients with FMS also have overlapping pain conditions, such as chronic migraine, temporomandibular dysfunction, interstitial cystitis, and irritable bowel syndrome (IBS).[2] Following depression and other mood and anxiety disorders, IBS is the most common comorbid health condition in this population.[2] IBS is defined as a functional disorder of the gastrointestinal tract with chronic abdominal pain and altered bowel habits, such as constipation and diarrhea. The link between FMS and IBS has been attributed to central sensitization and small fiber neuropathy.[3,4] The prevalence of IBS in fibromyalgia patients was reported as high as 73%.[5] Fatigue and unrefreshing sleep are key symptoms in FMS, which are also present in patients with IBS. In addition, FMS is also a common comorbidity in patients with other chronic disease conditions, such as diabetes mellitus, rheumatoid arthritis, and inflammatory bowel disease.

As any physician caring for patients with FMS would attest, obtaining a thorough history from these patients is an arduous process that

requires attention to rule out any comorbid organic disease that may exacerbate or mimic symptoms related to FMS. When a patient with FMS complains of abdominal pain, physicians may be inclined to group these symptoms under somatic manifestations of FMS, which may result in the underdiagnosis of underlying organic gastrointestinal disease.

Irritable bowel syndrome is diagnosed in the presence of abdominal pain, changes in stool consistency and frequency, and absence of pathological laboratory findings indicating organic gastrointestinal disease.[6] Ideally every patient presenting with these symptoms should undergo laboratory testing, including complete blood count, markers of inflammation, stool parasite tests, and serologic testing for celiac disease. However it was reported that a small percentage of patients seek medical attention, and only 40% of those patients with IBS have a formal diagnosis.[7]

Fecal calprotectin (FC) is a biomarker elevated in inflammatory gut conditions and is widely employed for differentiating IBS from inflammatory disorders. Conventionally, its levels are not expected to rise in functional bowel disorders such as IBS. To our knowledge, there is no previous study assessing FC levels in patients with fibromyalgia. Hence, this study aimed to assess intestinal inflammation by measuring FC levels in FMS patients with or without gastrointestinal symptoms and compare their FC levels with healthy controls.

## PATIENTS AND METHODS

The cross-sectional study was carried out on patients with FMS and healthy controls at the Ege University Faculty of Medicine Hospital between January 2021 and February 2022. FMS patients without gastrointestinal symptoms constituted Group 1, patients with gastrointestinal symptoms were included in Group 2, and healthy controls were included in Group 3. FMS patients were allocated to the study from the physiatry and rheumatology outpatient clinics of the hospital.Groups 1 and 2 included patients diagnosed with FMS according to the 2016 American College of Rheumatology classification criteria.[8] All patients were older than 18 years of age. The exclusion criteria were as follows: patients with gastrointestinal symptoms with an onset after 50 years of age, a diagnosis of inflammatory rheumatic disorders, presence of organic gastrointestinal disorder, and a history of fever, infective gastroenteritis, endoscopy, or colonoscopy in the previous month. Furthermore, patients with a family history of inflammatory bowel disease and patients with significant symptoms that required further investigation, such as weight loss, nocturnal diarrhea, and rectal bleeding, were excluded from the study. Illiterate patients were not included as they would have difficulty answering the study questionnaires. FMS patients were allocated to Group 2 if they had any of the following symptoms: abdominal pain, constipation, diarrhea, tenesmus, and abdominal cramps. If none of these symptoms were present, subjects were allocated to Group 1. The study protocol was approved by the Ege University Faculty of Medicine Medical Research Ethics Committee (date: 01.10.2020, no: 20-10T/47). Written informed consent was obtained from all participants. All steps of the study were carried out in accordance with STROBE guidelines.

Patients were questioned about their personal and family history and symptoms, and all participants underwent musculoskeletal, neurological, and abdominal physical examination. Widespread pain index and symptom severity scale scores were calculated and recorded.

After initial assessment and physical examination, all subjects were asked to provide a stool sample for the study. They were instructed on sample collection and storage. Fecal calprotectin levels were measured using the Calprest NG test (Eurospital, Trieste, Italy), which is an enzymatic immunoassay that detects and quantifies the S100A8/A9 antigen in the stool. Patients were instructed not to take nonsteroidal anti-inflammatory drugs (NSAIDs) during the week preceding stool sample collection. Patients were asked to keep their stool samples refrigerated before bringing them to the hospital for analysis. Levels under 50 µg/g of feces were considered negative. Levels above 50 µg/g of feces were quantified and were considered positive. Stool testing was carried out by a separate researcher from the biochemistry department that did not take part in clinical assessment and was blinded regarding patient groups.

### Statistical analysis

The sample size was calculated using the G*Power version 3.1 software (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany). Depending on a previous study that compared FC levels between healthy individuals and patients with IBD, which reported mean levels of FC in organic bowel disorders and healthy individuals as 70±52 µg/g and 24±6 µg/g, respectively,[9] we calculated the minimum sample size required for each group as 15, with 95% power and an alpha level of 0.05.

Data was analyzed using IBM SPSS version 20.0 software (IBM Corp., Armonk, NY, USA). Descriptive statistical methods were used for presenting demographic and disease characteristics. For categorical data, the chi-square test was used. The Kolmogorov-Smirnov test was utilized to assess parameters for normal distribution. To compare numerical data among the three groups, analysis of variance was used. The independent sample t-test was employed for pairwise comparisons between groups if the parameter showed a normal distribution, whereas the Mann-Whitney U test was used for pairwise comparisons, and the Kruskal-Wallis test was used for the comparison of all three groups. Pearson correlation analysis was used to assess the possible correlation between FC levels and clinical parameters. A p-value <0.05 was considered statistically significant.

## RESULTS

A total of 100 subjects were included in the study. The study flowchart is presented in Figure 1. There were 33 patients (4 males, 29 females, mean age: 46.9±10.6; range, 22 to 69 years) in Group 1, 32 patients (2 males, 30 females, mean age: 48.5±11.0; range, 22 to 73 years) in Group 2, and 35 patients (11 males, 24 females, mean age: 39.2±13.1; range, 22 to 67 years) in Group 3. The mean age was significantly lower in Group 3 ($p<0.05$). The majority of patients in FMS groups were female (91%), whereas a lower number of subjects in Group 3 were female (68.6%; $p<0.05$). Most control subjects were recruited from the university staff, which explains the higher ratio of college education in Group 3. Demographic data are presented in Table 1.

Of the subjects in Group 2, all 32 had abdominal pain. In addition to pain, constipation was the most common gastrointestinal complaint (n=14), followed by diarrhea (n=7), abdominal

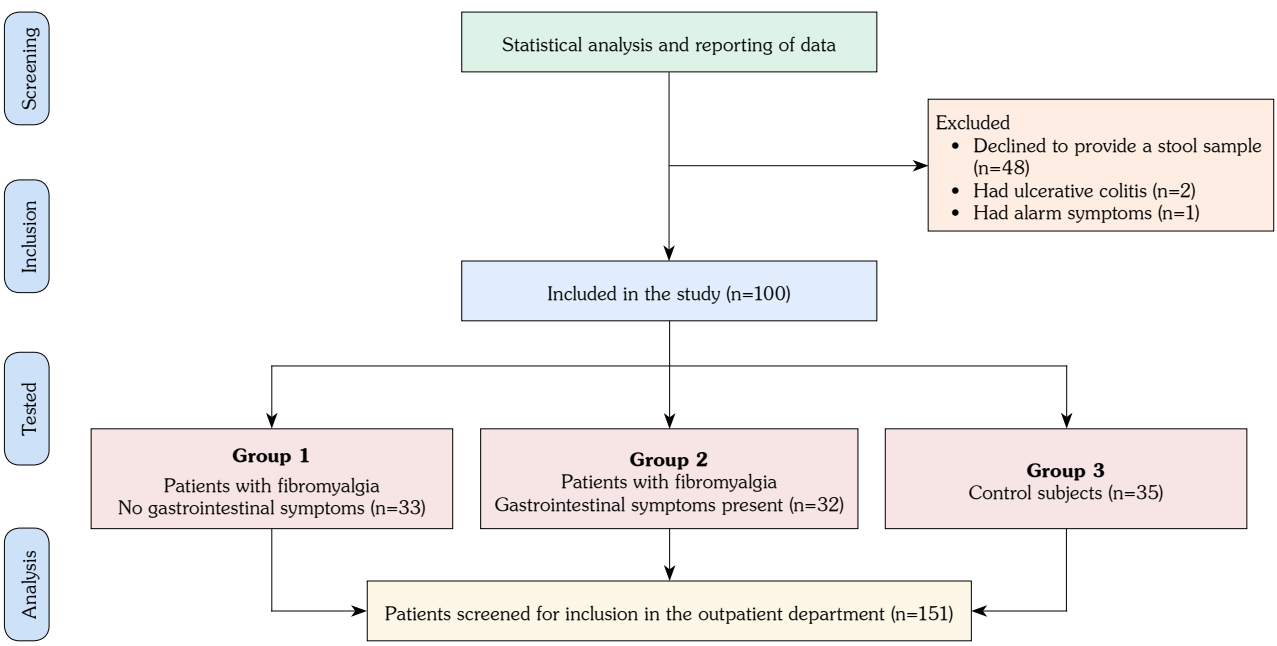


**Figure 1.** Flowchart of the study.

**Table 1.** Demographic characteristics

| | FMS group (n=33) | | FMS+GIS group (n=32) | | Control group (n=35) | |
|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | n | Mean±SD |
| Age (year) | | 46.9±10.6 | | 48.5±11.0 | | 39.2±13.1 |
| Sex | | | | | | |
| Female | 29 | | 30 | | 24 | |
| Male | 4 | | 2 | | 11 | |
| Level of education | | | | | | |
| Literate | 0 | | 1 | | 0 | |
| Primary education | 4 | | 9 | | 1 | |
| Secondary education | 10 | | 3 | | 1 | |
| Higher education | 11 | | 7 | | 26 | |
| Comorbidities | | | | | | |
| Asthma | 0 | | 1 | | 0 | |
| Diabetes | 1 | | 2 | | 2 | |
| Hypertension | 3 | | 4 | | 2 | |
| Hypothyroidism | 5 | | 2 | | 1 | |
| Nephrolithiasis | 0 | | 1 | | 0 | |
| Spinal stenosis | 0 | | 0 | | 1 | |
| Breast cancer | 1 | | 1 | | 0 | |
| Autoimmune hepatitis | 1 | | 0 | | 0 | |
| Ovarian cancer | 0 | | 1 | | 0 | |
| Prolactinoma | 0 | | 0 | | 0 | |
| Vitiligo | 0 | | 0 | | 1 | |
| Coronary artery disease | 1 | | 0 | | 1 | |
| FMS treatment | | | | | | |
| Duloxetine | 14 | | 13 | | | |
| Pregabalin | 1 | | 3 | | | |
| Amitriptyline | 0 | | 1 | | | |
| Venlafaxine | 1 | | 1 | | | |

FMS: Fibromyalgia syndrome; GIS: Gastrointestinal symptoms; SD: Standard deviation.

cramps (n=11), and tenesmus (n=6). FC results and comparisons of disease characteristics are presented in Table 2. Fibromyalgia symptoms in Group 2 were present for a significantly longer time compared to patients in Group 1 (7.4 *vs.* 4.4 years, p=0.01). Similarly, widespread pain index and symptom severity scores were significantly worse in Group 2 (p=0.04 and p=0.007, respectively). The number of patients with positive FC results were similar among the three groups (p=0.156). However, when quantitative results were compared pairwise between groups, FC levels in Group 2 were significantly higher than in Group 3 (p<0.05).

**Table 2.** Comparison of disease characteristics and FC levels among the groups

| | FMS group (n=33) | | | FMS+GIS group (n=32) | | | Control group (n=35) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean±SD | Median | Min-Max | Mean±SD | Median | Min-Max | Mean±SD | Median | Min-Max | *p* |
| Duration of FMS | 4.4±3.6 | | | 7.4±5.3 | | | | | | 0.01*† |
| Widespread pain index | 11.6±3.5 | | | 13.4±3.4 | | | | | | 0.04*† |
| Symptom severity scale | 8.0±2.0 | | | 12.0±4.8 | | | | | | 0.007*† |
| Number of fecal calprotectin positive subjects | | 10 | | | 12 | | | 7 | | 0.28# |
| Fecal calprotectin level (µg/g) | | 105.0 | 0-345.6 | | 199.0 | 0-1005.0 | | 66.5 | 0-156.0 | p1: 0.156<br>p2: 0.602<br>p3: 0.188<br>p4: 0.048* |

FMS: Fibromyalgia syndrome; GIS: Gastrointestinal symptoms, SD: Standard deviation, * p<0.05; # Chi-square test; † Independent samples t test; $p^1$: Comparison among the 3 groups, Kruskal Wallis test; $p^2$: Group 1 *vs.* Group 2, Mann Whitney U test; $p^3$: Group 1 *vs.* Group 3, Mann Whitney U test; $p^4$: Group 2 *vs.* Group 3 Mann Whitney U test.

We were not able to detect a correlation between FC levels and clinical parameters. A weak correlation was detected between FMS duration and symptom severity scale scores (r=0.435, p=0.015).

## DISCUSSION

In this study, we detected higher levels of FC in FMS patients who complained of gastrointestinal symptoms compared to healthy controls and FMS patients without gastrointestinal complaints. FC is a zinc- and calcium-binding microbicidal protein from the S100 family of proteins that is present in neutrophil granulocytes and becomes detectable after neutrophil migration and death in the gastrointestinal tract.[10] It is a clinical indicator of inflammation in the gastrointestinal system with high sensitivity and specificity and is widely used in scanning and follow-up of inflammatory bowel disease. In addition to ulcerative colitis and Crohn's disease, FC levels may also be elevated in other clinical conditions such as gastroenteritis, colonic polyps, colon cancer, cystic fibrosis, and systemic inflammation. Its high negative predictive value, ease of use, noninvasiveness, and low cost makes it a suitable surrogate for endoscopy in many conditions.[11] IBS is a functional disorder of the gastrointestinal system that is not correlated with elevated FC levels in the absence of coexistent inflammatory disorders.

Fibromyalgia syndrome is a widespread pain condition that often coexists with IBS. The nature of gastrointestinal symptoms in FMS and the relationship between widespread pain, IBS, anxiety, and mood disorders is a complex topic involving the interaction of central sensitization with pain perception and autonomic functions.[12] In the absence of alarming symptoms, such as rectal bleeding, unintentional weight loss, nocturnal diarrhea, and late onset after the age of 50, abdominal pain and an altered bowel habit in patients with FMS are often indicative of IBS.[13] In some cases, addition of new symptoms indicating underlying organic disease may be disregarded or unrecognized in a patient with FMS, particularly if these symptoms have been present for a long time and were previously associated with IBS. In this study, we detected similar numbers of FC-positive patients in each group; however, FC levels were significantly higher in FMS patients with gastrointestinal complaints compared to controls. By excluding patients with alarming symptoms, we aimed to target those with nonspecific abdominal symptoms that may be easily overlooked as gastrointestinal manifestations of FMS.

Fecal calprotectin levels were reported to be higher in the elderly population; however, these differences among different age groups were not as wide as the levels we observed between our FMS and control subjects.[14] None of the subjects in either Group 1 or Group 3 had FC levels in the ranges that were observed in Group 2. Healthy older adults were reported to have FC levels higher than younger adults, with a mean FC level of 160.3 µg/g (range, 0.93-545.9 µg/g) for subjects over 70 years of age.[14] In our study, patients with abdominal symptoms had a mean FC level of 262.9±181.2 µg/g, which was significantly higher compared to the other groups; however, the mean FC levels were not in the normal range in either age group. Therefore, the difference in FC levels was not considered to be a result of the age difference among the groups, which was different by approximately nine years between Groups 2 and 3.

Subjects in Group 2 were found to have higher widespread pain index and symptom severity scores. Although these constitute differences in baseline characteristics among the groups, abdominal pain and gastrointestinal symptoms are included in these scales; therefore, subjects in Group 2 having higher scores on those indices would be expected.

Fecal calprotectin was reported to have elevated levels in some systemic conditions other than gastrointestinal disease, such as ankylosing spondylitis, coronavirus disease 2019 (COVID-19), stroke, and Parkinson's disease.[15-18] In Parkinson's disease, it has been proposed that gastrointestinal inflammation is involved in the pathogenesis of neurodegeneration. Higher body mass index has also been correlated with higher FC levels.[19] NSAID use and proton pump inhibitors have also been reported to affect FC levels by stimulating neutrophils to migrate to the

gastrointestinal tract.[19] Patients with ankylosing spondylitis experienced a drop in FC levels after cessation of NSAID therapy. These findings suggest that many questions remain unanswered regarding FC and its role in the disease process and assessment.

This study had several limitations. This study aimed to detect FC levels in subjects with and without gastrointestinal symptoms. Although our goal was not to perform endoscopic evaluations for every patient, endoscopic examination, as the gold standard for diagnosing inflammatory and malignant gut conditions, would provide the most accurate assessment of symptoms. Another limitation of our study was its cross-sectional design. We were not able to follow the patients regarding their gastrointestinal symptoms. Some of the subjects with higher FC levels may go on to develop manifestations of inflammatory bowel disease. The control group had a significantly lower mean age ($p<0.05$). Although age was not found to be correlated to clinical parameters other than duration of FMS, this difference was not considered to be the reason for the difference in FC levels between groups, which constituted a limitation of the study. Nevertheless, the strength of this study lies in its being the first study to evaluate FC levels in patients with FMS.

In conclusion, FMS patients have a plethora of symptoms related to central sensitization and autonomic dysregulation. Most of their gastrointestinal symptoms may be explained by IBS, which has a higher incidence in these patients compared to healthy adults. FC levels were found to be higher in FMS patients with gastrointestinal complaints. Further studies are needed to determine whether this finding is due to the nature of the disease or accompanying organic pathologies.

**Data Sharing Statement:** The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Author Contributions:** Idea/concept, design, references and fundings: S.H., E.C.; Control/supervision: S.H.; Data collection and/or processing: E.C., M.B.T.A., B.B., M.B.; Analysis and/or interpretation: E.C., B.B.; Literature review, materials: E.C.; Writing the article: E.C., M.B.; Critical review: S.H., B.B.

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